

Supporting Information:

**Structural elucidation and isomeric differentiation/quantification
of monophosphorylated phosphoinositides using gas-phase ion/ion
reactions and dipolar DC activation**

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Table of Contents

Supplementary Figures.....Page S3-S7

Schematic S1. A simplified schematic of the modified Sciex 5600 q-TOF platform enabled for ion/ion reactions and dipolar DC activation.

Figure S1. The CID spectrum of [PI 16:0/18:1-H]⁻.

Figure S2. The CID spectra of charge inverted released fatty acyl chains, [18:1-H]⁻ and [16:0-H]⁻ with [Mg(Terpy)₂]²⁺.

Figure S3. CID spectrum of [18:1-H+MgTerpy]⁻ that resulted from CID of [PI3P 18:1/18:1-2H]²⁻, followed by an ion/ion reaction with [Mg(Terpy)₂]²⁺.

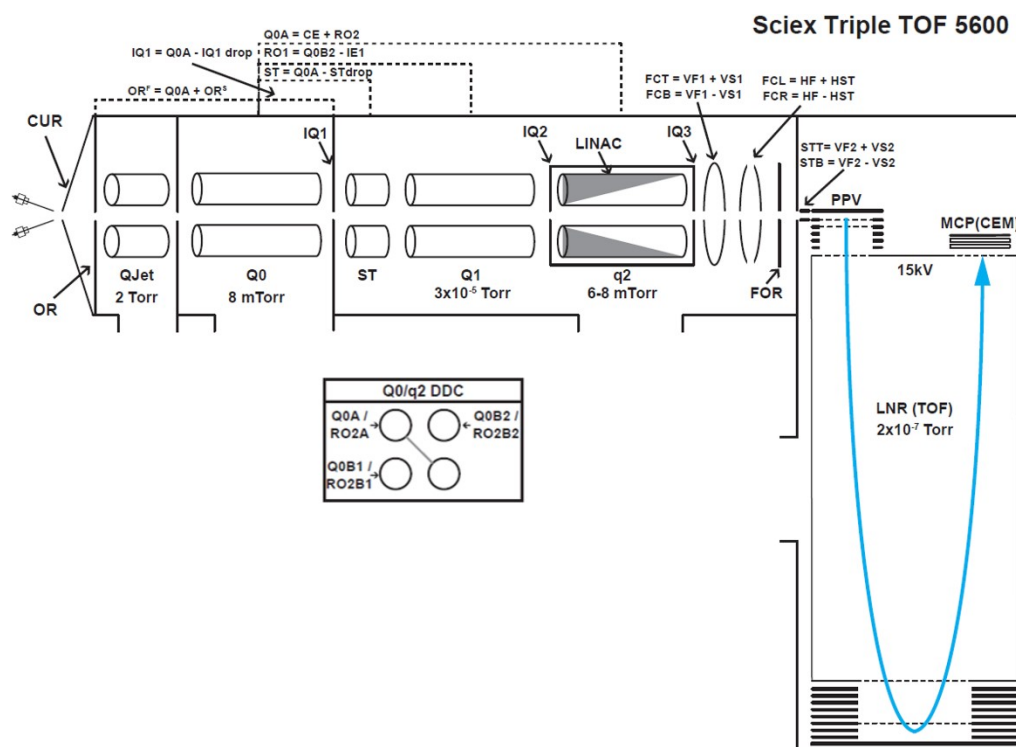
Figure S4. MS2 spectra of doubly-deprotonated monophosphorylated PIyPs.

Figure S5. CID Spectra of charge-reduced PIPs, (series PIyP 18:0/20:4 where y=3, 4, or 5), by a proton transfer ion/ion reaction.

Figure S6. CID Spectra of charge-inverted PIPs, (series PIyP 18:0/20:4 where y=3, 4, or 5), by sequential ion/ion reactions, first by a proton transfer reaction using [Terpy+H]⁺, then a charge-inverted reaction using [Mg(Terpy)₂]²⁺.

Supplementary Tables.....Page S4

Table S1. Ion identities from CID of [PI 16:0/18:1-H]⁻ (Figure S1). Observed fragments are in good agreement with those reported on LIPID MAPS®.



Schematic S1. A simplified schematic of the modified Sciex 5600 q-TOF platform enabled for ion/ion reactions and dipolar DC activation.

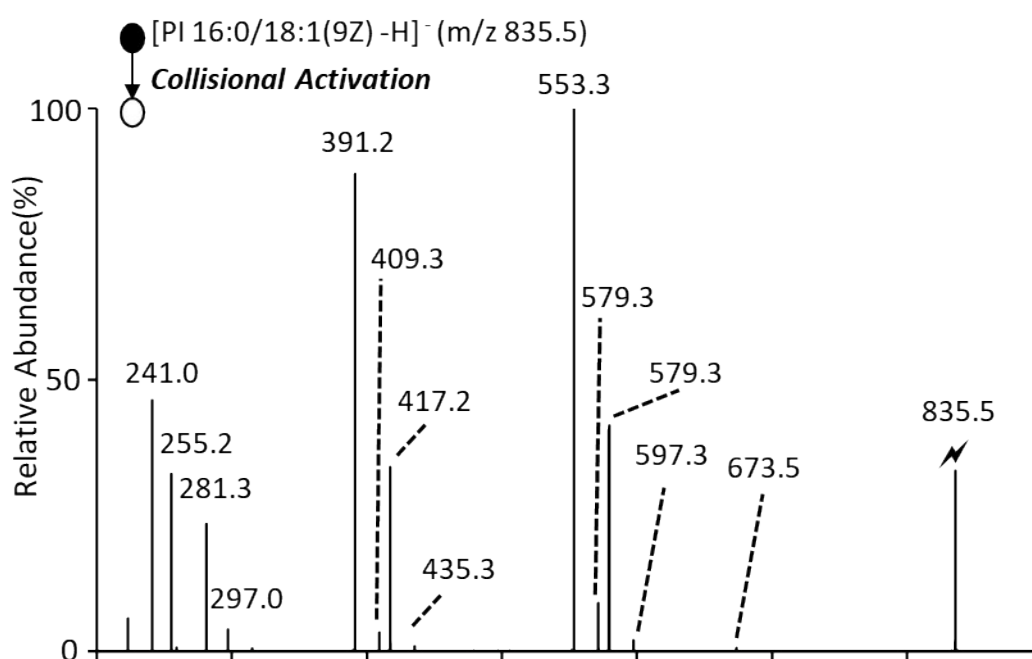


Figure S1. The CID spectrum of [PI 16:0/18:1-H]⁻.

Table S1. Ion identities from CID of [PI 16:0/18:1-H]⁻ (Figure S1). Observed fragments are in good agreement with those reported on LIPID MAPS®.

m/z	Ion Identify
835.5	[PI 16:0/18:1-H] ⁻ (precursor ion)
673.5	NL of inositol
597.3	NL of sn1 as a ketene (RCH=C=O)
579.3	NL of sn1 as an acid (RCOOH)
571.3	NL of sn2 as a ketene (RCH=C=O)
553.5	NL of sn2 as an acid (RCOOH)
435.3	NL of sn1 as a ketene (RCH=C=O) and inositol
417.2	NL of sn1 as an acid (RCOOH) and inositol
409.3	NL of sn2 as a ketene (RCH=C=O) and inositol
391.2	NL of sn2 as an acid (RCOOH) and inositol
297.0	Glycerophosphoinositol ion – NL of water
281.3	sn2 RCOO ⁻
255.2	sn1 RCOO ⁻
241.0	Inositol phosphate ion – NL of 2 water

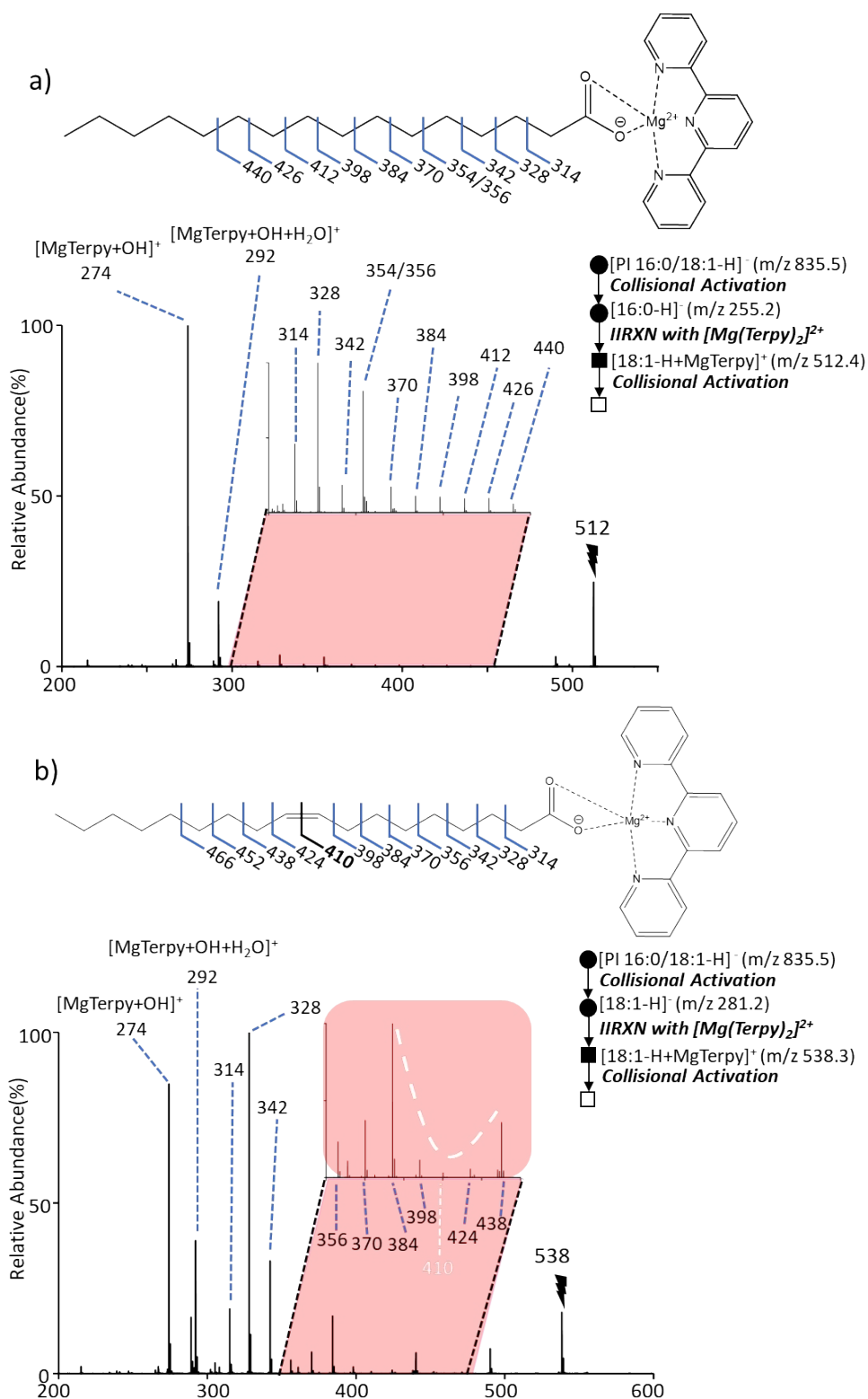


Figure S2. The CID spectra of charge inverted released fatty acyl chains, [18:1-H]⁻ and [16:0-H]⁻ with [Mg(Terpy)₂]²⁺. a) CID of [16:0-H+MgTerpy]⁺ (m/z 512.4). The insert is an enhanced spectra of m/z region 300-450. b) CID of [18:1-H+MgTerpy]⁺ (m/z 538.3). The insert is an enhanced spectra of m/z region 350-450. The white dotted line is indicative of the spectral gap obtained around the double bond position.

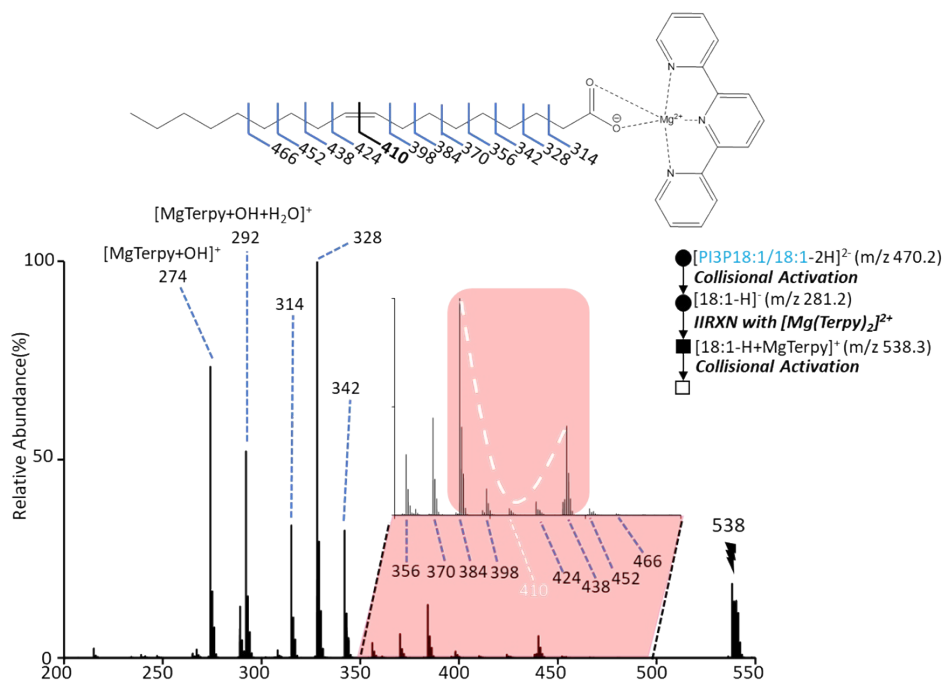


Figure S3. The CID spectrum resulting from an initial CID step of $[PI3P\ 18:1/18:1-2H]^{2-}$, to release its fatty acyl chains (they are identical for this series), $[18:1-H]^-$ ($m/z\ 281.2$) which is subsequently charge inverted to with $[Mg(Terpy)_2]^{2+}$ to yield the charge inverted complex, $[18:1-H+MgTerpy]^-$ that is then subjected to CID which is the shown spectrum.

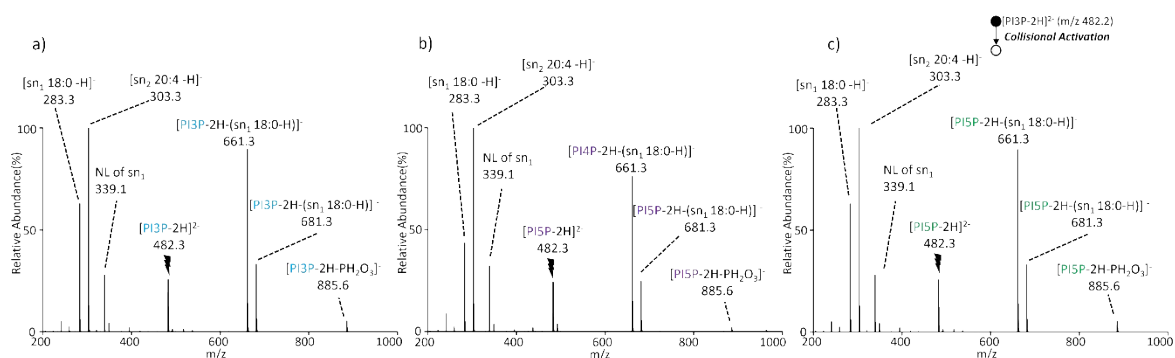


Figure S4. MS2 spectra of doubly-deprotonated monophosphorylated PI y Ps. 18:0/20:4 where $y=3, 4,$ or 5 and indicates the site of phosphorylation along the inositol ring. To simplify the nomenclature, the composition of the fatty acids is removed from the labeling of ions for this series (e.g PI3P 18:0/20:4 doubly deprotonated is denoted $[PI3P-2H]^{2-}$). (a) The CID spectrum of $[PI3P-2H]^{2-}$. (b) The CID spectrum of $[PI4P-2H]^{2-}$. (c) The CID spectrum of $[PI5P-2H]^{2-}$.

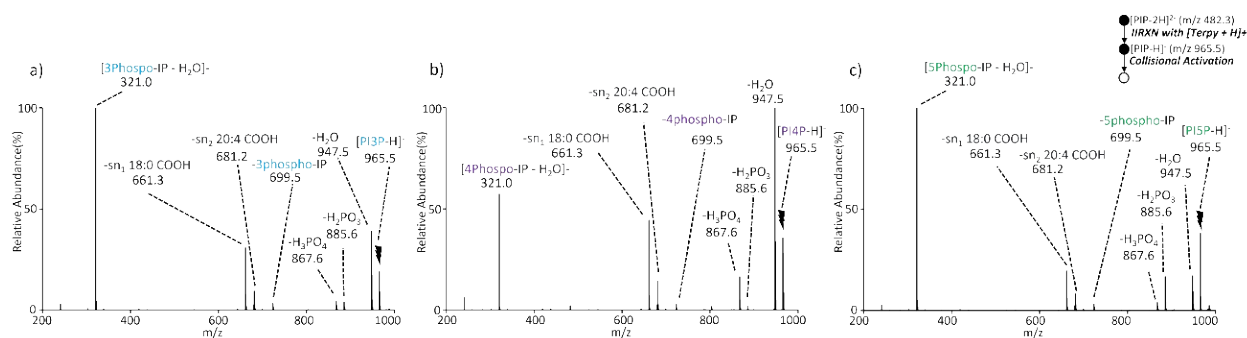


Figure S5. CID Spectra of charge-reduced PIPs, (series PIyP 18:0/20:4 where y=3, 4, or 5), by a proton transfer ion/ion reaction. (a) The CID spectrum of [PI3P-H]⁻. (b) The CID spectrum of [PI4P-H]⁻. (c) The CID spectrum of [PI5P-H]⁻.

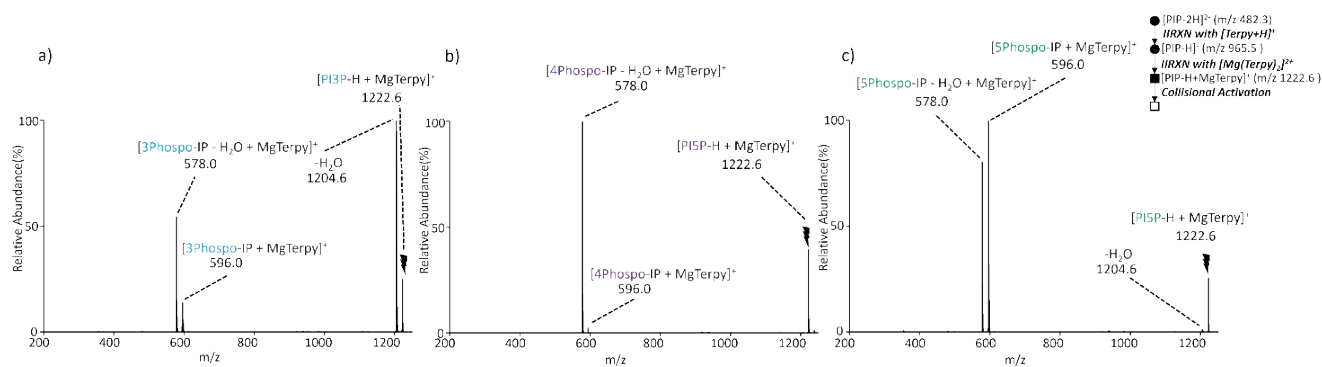


Figure S6. CID Spectra of charge-inverted PIPs, (series PIyP 18:0/20:4 where y=3, 4, or 5), by sequential ion/ion reactions, first by a proton transfer reaction using [Terpy+H]⁺, then a charge-inverted reaction using [Mg(Terpy)₂]²⁺. (a) The CID spectrum of [PI3P-H+MgTerpy]⁺. (b) The CID spectrum of [PI4P-H+MgTerpy]⁺. (c) The CID spectrum of [PI5P-H+MgTerpy]⁺.