

Mechanistic Insights into the Phosphatidyl Inositol Binding Properties of Pleckstrin Homology Domain of Lamellipodin

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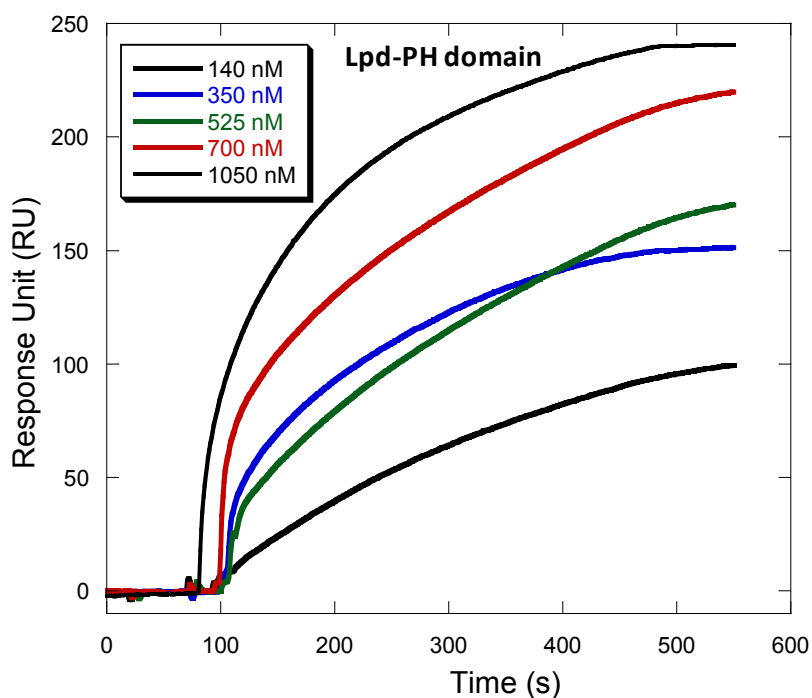


Figure S1: Representative equilibrium SPR sensograms for Lpd-PH domain interacting with PC/PE/PS/PI(3,4)P₂ (57:20:20:3) liposome immobilized on L1-sensor chip.

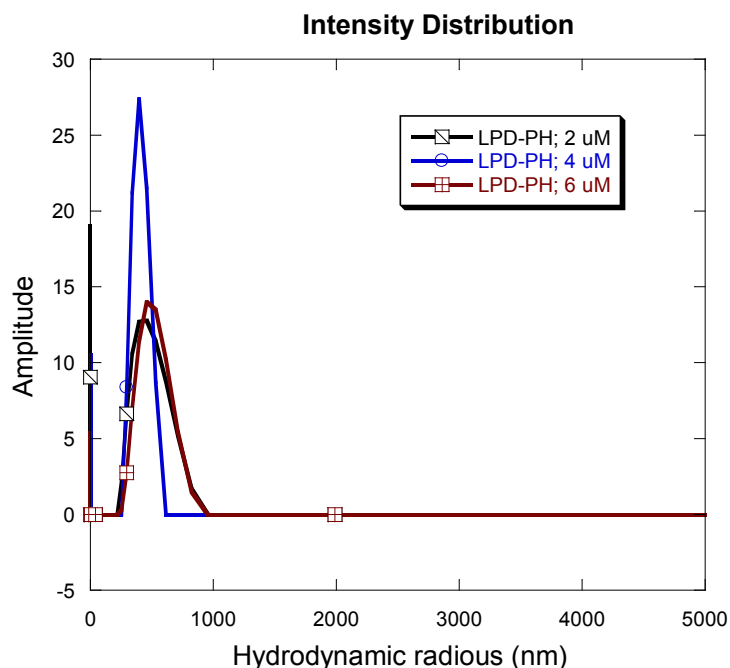
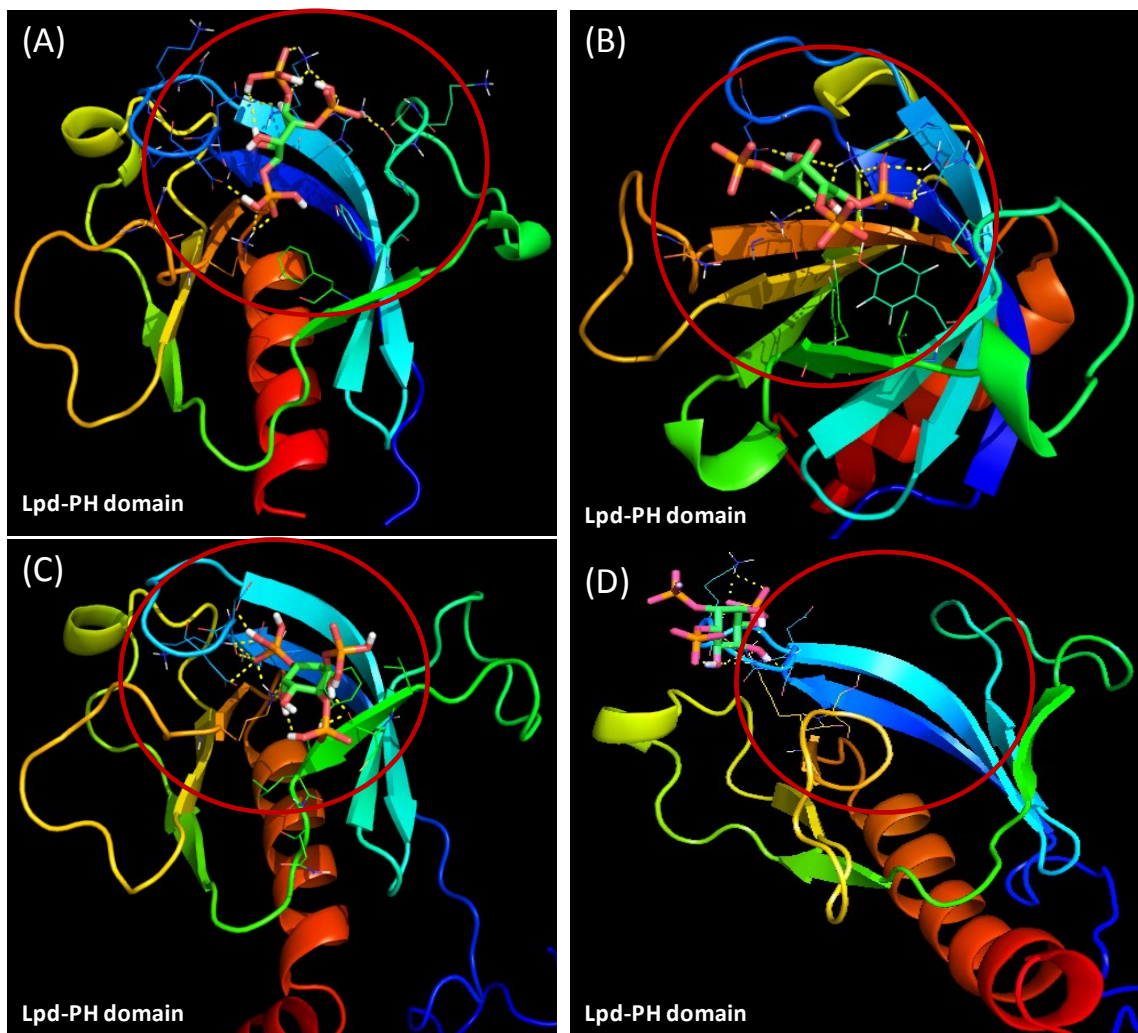


Figure S2: Oligomerization analysis of isolated Lpd-PH domain in buffer solution by dynamic light scattering measurements.

Molecular dynamics simulation method: Molecular dynamics (MD) simulations were performed using GROMACS 5.0.1 package with the standard GROMOS96 43a1 force field.^{1, 2} Topology file and force field parameters of the ligand were generated using PRODRG2.5 server.³ Some reports have shown that while bonded parameters and atom types assigned by PRODRG2.5 are usually correct, the generated topologies often have inaccuracies in the charges and charge groups.⁴ Partial charges for each docked conformation of the ligands were calculated using Automated Topology Builder (ATB) and Repository version 2.2 server using DFT (B3LYP/6-31G*) quantum mechanical method.⁵ Canonical and the optimized ligand structures were re-joined using the GROMACS “pdb2gmx” procedure at which point the hydrogen atoms were added. Each protein-ligand complexes were soaked in a cubic box of simple point charge (SPC) water molecules with a margin of at least 13 Å from the protein face. Chloride counter-ions were added to preserve electro-neutrality. The energy of these complexes was minimized using the steepest descent approach realizing in the GROMACS package. Subsequently two MD equilibration steps of 100 ps under NVT ensemble using 2.0 fs integration time step at temperature of 300 K was applied using the leap-frog integrator. A second 100 ps NPT equilibration step was executed at 1bar pressure to equilibrate the size of the system

using the same integrator. Finally, 10 ns MD simulations were performed using leap-frog integrator at the NPT canonical ensemble and the periodic boundary conditions were used in all three dimensions. The production phase was conducted using NPT ensemble at 300 K applying the V-rescale temperature coupling algorithm. The pressure of the system was adjusted at 1 atm under isotropic molecule-based scaling using Parrinello-Rahman pressure coupling method. The Particle-Mesh-Ewald (PME) method⁶ for long-range electrostatics, a 14 Å cutoff for van der Waals interactions and Coulomb interaction were used. The LINCS algorithm was applied to constrain all bonds while the non-bonded cutoff was set to 10 Å.⁷ The GRID method was used to search and update the neighbor list with a frequency set to 10 steps. The production simulation was run for 10 ns with a total of 5000000 steps.



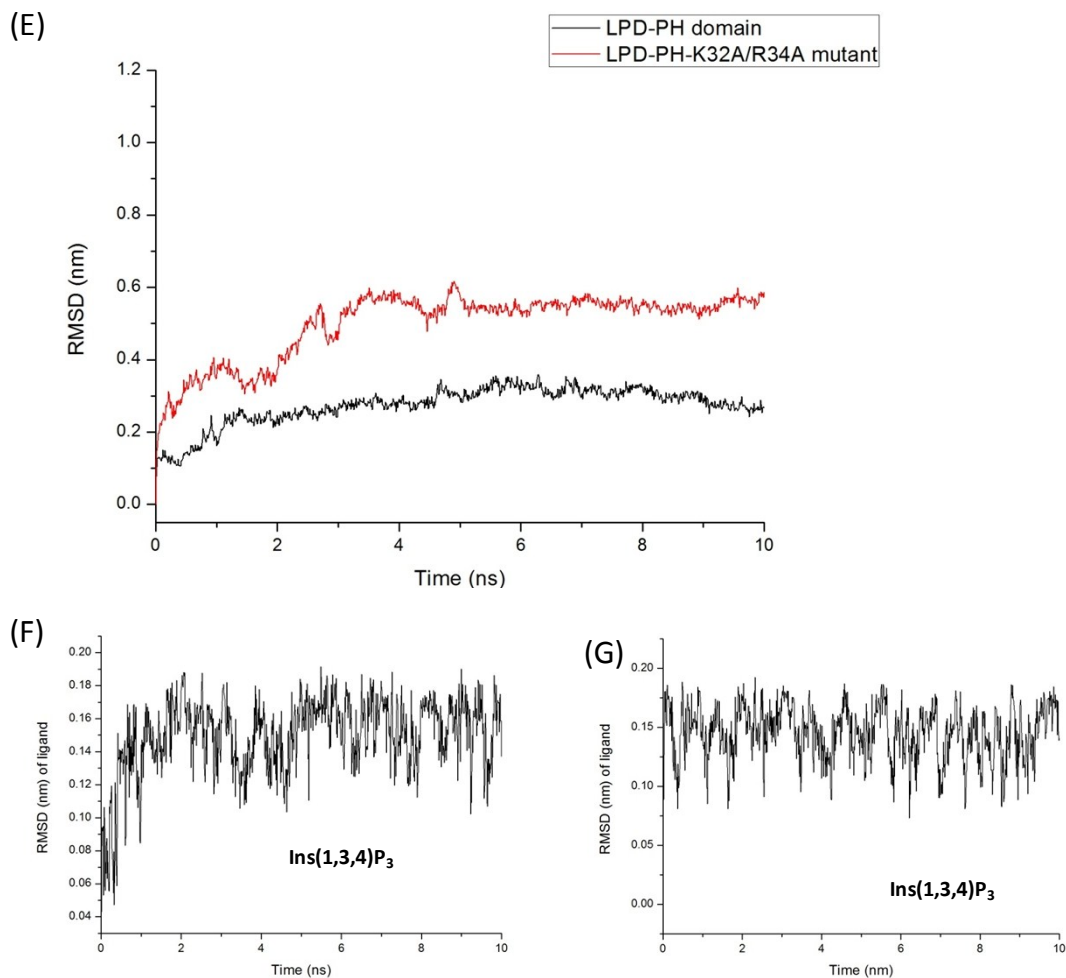


Figure S3: Binding mode of ligand, Ins(1,3,4)P₃ with Lpd-PH domain obtained after docking (A) and MD simulation (B). Binding mode of ligand, Ins(1,3,4)P₃ with Lpd-PH-K32A/R34A mutant obtained after docking (C) and MD simulation (D). RMSD of Ins(1,3,4)P₃ complexed with Lpd-PH domain and Lpd-PH-K32A/R34A mutant (E). RMSD of ligand, Ins(1,3,4)P₃ obtained during MD simulation with Lpd-PH domain (F) and Lpd-PH-K32A/R34A mutant (G).

Table S1: PIP specificity of Lpd-PH domain measured by kinetic SPR measurements

Lipid	k_a (M ⁻¹ S ⁻¹)	k_d (S ⁻¹)	K_d (M)	Fold change
PI(3,4)P ₂	4.3×10^4	1.62×10^{-4}	3.80×10^{-9}	1
PI(4,5)P ₂	5.5×10^4	1.12×10^{-3}	2.08×10^{-8}	5.5
PI(3,4,5)P ₃	5.3×10^4	6.19×10^{-4}	1.16×10^{-8}	3.1
PI(3,5)P ₂	7.34×10^4	5.36×10^{-4}	1.37×10^{-8}	539
PI(4)P	2.86×10^2	1.71×10^{-3}	5.97×10^{-6}	1571
PI(3)P	2.69×10^2	2.09×10^{-3}	7.78×10^{-6}	2047
PI(5)P	5.11×10^2	1.00×10^{-4}	1.96×10^{-7}	52
Tapp1-PH	8.49×10^4	1.11×10^{-4}	1.31×10^{-9}	-

Table S2. Apparent competitive inhibition constants [$K_i(IP_6)_{app}$] of Lpd-RA-PH domain for IP₆^a

Protein	PI	PI(3)P	PI(4)P	PI(5)P	PI(3,4)P ₂	PI(3,5)P ₂	PI(4,5)P ₂	PI(3,4,5)P ₃
LPD-RA-PH	76 ± 9	122 ± 11	105 ± 12	119 ± 11	805 ± 36	111 ± 27	160 ± 24	261 ± 33

^aAll FRET measurements were performed in 20 mM Tris buffer at pH 7.4 containing 160 mM NaCl. IP₆ was used as inhibitor of membrane-to-protein interactions. Protein, 1 μM was used for FRET analysis.

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