SUPPORTING INFORMATION FOR

Structure of phospholipase Ce reveals an integrated RA1 domain and novel regulatory elements

Ngango Y. Rugema^{1†}, Elisabeth E. Garland-Kuntz^{1†}, Monita Sieng¹, Kaushik Muralidharan², Michelle Van Camp¹, Hannah O'Neill¹, William Mbongo², Arielle F. Selvia¹, Andrea T. Marti², Emmanda McKenzie², Amanda Everly¹, and Angeline M. Lyon^{1,2*}

From the ¹Department of Chemistry and the ²Department of Biological Sciences, Purdue University, West Lafayette, IN 47907

*To whom correspondence should be addressed: Angeline M. Lyon, Departments of Chemistry and Biological Sciences, Purdue University, 560 Oval Drive, West Lafayette, Indiana 47907, Telephone: (765)-494-5291; email: lyonam@purdue.edu

[†]Authors contributed equally to the work.



Supplementary Figure 1. The asymmetric unit contains four copies of PLC ϵ EF3-RA1. Within the asymmetric unit, chains A and B (top) and chains C and D (bottom) form dimers through their TIM barrel–EF3/4 interfaces. Domains are colored as in Figure 1A. Fo-Fc difference Fourier maps generated peaks consistent with Ca²⁺ (black sphere) in each of the four chains, All four chains were similar, with an r.m.s.d. of 0.206 – 0.223 Å.



Supplementary Figure 2. PLCε EF3-RA1 is primarily monomeric and monodisperse in solution. (A) Size exclusion chromatogram showing the elution profile of PLCε EF3-RA1. The (B) raw scattering curve, (C) Guinier plot, and (D) pair-distance distribution function are consistent with a largely globular protein that is monomeric in solution²².

			$F3\alpha$ $E4\alpha$				
R.	norvegicus PLCE	1284	AAASIVINGTGUESTSLEIFGVGILOLNDFLVNCOGEHCTYDEIL	1328			
R.	norvegicus PLC δ	192	QTDSLEDEEIETFYKMLTQRAEIDRAFEEAAGS-AETLSVERLVTFLQHQQREEEAGPALAL	252			
н.	sapiens PLC β 2	199	NPEDFPEPVYKSFLMSLCPRPEIDEIFTSYHAKAKPYMTKEHLTKFINQKQRDSRLNSLLFPPARPDQVQ	268			
H.	sapiens PLC eta 3	204	RPDEFSLEIFERFLNKLCLRPDIDKILLEIGAKGKPYLTLEQLMDFINQKQRDPRLNEVLYPPLRPSQAR	273			
			$E4\alpha$ $E4\alpha'$ $F4\alpha$ $TB1$				
ъ	norwogique BLCc	1220		1200			
R.	norvegicus PLC	253	SITERVEPSETAKAOROMTKDGFLMVLI.SADGNAFSL-AHRRVYODMDOPI.SHVLVSSSHNTYLLEDOLT	321			
н.	sapiens PLC _{β2}	269	GLIDKYEPSGINAQRGQLSPEGMVWFLCGPENSVLAQ-DKLLLHHDMTQPLNHYFINSSHNTYLTAGQFS	337			
н.	sapiens PLC β 3	274	LLIEKYEPNQQFLERDQMSMEGFSRYLGGEENGILPL-EALDLSTDMTQPLSAYFINSSHNTYLTAGQLA	342			
			$- T_{\alpha 1} T_{R2} T_{R2} T_{R2} T_{\alpha 2} T_{R3}$				
_							
R.	norvegicus PLCE	1399	GESSVELYSQVLLQGCRSIELDCWDGDDGMPIIYHGHTLTTKIPFKEVVEAIDRSAFITSDLPIIISI	1466			
к.	saniens PLC ^B 2	338	GPSSIEATIKALCKGCKCLELDCWDGPNQEPIIINGIIFISKILFCDVLKAIKDIAFKASPIPVILSL GLSSAFMYROVIJSCCRCVELDCWKGKPDDEEDIITGGTFTSKILFCDVLKAIKDIAFKASPIPVILSL	405			
н.	sapiens PLC ₃	343	GTSSVEMYROALLWGCRCVELDVWKGRPPEEEPFIT GFTMTEVPLRDVLEAIAETAFKTSPYPVILSF	412			
			$\begin{array}{c} 103 \\ \cdot \\ $				
R.	norvegicus PLCE	1467	ENHC-SLPQQRKMAEIFKSVFGEKLVAKFLFETDFSDDPMLPSPDQLRRKVLLKNKKLKAHQTPVDILKQ	1535			
R.	norvegicus PLCo	390	ENHC-SLEQQRVMARHLRAILGPILLDQPLDGVTTSLPSPEQLKGKILLKGKKLGGLLPAG	449			
н. ч	sapiens PLCp2	408	ENHVDSPRUQAKMAEYCRTIFGDMLLTEPLEKFPLKPGVPLPSPEDLRGKILIKNKKNQFSGPTSSS	4/4			
п.	sapiens richs	413	ENINDSARQQARMAETCKSTFODALLTEFLDRTFLAFGVFLFSFQDLTGRTLVRRRRRINFSROGFDSAG	401			
			α _{x-Y}				
R.	norvegicus PLC ϵ	1536	KAHQLASMQTQAFTGGNANPPFASNEEEEDEEDEYDYDYESLSDDNILE	1584			
R.	norvegicus PLC δ	450	GENGSEATDVS	460			
н.	sapiens PLC β 2	475	KDTGGTVWAGEEGTELEE	507			
н.	sapiens PLC 3	483	RKRPLEQS-NSALSESSAATEPSSPQLGSP-SSDSCPGLSNGEEVGLEKPSLEPQKSLGDEGLNRGPYVL	550			
			Τα4				
R.	norvegicus PLCE	1585	DRPENKSCADKLOFENNEEVEKRIKKADNSSGNKGKVYDMELGEEFYLPONKKESRQIAPELSDLVIYCQ	1654			
R.	norvegicus PLC δ	461	DEVEAAEMEDEAVRSQVQHKPEDKLKLVPELSDMIIYCKS	501			
H.	sapiens PLC β 2	508	EEVEEEEEESGNLDEEEIKKMQSDEGTAGLEVTAYEEMSSLVNYIQ	554			
н.	sapiens PLC β 3	551	GPADREDEEEDE-EEEEQTDPKKPTTDEGTASSEVNATEEMSTLVNYIE	558			
R.	norvegicus PLCs	1655	AVKEPCT STLNSSGSGRGKERKSRKSTFGNNPGRMSPGETASFNRTSGKSSCEGTROTWEEPPI, SPNTSI,	1724			
R.	norvegicus PLC δ	502	VHFGGFSSPGT	512			
н.	sapiens PLC β 2	555	PTKFVSFEFSAQ	566			
н.	sapiens PLC β 3	559	PVKFKSFEAARK	610			
			$T\beta 6$ $T\alpha 5$ $T\alpha 5$ $T\beta 7$ $T\alpha 6$ $T\beta 8$				
		1705		1704			
R. P	norvegicus PLCE	513	SATTREFRETHISSLNENAAKRLCKRISQKLIQHTACQLLKTIPAATRIDSSNPNPLMFWLHGIQLVALN	579			
н.	sapiens PLC62	567	KNRSYVISSFTELKAYDLLSKASVOFVDYNKROMSRIYPKGTRMDSSNYMPOMFWNAGCOMVALN	631			
н.	sapiens PLC β 3	611	RNKCFEMSSFVETKAMEQLTKSPMEFVEYNKQQLSRIYPKGTRVDSSNYMPQLFWNVGCQLVALN	675			
R.	norvegicus PLCE	1795	YQTDDLPLHLNAAMFEANGGCGYVLKPPVLWDKSCPMYQKFSPL-ERDLDAMDPATYSLTIISGQNVCPS	1863			
к. ч	norvegicus PLCo	579	FOTPGPEMDVILGCFQDNGGCGIVLKPAFLKDPNTTFNSKALTQGPWWKPEKLKVKIISGQQLPKV	644			
н.	sapiens PLCB3	676	FOTINUZMOLNACVFEYNGRSGYLLKPEFMRRPDKSFDPFTEVIVDGTVANALRVKVISGOFLSDR	741			
	bupitons i Lopo	0.0					
R.	norvegicus PLC ϵ	1864	eq:n	1922			
R.	norvegicus PLCS	645	NKNKNSIVDPKVIVEIHGVGRDTGSRQTAVITNNGFNPRWDME-FEFEV-TVPDLALVRFMVEDYDSS	710			
н.	sapiens PLC ³ 2	698	SVRTYVEVELFGLPGDPKRRYRTKLSPSTNSINPVWKEEPFVFEKILMPELASLRVAVME	757			
н.	sapiens PLCp3	742	KE	800			
			$- C\beta6 C\alpha2 - C\beta7 - C\beta8$				
R.	norvegicus PLC ϵ	1923	NSSAITAQRIIPLKALKRGYRHLQLRNLHNEILEISSLFINSRRME 1968				
R.	norvegicus PLC δ	711	SKNDFIGQSTIPWNSLKQGYRHVHLLSKNGDQHPSATLFVKISIQD 756				
н.	sapiens PLC β 2	758	EGNKFLGHRIIPINALNSGYHHLCLHSESNMPLTMPALFIFLEMKD 803				
н.	sapiens PLC β 3	801	EGGKFVGHRILPVSAIRSGYHYVCLRNEANQPLCLPALLIYTEASD 846	_			
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Supplementary Figure 3. Sequence and secondary structure alignment of phospholipase C enzymes. The amino acid sequences of *R. norvegicus* PLCε (UNIPROT Q99P84), *R. norvegicus*

PLC8 (UNIPROT P10688), *H. sapiens* PLCβ2 (UNIPROT Q00722), and *H. sapiens* PLCβ3 (UNIPROT Q01970) spanning the EF3-C2 domains are shown. Sequence identity is highlighted in dark gray, and sequence similarity is highlighted in light gray. The observed secondary structure is shown above the alignment, with β strands shown as arrows, α helices as rounded rectangles, and disordered regions as dashed lines. The secondary structure elements are referred to as described in the structure of *R. norvegicus* PLC δ^{23} , with the exception of the α_{X-Y} helix. PLC ϵ residues shown in gray or white are disordered in the crystal structure. Residues in red are involved in coordinating the catalytic Ca²⁺ ion, and residues in blue correspond to catalytic histidines². The black circles above the alignment are spaced every ten amino acids for reference.



Supplementary Figure 4. Comparison of the PLC ϵ EF3-C2 core domains across PLC subfamilies. The crystal structures of the EF3-C2 domains from (A) *R. norvegicus* PLC ϵ (PDB ID 6PMP, this work), (B) *H. sapiens* PLC β 3 (PDB ID 3OHM³⁹), (C) *R. norvegicus* PLC γ 1 (PDB ID 6PBC²⁶), and (D) *R. norvegicus* PLC δ 1 (PDB ID 2ISD²³). Domains are colored as in (A), and the catalytic Ca²⁺ is shown as a black sphere. Asterisks indicate the first and last ordered residues within the X–Y linker (hot pink) in PLC ϵ , PLC β , and PLC δ , or the ends of the regulatory domains in PLC γ .



Supplementary Figure 5. The PLC ϵ active site and X–Y linker share similarities with PLC β . (A) The architecture of the PLC ϵ EF3-RA1 active site (yellow) is conserved with the PLC β 3 active site (gray, PDB ID 30HM³⁹). The acidic residues shown help coordinate the active site Ca²⁺ (black sphere), along with catalytic histidines. (B) The PLC ϵ EF3-RA1 linker was observed to form the α_{X-Y} helix and a β -hairpin. This shares some similarity to the "lid helix" in PLC β 3 (in blue), which must be displaced prior to substrate binding. (C) Interactions of the X–Y linker β hairpin with residues on the surface of the TIM barrel. Dashed yellow lines indicate hydrogen bonds or salt bridges ≤ 3.5 Å.



Supplementary Figure 6. The structure of PLC ϵ EF3-RA1 is compatible with the predicted location of the PH and EF1/2 domains. (A) Conserved residues on the surface of EF3/4 and the TIM barrel and C2 domains that could form canonical interactions with the PH domain and EF1/2 domains in the context of the complete PLC ϵ core structure instead mediate crystal contacts. The side chains of these residues are shown as balls and sticks, and domains colored as in Figure 1A. (B) Superimposing the PLC β 3 EF3-C2 domains (PDB ID 3OHM³⁹) with those of PLC ϵ shows the structure is compatible with the canonical positions of the PH domain, EF1/2, and the F3 α helix of EF3, as seen in other PLC structures. The r.m.s.d. for the PLC β 3 and PLC ϵ EF3-C2 domains is 1.079 Å for 355 C α atoms. (C) In the PLC ϵ EF3-RA1 structure, the hydrophobic face of the α_{X-Y} helix interacts with a hydrophobic surface on EF3/4 through an *in trans* crystal contact. In the presence of the PH domain, EF1/2, and the F3 α helix, (shown in B), the EF3/4 surface interacts with the F3 α helix, which is disordered in the EF3-RA1 structure.



Supplementary Figure 7. Sequence and secondary structure alignment of the PLCE RA1

domain. The amino acid sequences of the RA1 domain from *R. norvegicus* PLC ε (UNIPROT Q99P84), *H. sapiens* PLC ε (UNIPROT Q9P212), and *C. elegans* PLC ε (UNIPROT G5EFI8) are shown. Sequence identity is highlighted in royal blue, and sequence similarity is highlighted in light blue. The secondary structure of the domain, based on the PLC ε EF3-RA1 structure and the solution structure of the RA1 domain (PDB ID 2BYE¹⁸), is shown above the sequence. β strands shown as arrows, α helices are shown as rounded rectangles, and disordered regions are shown as a dashed line. Residues in gray are disordered in the crystal structure. The black circles above the alignment are spaced every ten amino acids for reference.



Supplementary Figure 8. Thermal stability and basal activity of PLC ϵ variants. (A) Representative DSF curves of the PLC ϵ PH-COOH point mutants and X–Y linker deletion variants. The T_m for each variant is determined from the inflection point of the denaturation curve. (B) Scatter plot of the specific activity of the PLC ϵ variants. Error bars represent SD. Significance was determined based on one-way ANOVA followed by Dunnett's multiple comparisons test vs. PLC ϵ PH-COOH (****, p \leq 0.0001, ***, p \leq 0.0005, **, p \leq 0.001, * p \leq 0.05). Data for PLC ϵ PH-COOH and PH-C2 was previously published²².



Supplementary Figure 9. Representative western blots of PLCE variants. PLCE variant expression in COS-7 cells was visualized and quantified by western blot, using empty vector as a negative control and actin as a loading control. The blots shown are the same as those shown in Figure 3C, but are not cropped. Cell lysates were divided into two pools, and analyzed on separate SDS-PAGE gels and western blots, due to the large size and technical difficulties in transferring the PLCE variants to the PVDF membrane. To minimize the amount of antibody needed for detecting the PLCE variants, the bottom one-third of the membrane was removed prior to incubation with the primary antibody.



Supplementary Figure 10. The EF3/4–RA1 interface blocks small GTPase binding. (A) The structure of the RA1 domain in the PLC ϵ EF3-RA1 structure is similar to the structure of the RA2 domain in complex with activated H-Ras (r.m.s.d. of 1.2 Å for 37 C α , PDB ID 2C5L¹⁸). However, the PLC ϵ RA1 domain is not known to contribute to GTPase binding. (B) Superimposing RA1 from the EF3-RA1 structure with RA2 in the H-Ras–RA2 structure shows that the vestigial GTPase binding surface on RA1 is blocked by EF3/4. H-Ras is shown in dark grey, Mg²⁺ as a black sphere, and GTP in red sticks.



Supplementary Figure 11. PLC ε and PLC β may share a conserved regulatory interface. The TIM barrel-C2 domain interface may be a conserved regulatory hot spot in some PLC enzymes. (A) In the PLC ε EF3-RA1 structure, the C2-RA1 linker docks at the TIM barrel-C2 interface. Deletion of this element, or mutations within this region decrease stability of the enzyme, but increase basal activity across assay formats (Figures 1, 4, Table 1). (B) The crystal structure of the PLC β 3 core and proximal C-terminal domain (CTD) in complex with the activated heterotrimeric G protein subunit G α_q (PDB ID 30HM³⁹). G α_q forms extensive interactions with the proximal CTD, EF3/4, and the C2 domain. These interactions occur on the same face of the PLC core as the C2-RA1 linker in the PLC ε EF3-RA1 structure. Activated G α_q is shown in tan, with Mg²⁺ shown in a black sphere, GTP shown in red sticks, and AlF4⁻ in tan and light blue spheres.

	PLCε EF3-RA1
Guinier analysis	
<i>I(0)</i> (Arb.)	113 ± 0.4
$R_{g}(A)$	35 ± 2.0
$q \min{(\text{\AA}^{-1})}$	0.0000805
q range (Å ⁻¹)	0.0000805-0.00139
P(r) analysis	
<i>I(0)</i> (Arb.)	114
R _g (Å)	36
D _{max} (Å)	126
Porod volume (Å ⁻³)	152,000
q range (Å ⁻¹)	0.0000805-0.230

Supplementary Table 1. SAXS parameters of PLCE EF3-RA1

(a) SAXS data collection parameters					
Instrument	BioCAT facility at the Advanced Photon Source beamline 18ID with Pilatus3 X 1M (Dectris) detector				
Wavelength (Å)	1.033				
Beam size (µm ²)	160 (h) x 75 (v)				
Camera length (m)	3.7				
q-measurement range (Å ⁻¹)	0.004-0.4				
Absolute scaling method	N/A or Glassy Carbon, NIST SRM 3600				
Basis for normalization to constant counts	To incident intensity, by ion chamber counter				
Method for monitoring radiation damage	Automated frame-by-frame comparison of relevant regions using CORMAP ⁵ implemented in BioXTAS RAW				
Exposure time, number of exposures	0.5 s exposure time with 2 s exposure period (0.5 s on, 1.5 s off) of entire SEC elution				
Sample configuration	SEC-SAXS. Separation by size using ÄKTA Pure with a Superdex 200 Increase 10/300 GL column. SAXS data was measured in a 1.5 mm ID quartz capillary with effective path length 0.542 mm.				
Sample temperature (°C)	20				
(b) Software employed for SAXS data reduction, analysis and interpretation					
SAXS data reduction	Radial averaging; BioXTAS RAW 1.4.0 ⁵⁰ and ATSAS ⁵⁴ used for frame comparison, averaging, and subtraction				
Basic analysis: Guinier, M.W., P(r)	BioXTAS RAW 1.4.0 ⁵⁰ used for Guinier fit and molecular weight; GNOM ⁵⁵ used for P(r) function				

Supplementary Table 2. SAXS Data Collection and Analysis Parameters