Supporting Information

Investigating the role of the stringent response in lipid modifications during the stationary phase in *E. coli* by direct analysis with ToF-SIMS

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ABSTRACT: *E. coli* is able to rapidly adjust the biophysical properties of its membrane phospholipids to adapt to environmental challenges including starvation stress. These membrane lipid modifications were investigated in glucose starved *E. coli* cultures and compared to a Δ*rel*AΔ*spo*T (ppGpp^0) mutant strain of *E. coli,* deficient in the stringent response, by means of time-of-flight secondary ion mass spectrometry (ToF-SIMS). Recent advances in ToF-SIMS, through the implementation of gas cluster ion beams (GCIBs), now permit the analysis of higher mass species from native, underivatized, biological specimen i.e. intact bacterial cells. Cultures in stationary phase were found to exhibit a radically different lipid composition as compared to cultures in exponential growth phase. Wild-type *E. coli* reacted upon carbon starvation by lipid modifications including elongation, cyclopropanation and increased cardiolipin formation. Observations are consistent with variants of cardiolipins (CL), phosphatidylglycerols (PG), phosphatidylethanolamines (PE), phosphatidic acids (PA), and fatty acids. Notably, despite having a proteomic profile and a gene expression profile somewhat similar to the wild-type during growth, the ppGpp^0 mutant *E. coli* strain was found to exhibit modified phospholipids corresponding to unsaturated analogues of those found in the wild-type. We concluded that the ppGpp^0 mutant reacts upon starvation stress by elongation and desaturation of fatty acyl chains, implying that only the last step of the lipid modification, the cyclopropanation, is under stringent control. These observations suggest alternative stress response mechanisms and illustrate the role of the RelA and SpoT enzymes in the biosynthetic pathway underlying these lipid modifications.

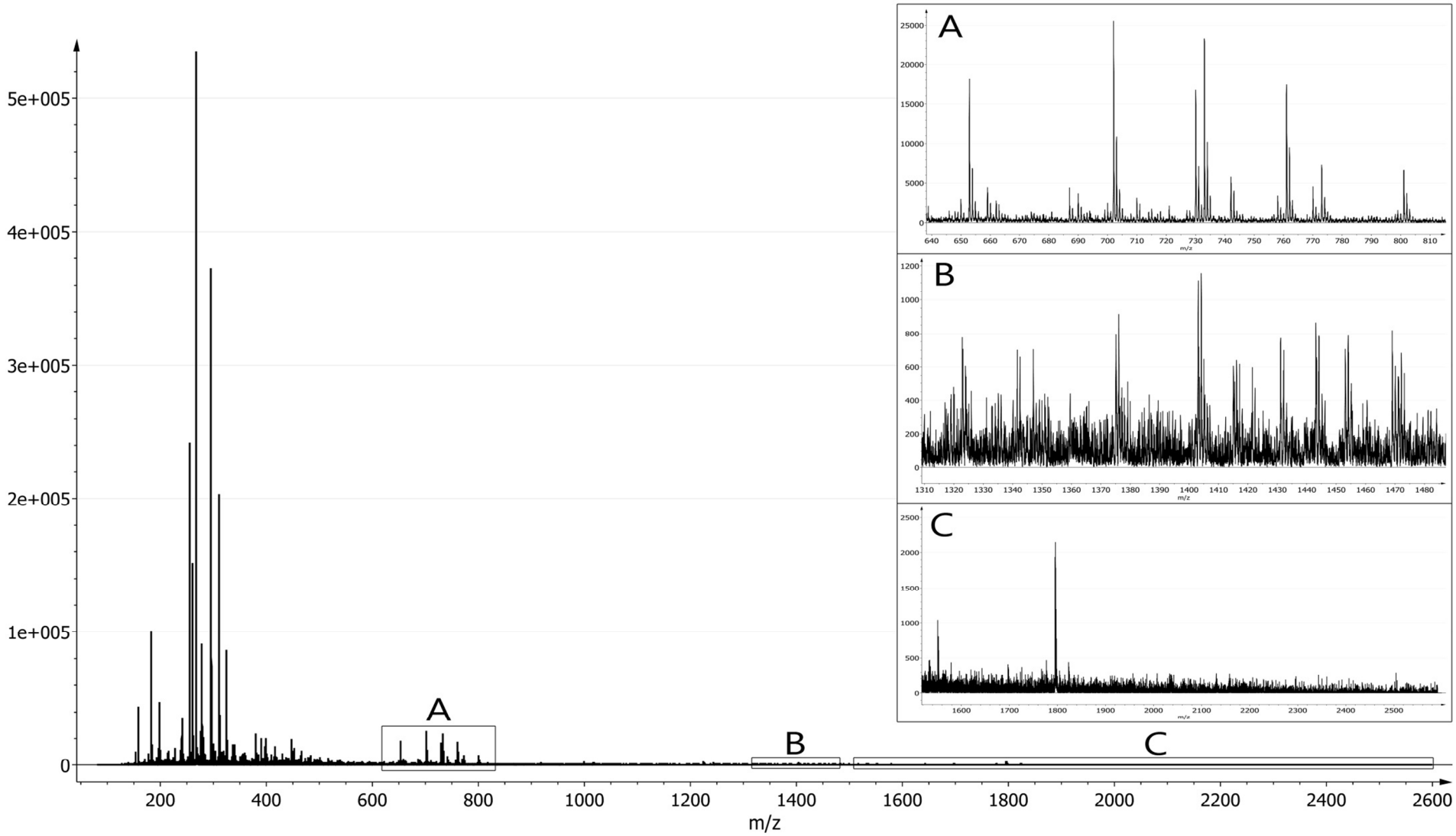

Figure S1. Typical ToF-SIMS spectrum in negative ion mode obtained from wild-type *E. coli* in stationary growth phase.

Table S1. Assignments of signals observed in ToF-SIMS spectra of wild-type and ppGpp^0 mutant *E. coli* in exponential and stationary growth phase.

		Negative ion mode					Positive ion mode				
Assignment	Formula	meas. m/z	exact mass	Δppm	Species	Ion formula	meas. m/z	exact mass	Δppm	Species	Ion formula
Head groups and fragments											
		78.9	78.95851	-	$[M-H]^-$	PO_3^-	-	-	-	-	-
Dihydrogenphosphate	$H_2PO_4^-$	96.9695	96.96907	4.4	$[M-H]^-$	$H_2PO_4^-$	-	-	-	-	-
	$C_3H_4O_4P^-$	134.9848	134.98472	0.6	$[M-H]^-$	$C_3H_4O_4P^-$	-	-	-	-	-
	$C_3H_6O_4P^-$	137.0029	137.00037	18.5	$[M-H]^-$	$C_3H_6O_4P^-$	-	-	-	-	-
Phosphoethanolamine	$C_2H_7NO_4P^-$	140.0136	140.01127	16.6	$[M-H]^-$	$C_2H_7NO_4P^-$	-	-	-	-	-
	$C_3H_6O_5P^-$	152.9975	152.99529	14.4	$[M-H]^-$	$C_3H_6O_5P^-$	-	-	-	-	-
	$HP_2O_6^-$	158.9260	158.92484	7.3	$[M-H]^-$	$HP_2O_6^-$	-	-	-	-	-
Phosphoglycerol	$C_3H_8O_6P^-$	171.0076	171.00585	10.2	$[M-H]^-$	$C_3H_8O_6P^-$	-	-	-	-	-
Pyrophosphate	$H_3P_2O_7^-$	176.9394	176.93540	22.6	$[M-H]^-$	$H_3P_2O_7^-$	-	-	-	-	-
	$C_3H_5OPO_4CH_2CH_2NH_2^-$	196.0387	196.03748	6.2	$[M-H]^-$	$C_3H_5OPO_4CH_2CH_2NH_2^-$	-	-	-	-	-
Fatty acids											
FA (10:0)	$C_{10}H_{20}O_2$	171.1084	171.13850	-175.9	$[M-H]^-$	$C_{10}H_{19}O_2^-$	-	-	-	-	-
FA (OH-10:0)	$C_{10}H_{20}O_3$	187.1000	187.13341	-178.6	$[M-H]^-$	$C_{10}H_{19}O_3^-$	-	-	-	-	-
FA (12:1)	$C_{12}H_{22}O_2$	197.1194	197.15415	-176.3	$[M-H]^-$	$C_{12}H_{21}O_2^-$	-	-	-	-	-
FA (12:0)	$C_{12}H_{24}O_2$	199.1348	199.16980	-175.8	$[M-H]^-$	$C_{12}H_{23}O_2^-$	-	-	-	-	-
FA (OH-12:0)	$C_{12}H_{24}O_3$	215.1294	215.16471	-164.1	$[M-H]^-$	$C_{12}H_{23}O_3^-$	-	-	-	-	-

FA (14:0)	$C_{14}H_{28}O_2$	227.2005	227.20109	-2.6	$[M-H]^-$	$C_{14}H_{27}O_2^-$	-	-	-	-	-
FA (16:1)	$C_{16}H_{30}O_2$	253.2154	253.21674	-5.3	$[M-H]^-$	$C_{16}H_{29}O_2^-$	-	-	-	-	-
FA (16:0)	$C_{16}H_{32}O_2$	255.2323	255.23239	-0.4	$[M-H]^-$	$C_{16}H_{31}O_2^-$	282.2559	282.25588	0.1	$[FA+26]^+$	$C_{18}H_{34}O_2^{\bullet+}$
FA (cp17:0)	$C_{17}H_{32}O_2$	267.2328	267.23239	1.5	$[M-H]^-$	$C_{17}H_{31}O_2^-$	294.2561	294.25588	0.7	$[FA+26]^+$	$C_{19}H_{34}O_2^{\bullet+}$
FA (18:1)	$C_{18}H_{34}O_2$	281.2476	281.24804	-1.6	$[M-H]^-$	$C_{18}H_{33}O_2^-$	308.2746	308.27153	10.0	$[FA+26]^+$	$C_{20}H_{36}O_2^{\bullet+}$
FA (cp19:0)	$C_{19}H_{36}O_2$	295.2640	295.26369	1.0	$[M-H]^-$	$C_{19}H_{35}O_2^-$	322.2894	322.28718	6.9	$[FA+26]^+$	$C_{21}H_{38}O_2^{\bullet+}$
Phosphatidic acids											
PA (26:0)	$C_{29}H_{57}O_8P$	563.3406	563.37126	-54.4	$[M-H]^-$	$C_{29}H_{56}O_8P^-$	467.4019	467.41004	-17.4	$[M+H-98]^+$	$C_{29}H_{55}O_4^+$
PA (OH-26:0)	$C_{29}H_{57}O_9P$	579.3196	579.36618	-80.4	$[M-H]^-$	$C_{29}H_{56}O_9P^-$	483.3837	483.40495	-44.0	$[M+H-98]^+$	$C_{29}H_{55}O_5^+$
PA (28:0)	$C_{31}H_{61}O_8P$	591.3553	591.40258	-80.0	$[M-H]^-$	$C_{31}H_{60}O_8P^-$	495.4194	495.44134	-44.3	$[M+H-98]^+$	$C_{31}H_{59}O_4^+$
PA (OH-28:0)	$C_{31}H_{60}O_9P$	606.37	606.38965	-32.4	$[M-H]^-$	$C_{31}H_{59}O_9P^-$	-	-	-	-	-
PA (30:0)	$C_{33}H_{65}O_8P$	619.4033	619.43386	-49.3	$[M-H]^-$	$C_{33}H_{64}O_8P^-$	523.4854	523.47264	24.4	$[M+H-98]^+$	$C_{33}H_{63}O_4^+$
PA (OH-30:0)	$C_{33}H_{65}O_9P$	635.3829	635.42877	-72.2	$[M-H]^-$	$C_{33}H_{64}O_9P^-$	-	-	-	-	-
PA (32:1)	$C_{35}H_{67}O_8P$	645.4574	645.44951	12.2	$[M-H]^-$	$C_{35}H_{66}O_8P^-$	549.5043	549.48829	29.1	$[M+H-98]^+$	$C_{35}H_{65}O_4^+$
PA (cp33:0)	$C_{36}H_{69}O_8P$	659.4670	659.46516	2.8	$[M-H]^-$	$C_{36}H_{68}O_8P^-$	563.5035	563.50394	-0.8	$[M+H-98]^+$	$C_{36}H_{67}O_4^+$
PA (34:2)	$C_{37}H_{69}O_8P$	-	-	-	-	-	575.5015	575.50394	-4.2	$[M+H-98]^+$	$C_{37}H_{67}O_4^+$
PA (34:1)	$C_{37}H_{71}O_8P$	673.4778	673.48081	-4.5	$[M-H]^-$	$C_{37}H_{70}O_8P^-$	577.5194	577.51959	-0.3	$[M+H-98]^+$	$C_{37}H_{69}O_4^+$
PA (cp35:0)	$C_{38}H_{73}O_8P$	687.4995	687.49645	4.4	$[M-H]^-$	$C_{38}H_{72}O_8P^-$	591.5370	591.53524	3.0	$[M+H-98]^+$	$C_{38}H_{71}O_4^+$
PA (cp36:0)	$C_{39}H_{73}O_8P$	699.4932	699.49645	-4.6	$[M-H]^-$	$C_{39}H_{72}O_8P^-$	603.5366	603.53524	2.3	$[M+H-98]^+$	$C_{39}H_{71}O_4^+$
PA (cp38:0)	$C_{41}H_{77}O_8P$	727.5284	727.52775	0.9	$[M-H]^-$	$C_{41}H_{76}O_8P^-$	631.5678	631.56653	2.0	$[M+H-98]^+$	$C_{41}H_{75}O_4^+$
Phosphatidylethanolamines											
PE (26:0)	$C_{31}H_{62}NO_8P$	606.3700	606.41346	-71.7	$[M-H]^-$	$C_{31}H_{61}NO_8P^-$	467.3890	467.41004	-45.0	$[M+H-141]^+$	$C_{29}H_{55}O_4^+$

PE (OH-26:0)	$C_{31}H_{62}NO_9P$	622.3666	622.40837	-67.1	$[M-H]^-$	$C_{31}H_{61}NO_9P^-$	483.3837	483.40495	-44.0	$[M+H-141]^+$	$C_{29}H_{55}O_5^+$
PE (28:0)	$C_{33}H_{66}NO_8P$	634.3988	634.44476	-72.4	$[M-H]^-$	$C_{33}H_{65}NO_8P^-$	495.4194	495.44134	-44.3	$[M+H-141]^+$	$C_{31}H_{59}O_4^+$
PE (OH-28:0)	$C_{33}H_{66}NO_9P$	650.3968	650.43967	-65.9	$[M-H]^-$	$C_{33}H_{65}NO_9P^-$	652.4215	652.45535	-51.9	$[M+H]^+$	$C_{33}H_{67}NO_9P^+$
							511.4145	511.43625	-42.5	$[M+H-141]^+$	$C_{31}H_{59}O_5^+$
PE (30:0)	$C_{35}H_{70}NO_8P$	662.4624	662.47605	-20.6	$[M-H]^-$	$C_{35}H_{69}NO_8P^-$	664.4884	664.49173	-5.0	$[M+H]^+$	$C_{35}H_{71}NO_8P^+$
							523.4854	523.47264	24.4	$[M+H-141]^+$	$C_{33}H_{63}O_4^+$
PE (OH-30:0)	$C_{35}H_{70}NO_9P$	678.4913	678.47097	30.0	$[M-H]^-$	$C_{35}H_{69}NO_9P^-$	-	-	-	-	-
PE (32:1);	$C_{37}H_{72}NO_8P$	688.4936	688.49170	2.8	$[M-H]^-$	$C_{37}H_{71}NO_8P^-$	690.5148	690.50738	10.7	$[M+H]^+$	$C_{37}H_{73}NO_8P^+$
PE (cp33:0)	$C_{38}H_{74}NO_8P$	702.5097	702.50735	3.3	$[M-H]^-$	$C_{38}H_{73}NO_8P^-$	704.5321	704.52303	12.9	$[M+H]^+$	$C_{38}H_{75}NO_8P^+$
							563.5035	563.50394	-0.8	$[M+H-141]^+$	$C_{36}H_{67}O_4^+$
							742.4944	742.47892	20.9	$[M+K]^+$	$C_{38}H_{74}KNO_8P^+$
PE (34:2)	$C_{39}H_{74}NO_8P$	714.5061	714.50735	-1.7	$[M-H]^-$	$C_{39}H_{73}NO_8P^-$	716.5088	716.52303	-19.9	$[M+H]^+$	$C_{39}H_{75}NO_8P^+$
PE (cp34:0)	$C_{39}H_{74}NO_8P$	714.5061	714.50735	-1.7	$[M-H]^-$	$C_{39}H_{73}NO_8P^-$	716.5088	716.52303	-19.9	$[M+H]^+$	$C_{39}H_{75}NO_8P^+$
PE (34:1)	$C_{39}H_{76}NO_8P$	716.5240	716.52300	1.4	$[M-H]^-$	$C_{39}H_{75}NO_8P^-$	718.5441	718.53868	7.5	$[M+H]^+$	$C_{39}H_{77}NO_8P^+$
							577.5194	577.51959	-0.3	$[M+H-141]^+$	$C_{37}H_{69}O_4^+$
PE (cp35:0)	$C_{40}H_{78}NO_8P$	730.5421	730.53865	4.7	$[M-H]^-$	$C_{40}H_{77}NO_8P^-$	591.5370	591.53524	3.0	$[M+H-141]^+$	$C_{38}H_{71}O_4^+$
							770.5214	770.51022	14.5	$[M+K]^+$	$C_{40}H_{78}KNO_8P^+$
PE (36:2)	$C_{41}H_{78}NO_8P$	742.5395	742.53865	1.1	$[M-H]^-$	$C_{41}H_{77}NO_8P^-$	603.5366	603.53524	2.3	$[M+H-141]^+$	$C_{39}H_{71}O_4^+$
PE (cp36:0)	$C_{41}H_{78}NO_8P$	742.5395	742.53865	1.1	$[M-H]^-$	$C_{41}H_{77}NO_8P^-$	603.5366	603.53524	2.3	$[M+H-141]^+$	$C_{39}H_{71}O_4^+$
PE (cp38:0)	$C_{43}H_{82}NO_8P$	770.5683	770.56995	-2.1	$[M-H]^-$	$C_{43}H_{81}NO_8P^-$	631.5678	631.56653	2.0	$[M+H-141]^+$	$C_{41}H_{75}O_4^+$
Phosphatidylglycerols											
PG (26:1)	$C_{32}H_{61}O_{10}P$	635.3829	635.39239	-14.9	$[M-H]^-$	$C_{32}H_{60}O_{10}P^-$	465.3890	465.39439	-11.6	$[M+H-172]^+$	$C_{29}H_{53}O_4^+$
PG (26:0)	$C_{32}H_{63}O_{10}P$	637.3713	637.40804	-57.6	$[M-H]^-$	$C_{32}H_{62}O_{10}P^-$	467.3890	467.41004	-45.0	$[M+H-172]^+$	$C_{29}H_{55}O_4^+$

PG (OH-26:0)	$C_{32}H_{63}O_{11}P$	653.3404	653.40295	-95.7	$[M-H]^-$	$C_{32}H_{62}O_{11}P^-$	483.3837	483.40495	-44.0	$[M+H-172]^+$	$C_{29}H_{55}O_5^+$
PG (28:0)	$C_{34}H_{67}O_{10}P$	665.4012	665.43934	-57.3	$[M-H]^-$	$C_{34}H_{66}O_{10}P^-$	495.4194	495.44134	-44.3	$[M+H-172]^+$	$C_{31}H_{59}O_4^+$
PG (OH-28:0)	$C_{34}H_{67}O_{11}P$	681.3929	681.43425	-60.7	$[M-H]^-$	$C_{34}H_{66}O_{11}P^-$	511.4145	511.43625	-42.5	$[M+H-172]^+$	$C_{31}H_{59}O_5^+$
PG (30:0)	$C_{36}H_{71}O_{10}P$	693.4567	693.47064	-20.1	$[M-H]^-$	$C_{36}H_{70}O_{10}P^-$	523.4854	523.47264	24.4	$[M+H-172]^+$	$C_{33}H_{63}O_4^+$
PG (OH-30:0)	$C_{36}H_{71}O_{11}P$	709.4159	709.46555	-70.0	$[M-H]^-$	$C_{36}H_{70}O_{11}P^-$	-	-	-	-	-
PG (32:1)	$C_{38}H_{73}O_{10}P$	719.4913	719.48628	7.0	$[M-H]^-$	$C_{38}H_{72}O_{10}P^-$	549.5043	549.48829	29.1	$[M+H-172]^+$	$C_{35}H_{65}O_4^+$
PG (32:0)	$C_{38}H_{75}O_{10}P$	721.4998	721.50193	-3.0	$[M-H]^-$	$C_{38}H_{74}O_{10}P^-$	551.5003	551.50394	-6.6	$[M+H-172]^+$	$C_{35}H_{67}O_4^+$
PG (cp33:0)	$C_{39}H_{75}O_{10}P$	733.5066	733.50193	6.4	$[M-H]^-$	$C_{39}H_{74}O_{10}P^-$	563.5035	563.50394	-0.8	$[M+H-172]^+$	$C_{36}H_{67}O_4^+$
PG (34:2)	$C_{40}H_{75}O_{10}P$	745.5059	745.50193	5.3	$[M-H]^-$	$C_{40}H_{74}O_{10}P^-$	575.5015	575.50394	-4.2	$[M+H-172]^+$	$C_{37}H_{67}O_4^+$
PG (cp34:0)	$C_{40}H_{75}O_{10}P$	745.5059	745.50193	5.3	$[M-H]^-$	$C_{40}H_{74}O_{10}P^-$	575.5015	575.50394	-4.2	$[M+H-172]^+$	$C_{37}H_{67}O_4^+$
PG (34:1)	$C_{40}H_{77}O_{10}P$	747.5205	747.51758	3.9	$[M-H]^-$	$C_{40}H_{76}O_{10}P^-$	577.5194	577.51959	-0.3	$[M+H-172]^+$	$C_{37}H_{69}O_4^+$
PG (cp35:0)	$C_{41}H_{79}O_{10}P$	761.5337	761.53323	0.6	$[M-H]^-$	$C_{41}H_{78}O_{10}P^-$	591.5370	591.53524	3.0	$[M+H-172]^+$	$C_{38}H_{71}O_4^+$
PG (36:2)	$C_{42}H_{79}O_{10}P$	773.5336	773.53323	0.5	$[M-H]^-$	$C_{42}H_{78}O_{10}P^-$	603.5366	603.53524	2.3	$[M+H-172]^+$	$C_{39}H_{71}O_4^+$
PG (cp36:0)	$C_{42}H_{79}O_{10}P$	773.5336	773.53323	0.5	$[M-H]^-$	$C_{42}H_{78}O_{10}P^-$	603.5366	603.53524	2.3	$[M+H-172]^+$	$C_{39}H_{71}O_4^+$
PG (cp38:0)	$C_{44}H_{83}O_{10}P$	801.5586	801.56453	-7.4	$[M-H]^-$	$C_{44}H_{82}O_{10}P^-$	631.5678	631.56653	2.0	$[M+H-172]^+$	$C_{41}H_{75}O_4^+$
Cardiolipins											
CL (cp65:0)	$C_{74}H_{142}O_{17}P_2$	1363.9178	1363.96435	-34.1	$[M-H]^-$	$C_{74}H_{141}O_{17}P_2^-$	-	-	-	-	-
CL (cp66:0)	$C_{75}H_{142}O_{17}P_2$	1375.9391	1375.96435	-18.4	$[M-H]^-$	$C_{75}H_{141}O_{17}P_2^-$	-	-	-	-	-
CL (cp67:1)	$C_{76}H_{144}O_{17}P_2$	1389.9347	1389.98	-32.6	$[M-H]^-$	$C_{76}H_{143}O_{17}P_2^-$	-	-	-	-	-
CL (cp68:1)	$C_{77}H_{144}O_{17}P_2$	1401.9524	1401.98	-19.7	$[M-H]^-$	$C_{77}H_{143}O_{17}P_2^-$	-	-	-	-	-
CL (cp86:0)	$C_{77}H_{146}O_{17}P_2$	1404.0054	1403.99565	6.9	$[M-H]^-$	$C_{77}H_{145}O_{17}P_2^-$	-	-	-	-	-
CL (cp69:0)	$C_{78}H_{146}O_{17}P_2$	1416.0159	1415.99565	14.3	$[M-H]^-$	$C_{78}H_{145}O_{17}P_2^-$	-	-	-	-	-
CL (cp69:1)	$C_{78}H_{148}O_{17}P_2$	1417.9910	1418.0113	-14.3	$[M-H]^-$	$C_{78}H_{147}O_{17}P_2^-$	-	-	-	-	-

CL (cp70:1)	$C_{79}H_{148}O_{17}P_2$	1429.9609	1430.0113	-35.2	$[M-H]^-$	$C_{79}H_{147}O_{17}P_2^-$	-	-	-	-	-
CL (cp70:0)	$C_{79}H_{150}O_{17}P_2$	1432.0219	1432.02695	-3.5	$[M-H]^-$	$C_{79}H_{149}O_{17}P_2^-$	-	-	-	-	-
CL (cp72:1); CL (71:0)	$C_{80}H_{150}O_{17}P_2$	1444.0205	1444.02695	-4.5	$[M-H]^-$	$C_{80}H_{149}O_{17}P_2^-$	-	-	-	-	-
CL (cp73:0)	$C_{82}H_{155}O_{17}P_2$	1473.0373	1473.06607	-19.5	$[M-H]^-$	$C_{82}H_{154}O_{17}P_2^-$	-	-	-	-	-
CL (cp74:0)	$C_{83}H_{154}O_{17}P_2$	1484.0320	1484.05831	-17.7	$[M-H]^-$	$C_{83}H_{153}O_{17}P_2^-$	-	-	-	-	-