# Lipid Separation and Structural Characterization Using Travelling Wave Cyclic Ion Mobility

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## Introduction

- Phosphatidyl inositol monophosphate (PIP) contains one phosphate moiety at the 3<sup>rd</sup>, 4<sup>th</sup>, or 5<sup>th</sup> position of the sugar and phosphatidyl inositol diphosphate (PIP2) contains two phosphates at (3,4), (3,5) and (4,5) positions of the sugar. The position of the phosphate group might have a biological implication and it is therefore important to separate these isomers.
- Here we demonstrate how the scalable ion mobility of the SELECT SERIES™ Cyclic™ ion mobility spectrometer (IMS) enhances the isomeric separation and aids structural characterization for PIP and PIP2 isomers.



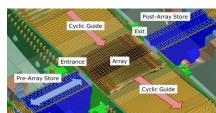


Figure 1. Geometry and design of the cyclic ion mobility (clM) device. It provides the option to perform either a single pass, or multiple passes until the desired resolution is achieved.

# **Experimental**

Product #	Product Name	Elemental formula	Expected m/z [M-H] <sup>-</sup>	
850150	18:1 PI(3)P	C <sub>45</sub> H <sub>84</sub> O <sub>16</sub> P <sub>2</sub>	941.5156	
850151	18:1 PI(4)P	C <sub>45</sub> H <sub>84</sub> O <sub>16</sub> P <sub>2</sub>	941.5156	
850152	18:1 PI(5)P	C <sub>45</sub> H <sub>84</sub> O <sub>16</sub> P <sub>2</sub>	941.5156	
850153	18:1 PI(3,4)P2	C <sub>45</sub> H <sub>85</sub> O <sub>19</sub> P <sub>3</sub>	1021.4820	
850154	18:1 PI(3,5)P2	C <sub>45</sub> H <sub>85</sub> O <sub>19</sub> P <sub>3</sub>	1021.4820	
850155	18:1 PI(4.5)P2	CasHosO10P2	1021,4820	

Table 1. PIP and PIP2 isomers purchased from Avanti Polar Lipids.

The PIP and PIP2 samples were purchased from Avanti Polar Lipids and a final concentration of 500pg/µL was prepared. Samples were infused at 5µL/min into the ESI source of the mass spectrometer.

## Results

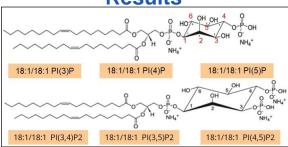


Figure 2. Structure of PIP and PIP2 isomers.

# **PIP Isomer Separation**

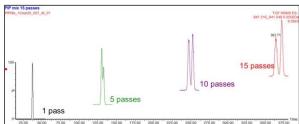


Figure 3. Separation of PI(3)P, PI(4)P and PI(5)P mixture; 1 to 15 passes of cIM (resolution ~65 to 250)

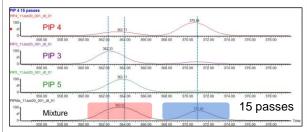


Figure 4. Identification of individual PIP species after 15 passes of cIM. The PI(4)P ions (highlighted in blue) were discarded, and the co-eluting PI(3)P & PI(5)P ions (highlighted in red) are sliced, stored in the Pre-Array store and transmitted to the cIM device for additional passes.

## Results

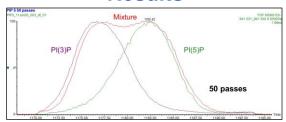


Figure 5. Identification of individual species of PI(3)P and PI(5)P isomers after 50 passes of cIM (resolution ~450  $\Omega/\Delta\Omega$ ).

#### **PIP2 Isomer Separation**



Figure 6. Separation of all PIP2 isomers PI(3,5)P2, PI(3,4)P2 and PI(4,5)P2 after 50 passes of cIM (resolution ~450  $\Omega/\Delta\Omega$ ).

#### HD MS/MS of PI(4,5)P2 fragment ion spectrum

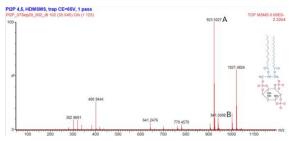


Figure 7. Loss of  $H_3PO_4$  (m/z 923.5, A) or  $HPO_3$  (m/z 941.5, B) groups can be from either the 4 or 5 position on the hexose ring resulting in the formation of the M- $H_2O$  of either PI(4)P or PI(5)P.

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#### Results

# Separation of the two di-phosphate species from figure 7 by IMS

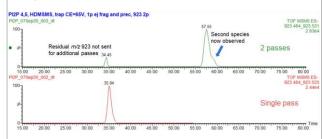


Figure 8. HD MS/MS ATD of pre-IMS fragment ions of PI(4,5)P2 *m/z* 923 (-H<sub>3</sub>PO<sub>4</sub>) selected for additional passes of cIM.



Figure 9. HD MS/MS ATD of pre-IMS fragment ions of PI2P at m/z 923 (-H<sub>3</sub>PO<sub>4</sub>) selected for 10 passes of cIM resulting in two discreet species being observed corresponding to the M-H<sub>2</sub>O of PI(4)P and PI(5)P.

### **Conclusions**

- Cyclic IMS provides novel, scalable ion mobility resolution.
- The increased resolution provides baseline separation of PI(4)P from PI(3)P and PI(5)P after 15 passes of the cIM device.
- Advanced modes of operation with ion activation followed by ion mobility separation offers new insights into lipid structural characterization.