Electronic Supplementary Information

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1 Additional Methods

1.1 Electronic structure calculations

Electronic structure calculations were carried out with the `Gaussian 09`[1] program to determine initial molecular structures for hybrid QM/MM simulations. A geometry optimization and partial charge distribution were computed using the Hartree-Fock/6-31G(d,p) level of theory in vacuum first and then a polarized continuum solvent.

1.2 Hybrid QM/MM MD simulations

To match the match the result of the electronic structure calculations in the using the CHARMM C36 force field, the residue INI1 was patched with I3P1 and I5P2 to reproduce PtdIns(3,5)P_2 with a proton added to the 5-phosphate patch (PO_3H^{1-}) and no proton added to the 3-phosphate patch (PO_4^{2-}). Patches I4P1 and I5P2 were applied for PtdIns(4,5)P_2.

An example system is shown in Figure 1. Both systems (PtdIns(3,5)P_2 and PtdIns(4,5)P_2 in separate water spheres) were subjected to 10 ps of equilibration before the production runs. Umbrella sampling QM/MM proton transfer simulations were completed with Na^+ ions fixed around the periphery of the water sphere. A custom implementation of the weighted histogram analysis method was used with a convergence tolerance of 10^{-7} to calculate the potentials of mean force.

To determine the effect of divalent ions, additional systems were created with either Ca^{2+} or Mg^{2+} ions in place of Na^+. In these simulations, one of the two divalent ions was constrained near the boundary of the water sphere and one of the divalent ions was free to diffuse and interact, with the PPI isomer.

The full distribution of head-tail angle for PtdIns(3,5)P_2 and PtdIns(4,5)P_2 in the presence of Na^+ ions, corresponding to Figure1b in the main text, is shown in Figure 2a. Whereas all the other head-tail angle distributions are best fit by a single Gaussian curve, the head-tail angle in simulations of PtdIns(4,5)P_2 and Na^+ seems to be composed of two separate Gaussian curves, with means of 90.8° and 112.0°. An additional simulation was completed with PtdIns(3,5)P_2 in the presence of K^+, which has a greater intracellular concentration than Na^+, and is also best fit by a single Gaussian with a mean of approximately 105° (Figure 2).

Whereas the most stable form of the inositol sugar ring of PtdIns(4,5)P_2 is a chair conformation (Figure 3a and c), the most stable form of the inositol sugar ring of PtdIns(3,5)P_2 is a twist ring conformation (Figure 3b and d). The 2-hydroxyl of PtdIns(3,5)P_2 is partially equatorial and partially axial due to the presence of the 3-phosphate group and surrounding solvent. For PtdIns(3,5)P_2, the O2-C2-C3 angle is 101.9° and the O2-C2-C3-O3P torsion is −4.8°; for PtdIns(4,5)P_2 the O2-C2-C3 angle is 116.0° and the O2-C2-C3-O3 torsion is 57.5°. In addition to being larger, these conformation differences may also have implications for how tightly protein effector domains – that also recognize PtdIns(4,5)P_2 – are able to bind the PtdIns(3,5)P_2 head group.

1.3 Classical MD simulations

All-atom classical MD simulations were carried out with the `Gromacs` simulation package version 4.5 and 4.6. Prepatched versions of PtdIns(3,5)P_2 and PtdIns(4,5)P_2 were incorporated into the CHARMM C36 force field implementation using CHARMM C36 atom types. Bilayer simulations were initially equilibrated in the NVT ensemble and then simulated as described in the main text. The divalent ions interact with the lipids through a variety of modes, including: one ion binding to a single lipid, multiple ions binding to a single lipid, one ion bridging two lipids, and multiple ions bridging two lipids. Two scenarios are shown in Figure 4.

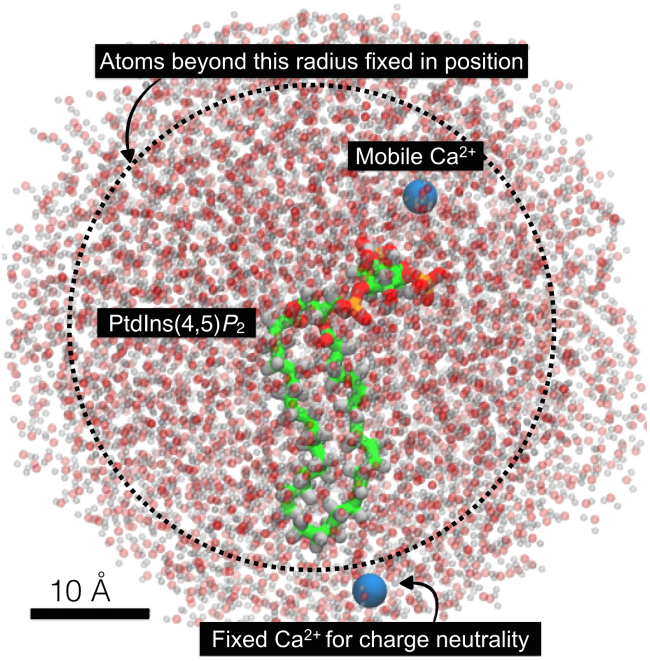

Fig. 1 The simulation setup for QM/MM and classical MD simulations of a single phospholipid in the presence of various counterions. The molecule of interest is placed in the center of a water sphere (typically about 10,000 atoms) and one or more counterions are available to diffuse and interact with the molecule of interest, which is also free to diffuse in the water sphere. To prevent the water sphere from dissolving in the vacuum, the outer layer of water atoms are fixed in place.

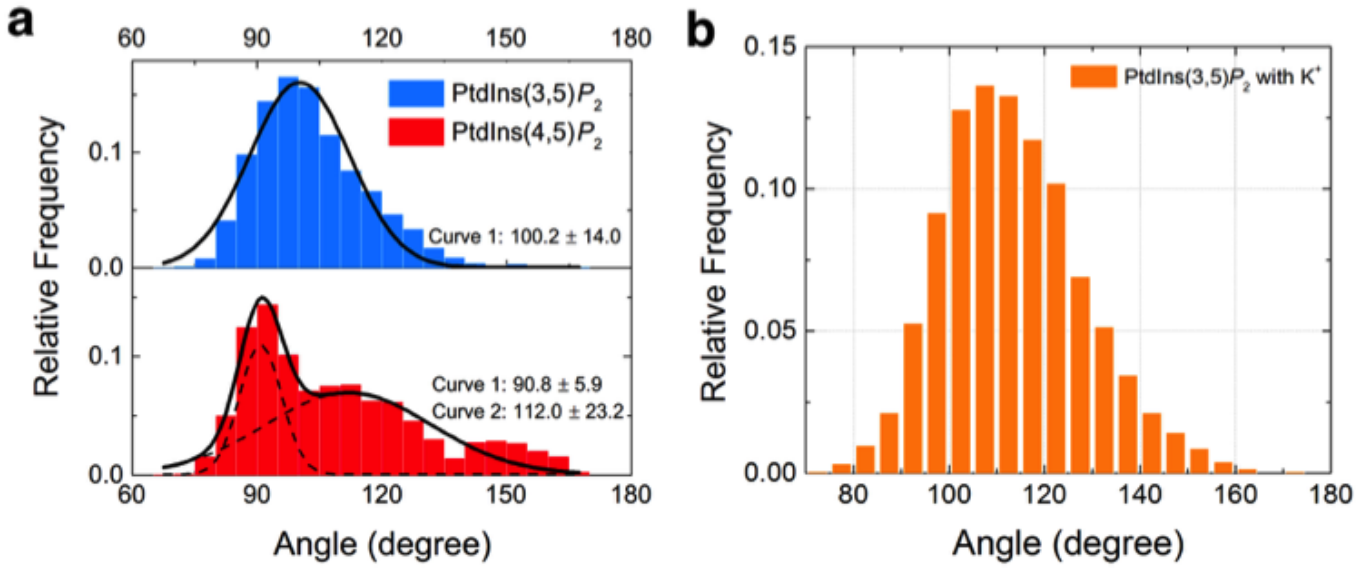

Fig. 2 Detailed head-tail angle distributions for PtdIns(3,5)P_2 and PtdIns(4,5)P_2 in the presence of Na^+ and K^+. a, the head-tail angle of PtdIns(3,5)P_2 in the presence of Na^+ is well fit by a single Gaussian curve, but not the head-tail angle of PtdIns(4,5)P_2. b, The head-tail angle of PtdIns(3,5)P_2 with K^+, with a mean around 105°.

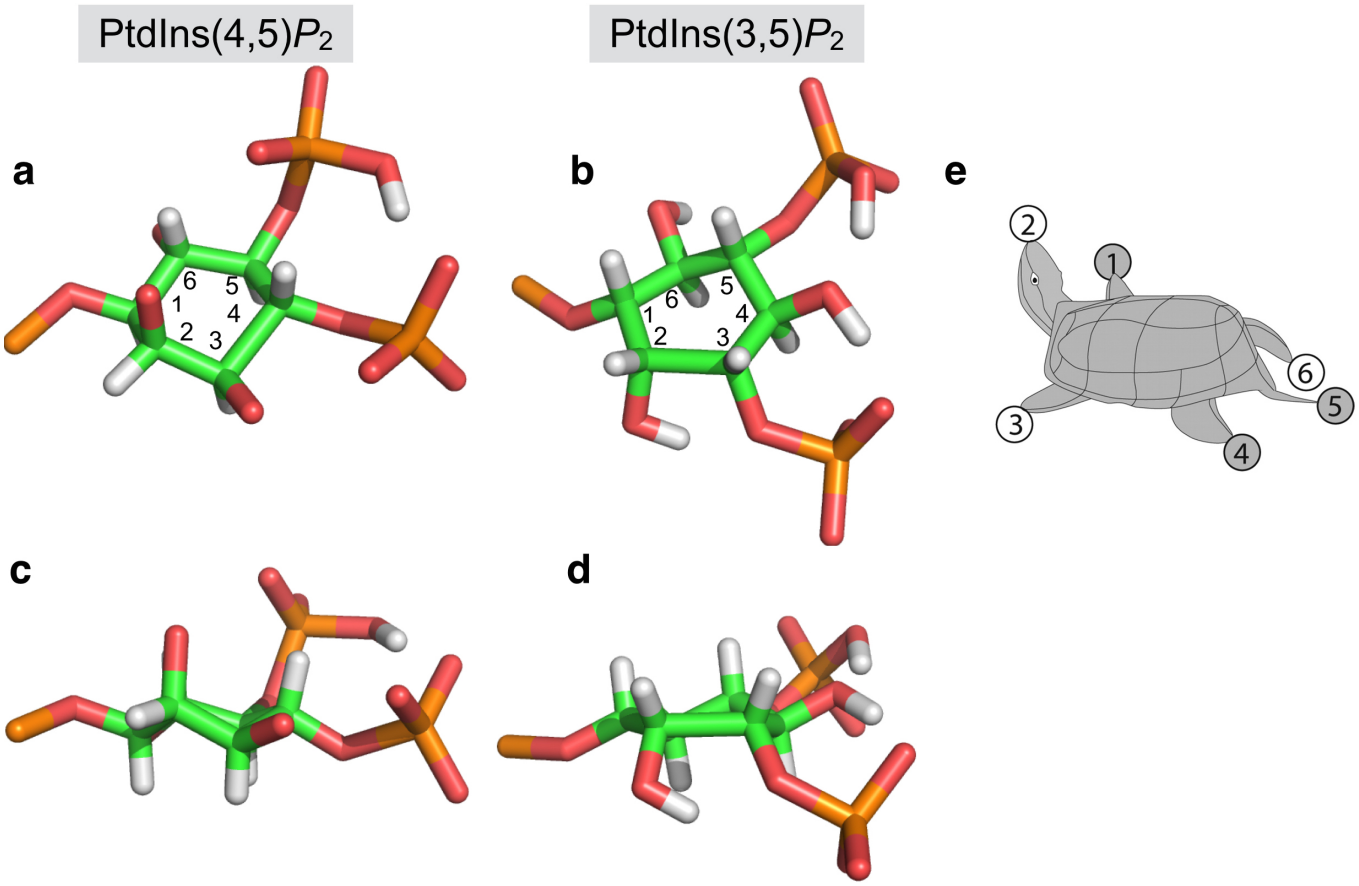

Fig. 3 Perspective and side views of the inositol ring of PtdIns(3,5)P_2 and PtdIns(4,5)P_2 with most hydrogens hidden for clarity. a and c, the inositol ring of PtdIns(4,5)P_2 is in chair form and the axial 2-hydroxyl is clear. b and d, the inositol ring of PtdIns(3,5)P_2 is twisted and the axial 2-hydroxyl is has some equatorial character along with lengthening of the carbon-carbon bond between carbons 2 and 3. e, The *myo*-inositol ring numbering scheme according to Agranoff [2] and the International Union of Biochemistry Nomenclature Committee, adapted from Figure 1 in Agranoff [2], by placing the axial hydroxyl group at the turtle head and proceeding clounterclockwise from above.

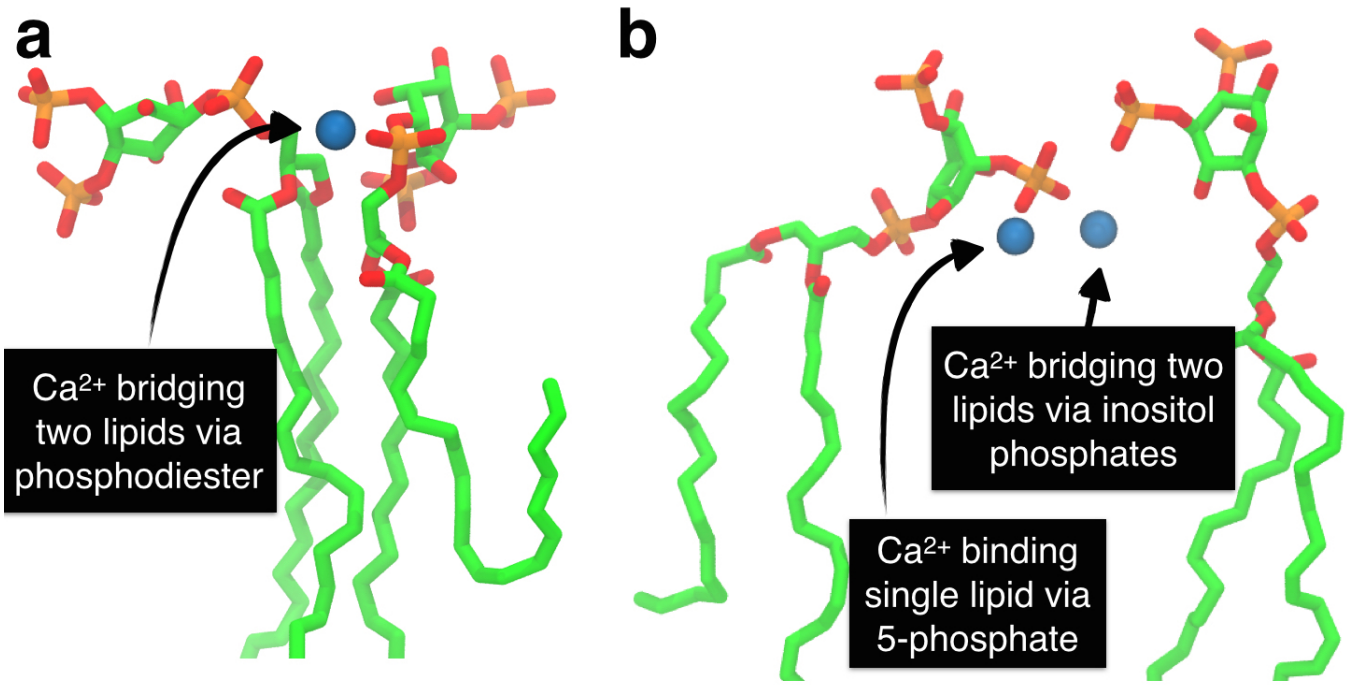

Fig. 4 An example of Ca^{2+} binding to two lipids embedded in the bilayer system shown in Figure 4a of the main text. a, two PtdIns(4,5)P_2 lipids bridged through their phosphodiester groups by a single Ca^{2+} atom. b, two PtdIns(4,5)P_2 lipids from the same simulation with one lipid binding to two Ca^{2+} ions and the second lipid binding (weakly) to a single Ca^{2+} ion.

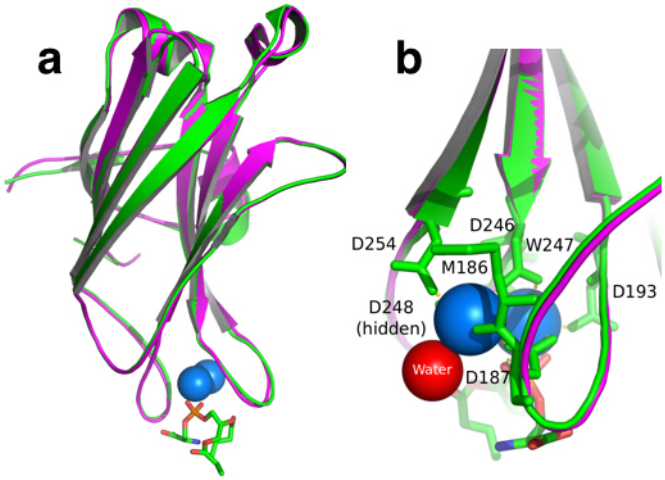

Fig. 5 The structural alignment of two crystal structures of protein kinase C alpha (PKCa) under different conditions. a, PKCa (green; PDB 1DSY) crystallized in the presence of phosphatidylserine (sticks) and Ca^{2+} (blue spheres), or without phosphatidylserine and Na^{+} (purple; PDB 4DNL). The root mean square deviation of the alignment is 0.64 Å over 136 residues, indicating there is little structural difference between the two structures. b, A more detailed view of the binding interface, showing the interactions mediated by the Ca^{2+} ions in PDB 1DSY.

References

1 *Gaussian 09* Revision B03; Gaussian, Inc.: Wallingford CT, **2009**.

2 Agranoff, B. W. *J. Biol. Chem.* **2009**, *284*, 21121–21126.