Electronic Supplementary Material (ESI) for Physical Chemistry Chemical Physics.

Electronic Supporting Information for: "Interfacial electrostatic potential modulates the insertion of cell-penetrating-peptides into lipid bilayers"

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Movie: The file `cpp-transfer.mpg` shows a CPP molecule (R_9) moving back and forth between solution and the centre of the membrane, in the absence of ionic imbalance across the bilayer. Notice that this is a fragment of a biased Molecular Dynamics (Metadynamics) trajectory covering only a couple of translocation events.

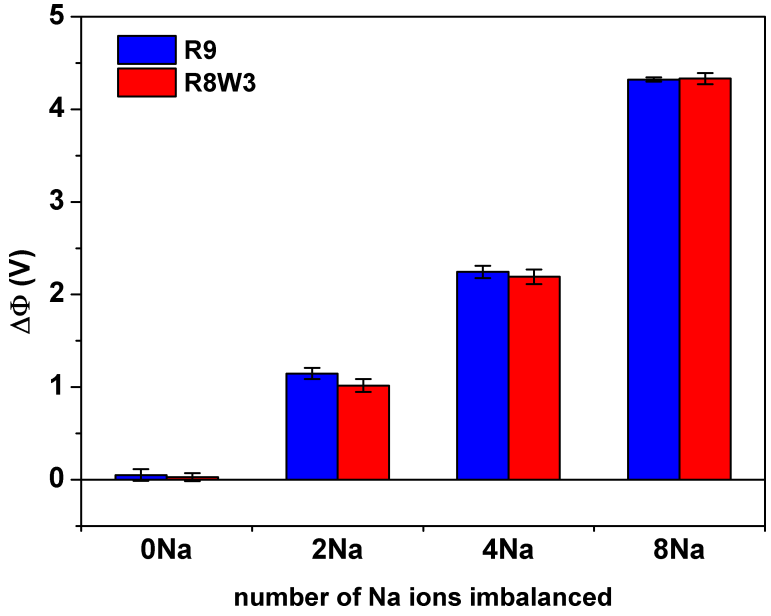

Figure 1 Electrostatic potential differences ($\Delta\Phi$) obtained for an imbalance of zero, two, four or eight sodium ions between the electrolytic solutions in contact with the DOPC bilayer. Blue and red columns represent the R_9 and R_8W_3 systems, respectively. $\Delta\Phi$ values were obtained by integrating Poisson's equation for the charge density perpendicular to the bilayer.

Comments on Figures S2, S3 and S4. As stated in the paper, the repulsive potential acting on Na^+ and Cl^- is applied within a very restricted region of space, i.e. within ± 2Å from the centre of mass of a 50Å wide membrane. In other words, the external potential acts only on the ions that manage to get near to the centre of the

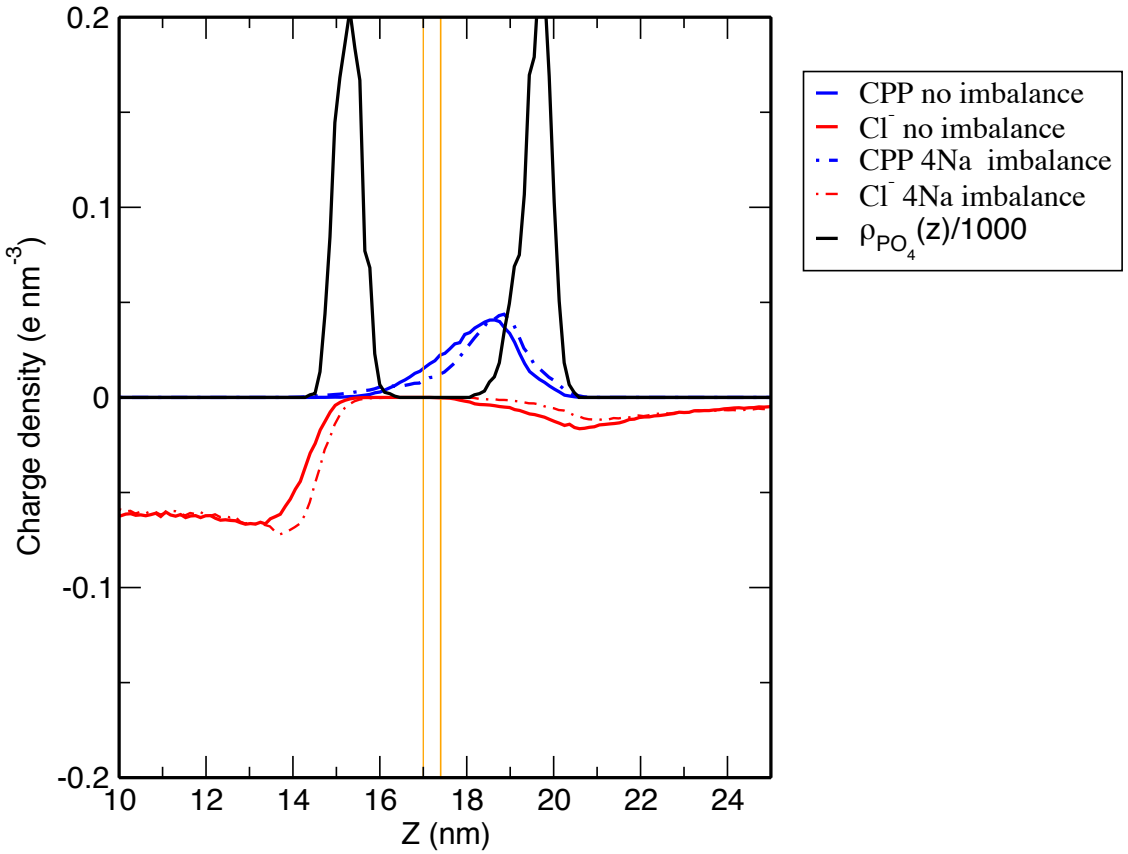

Figure 2 Cl^- charge density when the centre of mass of the peptide is half-way between the surface and the centre of mass of the membrane, both in the absence (full red line) and presence of ionic imbalance ($4Na^+$, red dot-dashed line) across the bilayer. The blue lines denote the charge density supplied by the CPP (R_9). The black line is the scaled mass density of phosphate groups, that highlights the borders of the bilayer. The vertical orange lines delimit the ions exclusion zone.

bilayer and, consequently, it is not directly responsible for the fact that most of the peptide's counter-ions (Cl^-) remain in solution.

Supplementary Figure 2 shows the Cl^- charge density when the centre of mass of the peptide is approximately half-way between the surface and the centre of mass of the membrane, both in the absence (full red line) and presence of ionic imbalance ($4Na^+$, red dot-dashed line). The blue lines denote the charge density supplied by the CPP (R_9). The black line is the scaled mass density of phosphate groups, which highlights the borders of the bilayer. The vertical orange lines delimit the $NaCl$ exclusion zone. Notice that, for the current value of the reaction coordinate, the peptide elastically deforms the membrane but there is no transmembrane pore. As can be observed, the Cl^-s do not get close enough to the ions exclusion zone, as to experience a direct repulsive force. Instead, either in the presence or absence of ionic imbalance, 2 Cl^- remain near the CPP (Z = 18-20nm), while the rest of the ions stay in solution or associated to the membrane surface, but far away from the pore. This is possibly driven by a combina-

tion of factors: low level of hydration inside the pore, translational entropy of Cl^-, and screening of the $4Na^+$ excess charge in the upper compartment.

Supplementary Figure 3 shows the same quantities (with the same colour code) of Figure 2, but now for the CPP located near the centre of the membrane. In this case there is a pore across the bilayer (as reflected by the phosphate density). Again, the Cl^- charge density within the bilayer is very low and, on the average, no counter-ions appraoch the exclusion zone. Still, the Cl^- are able to screen the tails of the peptide charge density.

In support of the previous statements, we performed simulations in which the CPP (R_9) was harmonically restrained to the centre of the bilayer, while its counter-ions were allowed to move (no ions exclusion zone) from one compartment to the other. Figure 4 shows the CPP and Cl^- charge densities. The CPP still nucleates and resides inside a transmembrane pore. Clearly the counter-ions dissolve and distribute, presumably evenly after a long time, between the two aqueous compartments.

In summary, as stated in the manuscript, in the absence or presence of ionic imbalance most of the CPP counter-ions (Cl^-) spontaneously remain in solution and far away from the *NaCl* exclusion zone, whose only purpose is to prevent the short-circuit between the upper and lower compartments. Naturally, a few Cl^- accompany the partially dehydrated peptide during its transit across the membrane. These counter-ions certainly feel the repulsive wall when they get close enough to the membrane centre ($\pm$2Å). However, this is not a serious problem given that, in the presence of a pore, the Cl^-s that are forced to remain in the upper compartment can be replaced, in the proximities of the filter, by ions coming from the lower compartment (and vice-versa). In this way, the overall Cl^- coordination of the CPP inside the pore can be satisfied.

Finally, it is worth speculating what would happen when a charge imbalance is applied across the membrane in the absence of *NaCl* filter. In our metadynamics simulations the CPP goes back and forth between the solution and the membrane space, creating transient pores and potentially dragging a few counter-ions per cycle from one compartment to the other. Over a long run the two compartments would be effectively short-circuited, and the desired transmembrane potential would disappear.

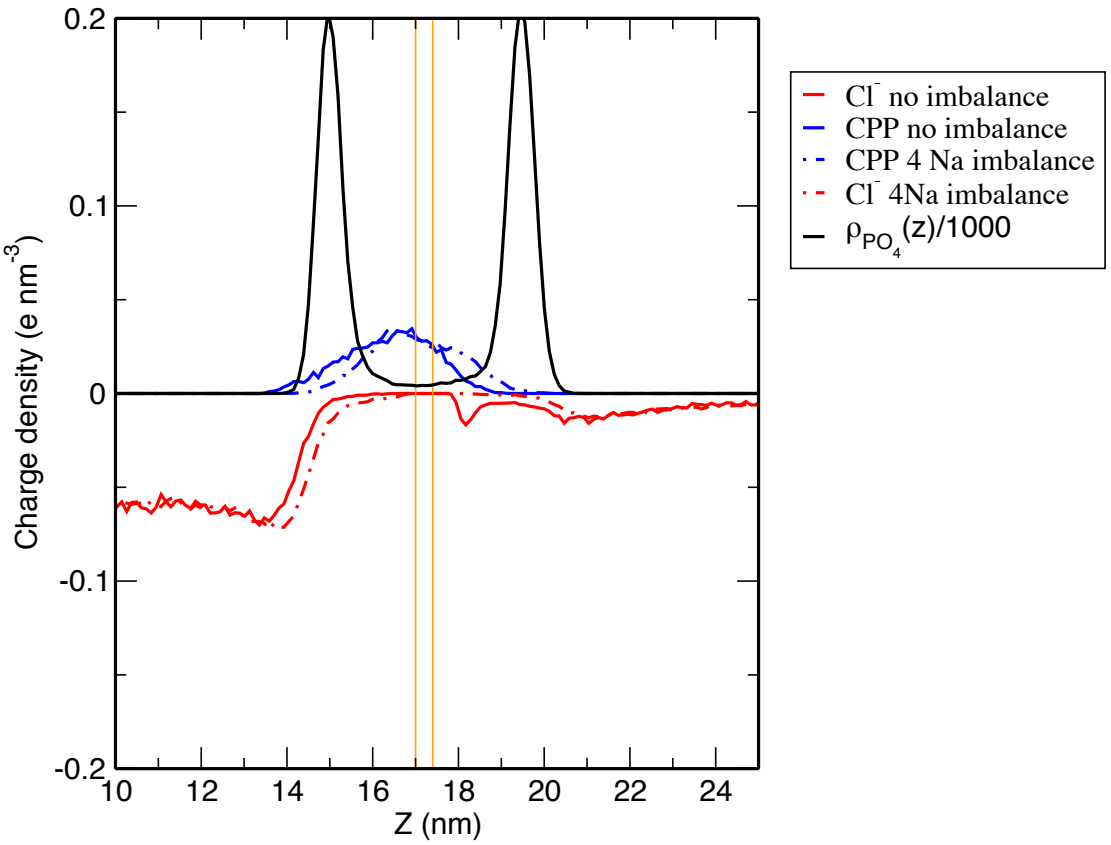

Figure 3 Same quantities (with the same color code) as in Figure 2, but now when the centre of the CPP (R_9) is located at the centre of the membrane and there is a pore across the bilayer (as reflected by the phosphates density). Again, the Cl^- charge density is very low inside the bilayer and, on the average, no counter-ions approach the repulsive walls. Still, the Cl^-s are able to screen the tails of the peptide charge density.

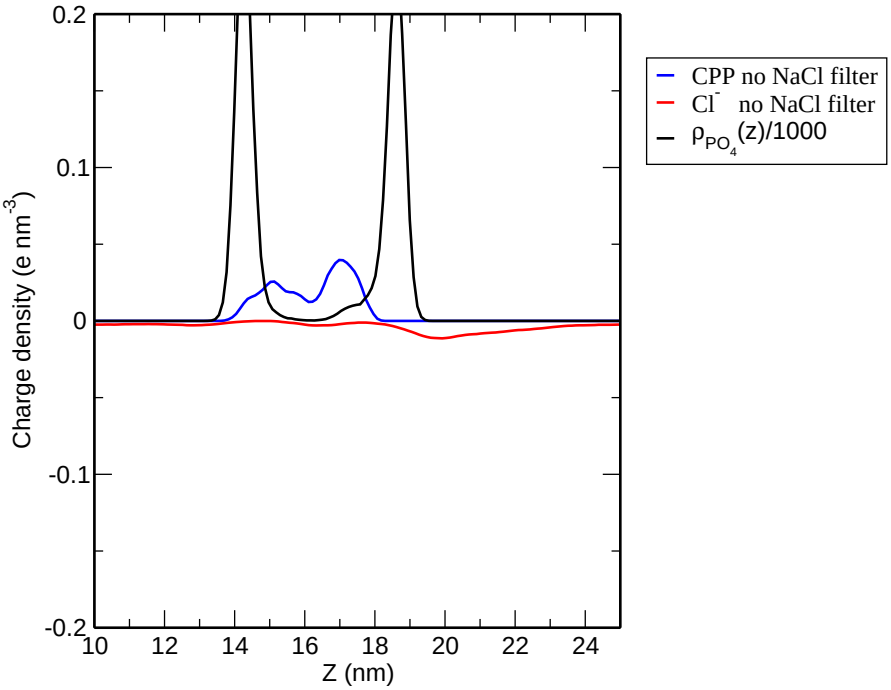

Figure 4 Charge densities obtained from a simulation in which the CPP (R_9) was harmonically restrained to the centre of the bilayer (same color code as in Figures 2 and 3). In this case the peptide counter-ions were allowed to move (no repulsive walls) from the one compartment to the other. The CPP still nucleates and resides inside a transmembrane pore. As can be observed, most of the counter ions remain in solution and distribute between the two compartments.

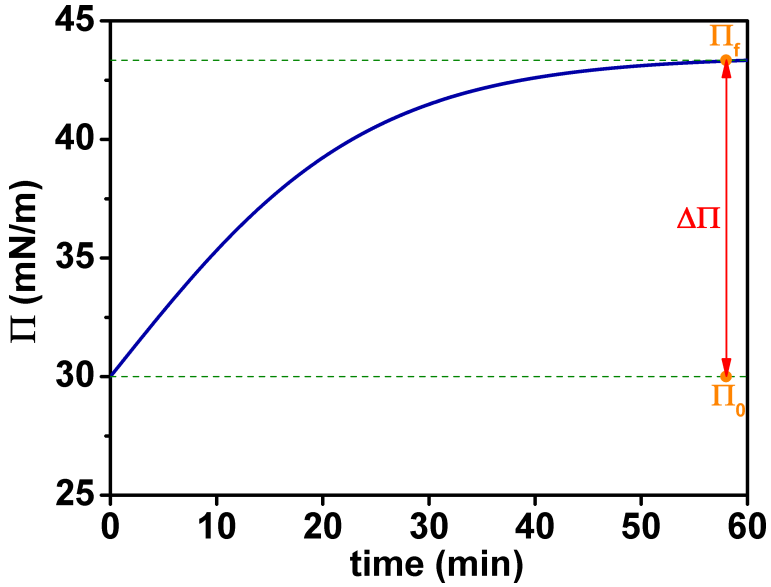

Figure 5 Penetration curve of KR_8W_3C in a perfluorotetradecanoic acid (PFTD) film on an aqueous subphase of NaCl 150 mM at pH5, with Π_0 = 30 mN. The dashed green lines represent the initial and final surface pressures (orange dots) that are used to calculate $\Delta\Pi$ (red arrow).