Cation-π interactions as lipid specific anchors for phosphatidylinositol-specific phospholipase-C

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Supporting Information

Table 1. Decomposition of the IMM1-GC membrane binding energy. Only favorable contributions are reported (in kcal/mol).

SSE	aa #	$\Delta W_{tr(aa)}$ [b] (kcal/mol)
β1-αB	K38	0.2±0.2/-**1.7±0.4** [a]
αB	P42	-1.0±0.3
	I43	-1.7±0.3
	K44	0.6±0.3/-**2.1±0.3**[a]
	V46	-1.1±0.3
	W47	-0.3±0.3
β6-αF	K201	0.0±0.0/-**1.1±0.2** [a]
β8-αH	K279	0.2±0.2/-**1.7±0.3** [a]

[a] for lysines, the electrostatic contribution (Gouy Chapman term) is given in bold.

[b] Mean values of $\Delta W_{tr(aa)}$ are given in kcal/mol, per amino acid, together with the standard deviation calculated along the last 1.5 ns of the simulation time

Table 2. Analysis of PI-PLC secondary structure and thermostability from far-UV circular dichroism (CD) data. Secondary structure content was determined from the CD spectra using the program CDNN[1].

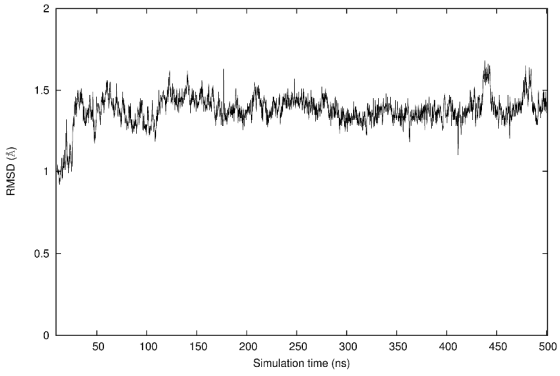
Variant[a]	Alpha Helix (%)	Beta Sheet (%)	Beta Turn (%)	Random Coil (%)	Tm (°C)
WT*	22.2	30.0	17.5	29.9	56.7
Y86/88A*	19.1	34.1	17.1	29.7	56.5
Y204/251A*	18.6	33.6	16.8	31.1	53.7
Y204S/Y248A*	21.8	31.5	17.5	29.1	52.9
Y246A*	20.9	31.6	17.2	30.2	53.1
Y247A*	19.5	34.3	17.2	29.1	52.6
Y246A/Y247A*	19.6	34.0	17.2	29.3	51.9

[a] The asterisk indicates that all variants contain the N168C mutation required for fluorescent labeling.

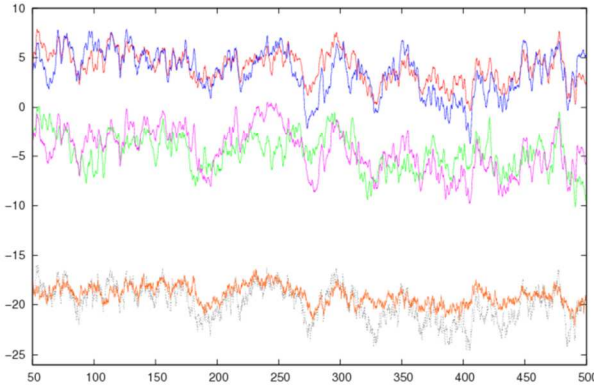
The CD data show that all of the variants are well folded with no significant changes in secondary structure. There is a small decrease in thermostability for both variants containing the Y204A mutation.

Figure 1. **(A)** RMSD versus simulation time for the WT PI-PLC backbone, calculated with respect to the minimized starting conformation. **(B)** Evolution during the simulation of the depth of anchoring of the center of mass of the residues Ile43 (red), Trp242 (blue), Tyr88 (green) and Tyr246 (magenta), and of the center of mass of the protein (dashed grey). The reference (Z=0) corresponds to the average Z position of the phosphate groups. The orange line shows the evolution of the position of the center of mass of the protein using as a reference only the Z position of the phosphate groups closest to the protein (15 Å or less). **(C)** Occurrences of hydrogen bonds along the simulation time, only those with occupancy over simulation time higher than 30% are shown. SC and BB indicate if the residue is interacting with bilayer lipids via its side chain or its backbone, respectively. Blue and magenta are used for interactions with oxygen atoms in phosphate groups and carbonyls, respectively. **(D)** Evolution during the trajectory of hydrophobic contacts for protein residues mediating on average at least two contacts per frame with the bilayer lipids. Each dot on Figures C and D corresponds to the center of 1-ns long windows in which the interaction is observed for more than 50% of the conformations within the window (see Methods section of the manuscript).

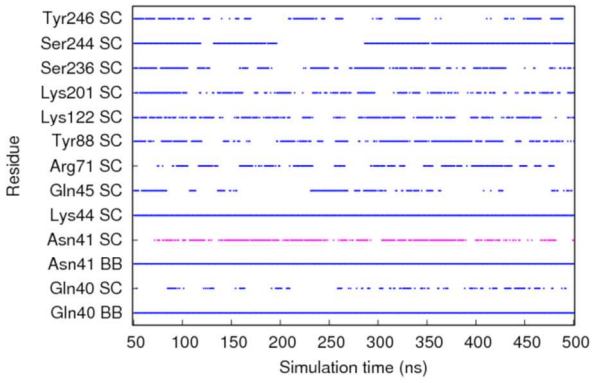
A.



B.



C.



D.

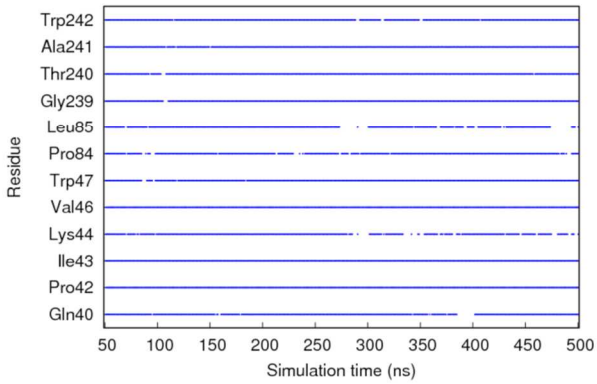


Figure 2. Effects of the Y248A mutation on PI-PLC dynamics from multiple 20 ns simulations. Root mean square fluctuations (RMSF) per amino acid for WT and Y248A PI-PLC in solution.

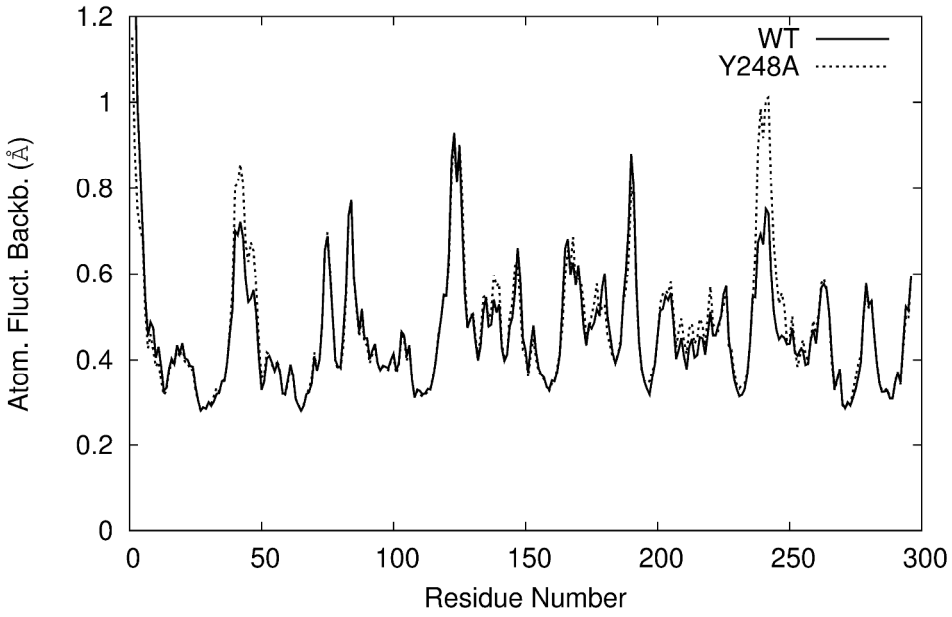
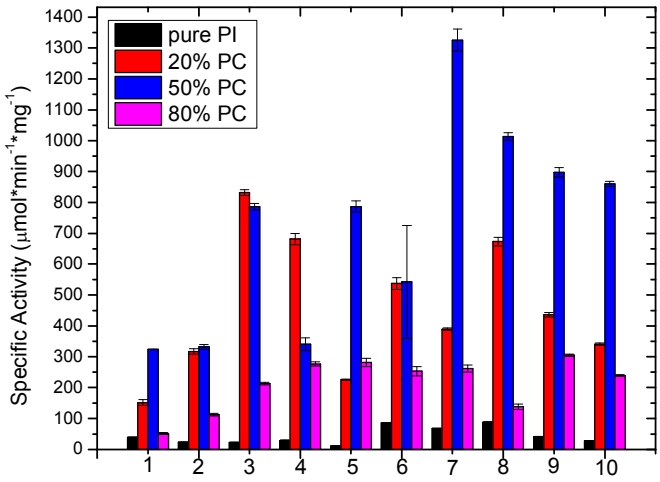
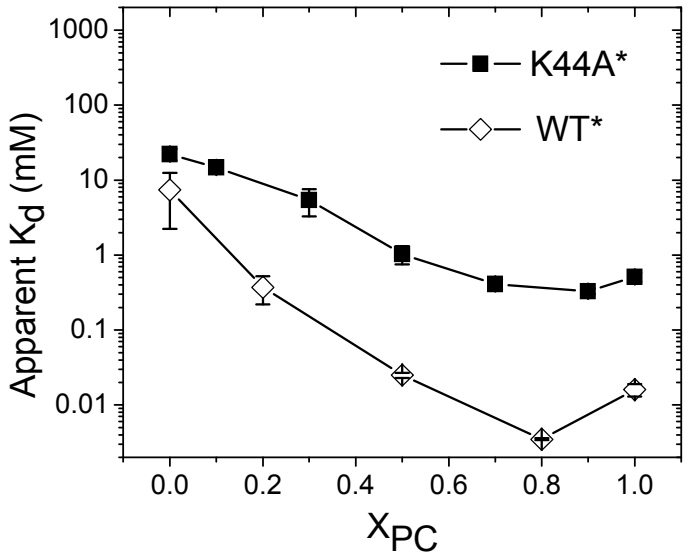


Figure 3. Specific activity of unlabeled PI-PLC variants with 4 mM PI and increasing concentrations of PC: (1) WT*; (2) Y86A*; (3) Y88A*; (4) Y86A/Y88A*; (5) Y246A*; (6) Y247A*; (7) Y246A/Y247A*; (8) Y204S*; (9) Y248A*; (10) Y204S/Y248A*. All of these PI-PLC variants contain the N168C mutation used for fluorophore attachment.



Enzymatic assays were performed using lipid concentrations that are 1.79 to 1250 times higher than the apparent K_ds , insuring that all of the protein partitions to the vesicles in the presence of PC. All of the PI-PLC Tyr variants show the same activity pattern as WT*. Activity is low towards pure PI SUVs and increases significantly once PC is present. The decrease in specific activity at 80% PC is the result of protein sequestration from substrate in PC-rich vesicles.[2] This surface dilution inhibition is not as significant for the tyrosine variants, suggesting that weakening vesicle binding at high X_{PC} increases the probability that substrate can access the enzyme active site, as previously observed for other PI-PLC variants.[3]

Figure 4. Binding affinities of K44A* mutation and WT* PI-PLC to PG/PC SUVs as a function of mole fraction of PC (X_{PC}).



The original FCS data for the K44A* variant[3] were reanalyzed, using the fitting procedure described in the main text, to account for the possibility that more than one protein binds per vesicle.

REFERENCES:

(1) Bohm, G.; Muhr, R.; Jaenicke, R. *Protein Eng* **1992**, *5*, 191.

(2) Pu, M.; Fang, X.; Redfield, A. G.; Gershenson, A.; Roberts, M. F. *J Biol Chem* **2009**, *284*, 16099.

(3) Pu, M.; Roberts, M. F.; Gershenson, A. *Biochemistry* **2009**, *48*, 6835.