Molecular basis for cooperative binding of anionic phospholipids to the PH domain of the Arf GAP ASAP1

Xiaoying Jian*[,1], Wai-Kwan Tang[&,1], Peng Zhai[*], Neeladri Roy[*], Ruibai Luo[*], James M. Gruschus[@], Marielle E. Yohe[%], Pei-Wen Chen*, Yifei Li[#], R. Andrew Byrd[#], Di Xia[&,2], Paul A. Randazzo*[,2]

Laboratory of Cellular and Molecular Biology, National Cancer Institute[*], Laboratory of Cell Biology, National Cancer Institute[&], Laboratory of Structural Biophysics, National Heart, Lung and Blood Institute[@], Genetics Branch, National Cancer Institute[%] and Structural Biophysics Laboratory, National Cancer Institute[#], National Institutes of Health, Bethesda, MD 20892. [1], co-first author; [2], co-corresponding author.

Supplemental Information

Figure S1, related to Figure 1. Bound dibutyryl-PtdIns(4,5)P_2 and their binding sites. (A) Stereo pair showing the atomic detail of the atypical PtdIns(4,5)P_2 -binding site. The bound diC4-PtdIns(4,5)P_2 is shown as the stick model with carbon in black, phosphor in orange, and oxygen in red. Superimposed on the phosphate moieties are anomalous electron density maps calculated based on Fourier coefficients of F(+)-F(-) and contoured at 3.5σ. Superimposed on the diC4-PtdIns(4,5)P_2 is the 2Fo-Fc electron density contoured at 1.5σ. Residues that are in contact with the ligand are labeled. (B) Stereo pair showing the atomic details of the canonical PtdIns(4,5)P_2 -binding site. The bound diC4-PtdIns(4,5)P_2 is shown as the stick model and is superimposed with the anomalous difference Fourier map contoured at 2.2σ and the 2Fo-Fc map contoured at 1.0σ. Contacting residues are labeled. (C) Titration of PH[325-451] with diC4-PtdIns(4,5)P2 . PH domain (50 μM in 20 mM phosphate buffer with 150 mM NaCl, 0.5 mM TCEP, and 0.2 mM NaN3) was titrated with molar ratios of 0.3, 0.75, 1.0, 1.5, 2.0, 3.0 of diC4-PtdIns(4,5)P_2 . ^{15}N HSQC spectra were obtained at 600 MHz (Bruker Avance III spectrometer with a TCI cryoprobe) for each titration point and superimposed. Assignments were obtained from

standard triple resonance three-dimensional NMR spectra on a uniformly $^{15}N/^{13}C$-labeled sample of PH[325-451]. Expansions of regions of interest are displayed to highlight the residues that experienced chemical shift pertubations, reflecting binding interactions, through the titration. BMRB accession number 26625

Figure S2, related to Figure 1. Flexibility of PtdIns(4,5)P2 binding to ASAP1 PH domain (A) Stereoscopic pair showing the interaction of diC4-PtdIns(4,5)P_2 with molecule A. (B) Stereoscopic pair showing the interactions of diC4-PtdIns(4,5)P_2 with molecule B, which has the inositol ring flipped 180°.

Figure S3, related to Figure 1. Structure of ASAP1 PH domain in complex with dibutyryl PtdIns(4,5)P_2 (A) Superposition of the two ASAP1 PH domain/diC4-PtdIns(4,5)P_2 complexes in the crystallographic asymmetric unit. The molecule A is colored green and molecule B cyan. As the two proteins are superimposed, so are the two bound PtdIns(4,5)P_2 moieties with near perfect overlap of the phosphate groups at the 4- and 5-positions. (B) Superposition of structures of various PH domains with bound ligands at the C site. The following coordinates are used: Black, ASAP1 PH domain with bound diC4-PtdIns(4,5)P_2 ; Green, PH domains of PDK bound with Ins(1,3,4,5)P_4 (PDB:1W1D) and orange, diC4-Ptd(3,4,5)P_3 (PDB:1W1G); Blue, PH domain of protein kinase B alpha bound with benzene-1,2,3,4-tetraayl tetrakisphosphate (PDB:2UVM); Brown, PH domain of Bruton's tyrosine kinase with bound Ins(1,3,4,5)P_4 (PDB:1B55); Yellow, Grp1 PH domain with bound PtdIns(3,4,5)P_3 (PDB:4KAX); Magenta, PH domain of centaurin alpha-1 bound with Ins(1,3,4,5)P_4 (PDB:3LJU). (C) Superposition of structures of various PH domains with bound ligands at the A site: Black, ASAP1 PH domain with bound diC4-PtdIns(4,5)P_2 ; Light blue, Slm1-PH domain in complex with Ins(4)P (PDB:4A6H); Green, Slim1-PH domain in complex with Ins (4)P (PDB:4A6K); Magenta, ArhGAP9 PH domain in complex with PtdIns(3,4)P_2 (PDB: 2P0H); Cyan, ArhGAP9 PH domain in complex with Ins(1,4,5)P_3 (PDB: 2p0d)

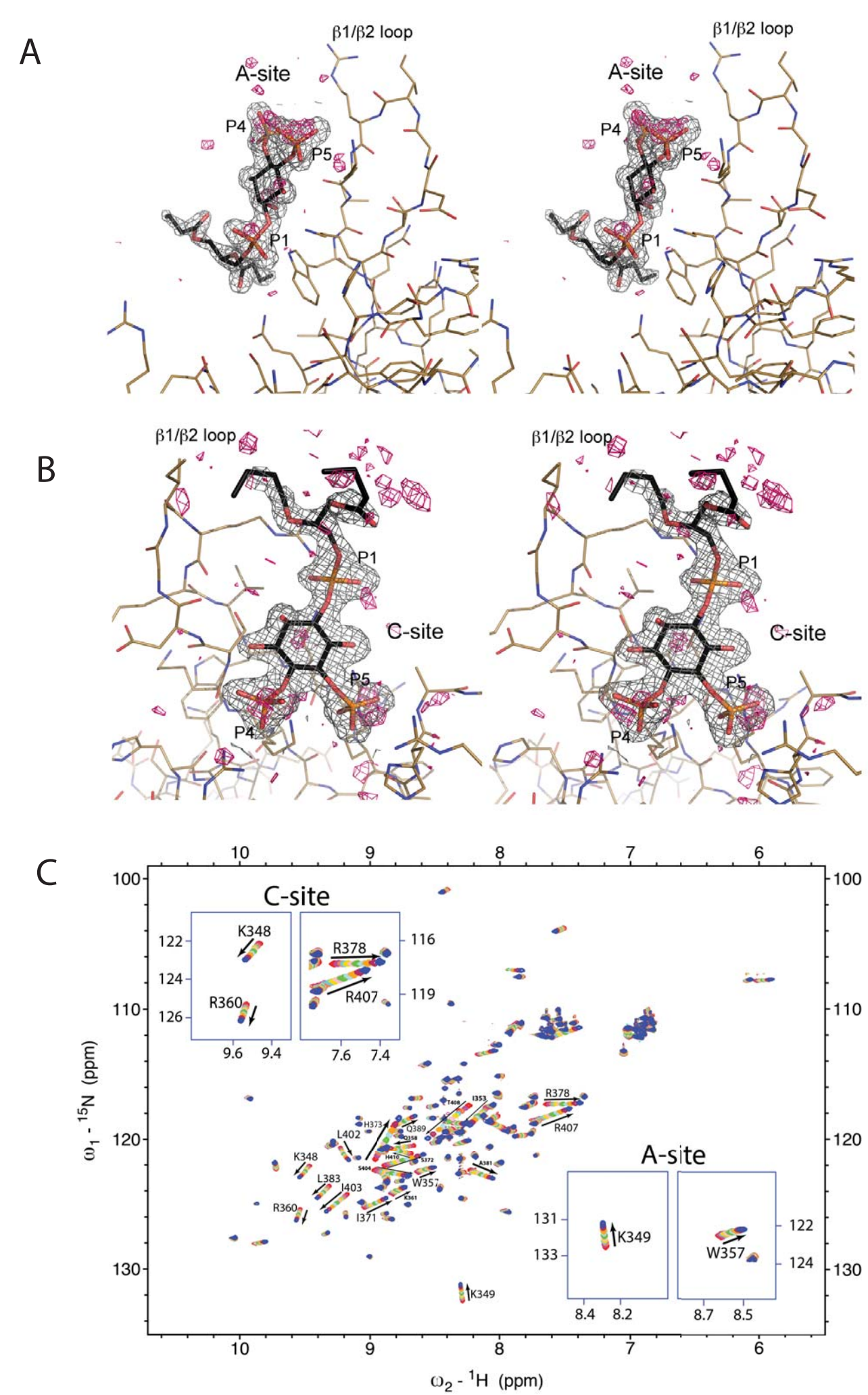

Figure S1. Jian, Tang et al.

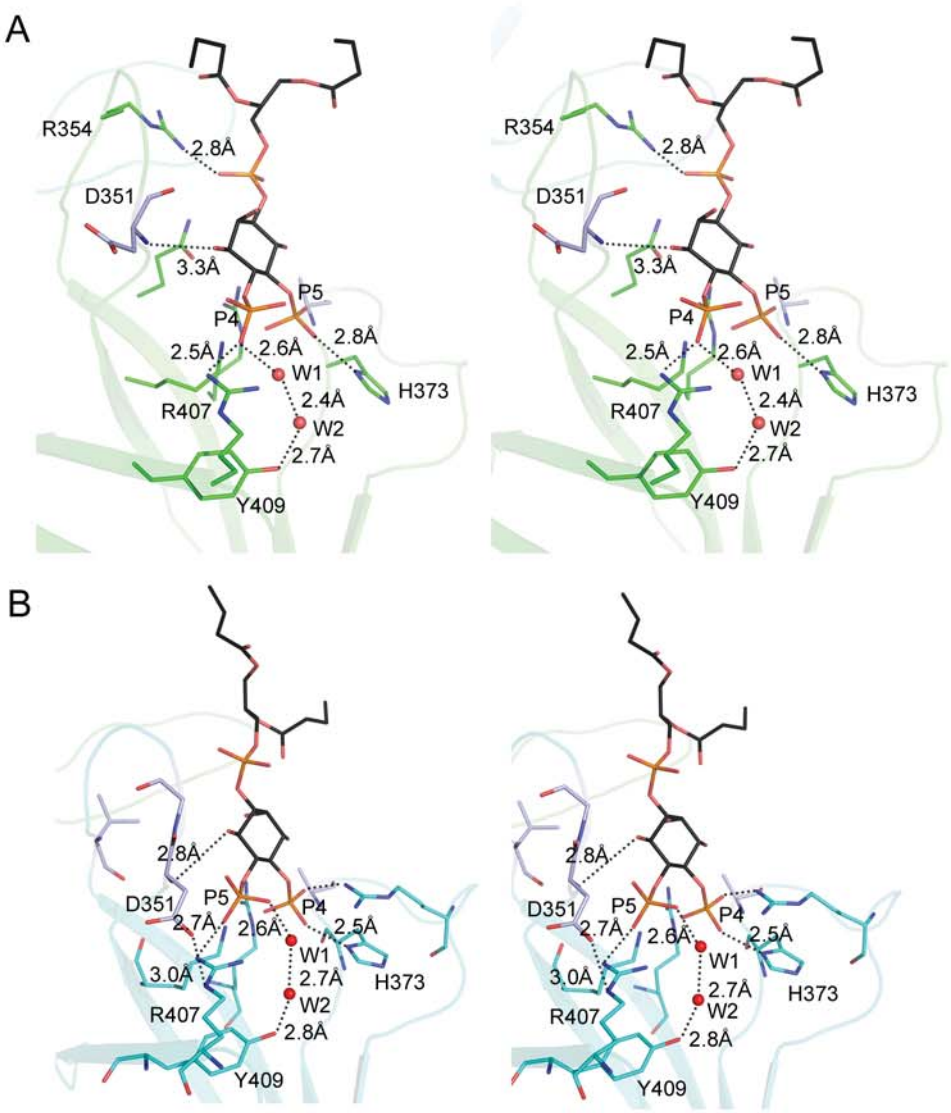

Figure S2. Jian, Tang et al.

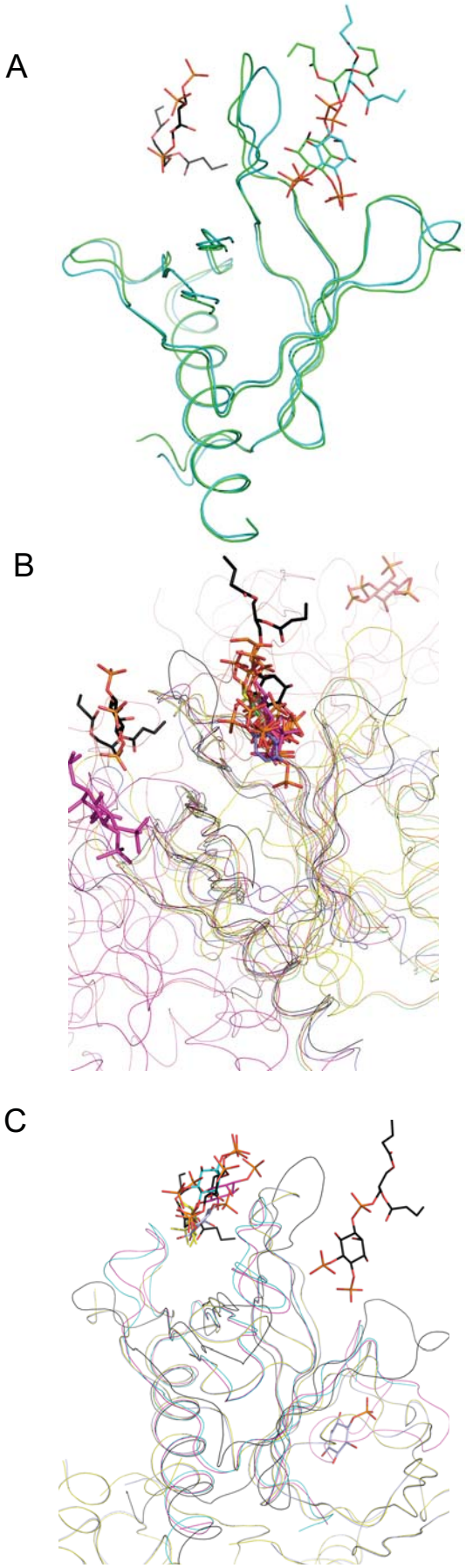

Figure S3. Jian, Tang et al.