

# Tandem mass spectrometry analysis of prostaglandins and isoprostanes

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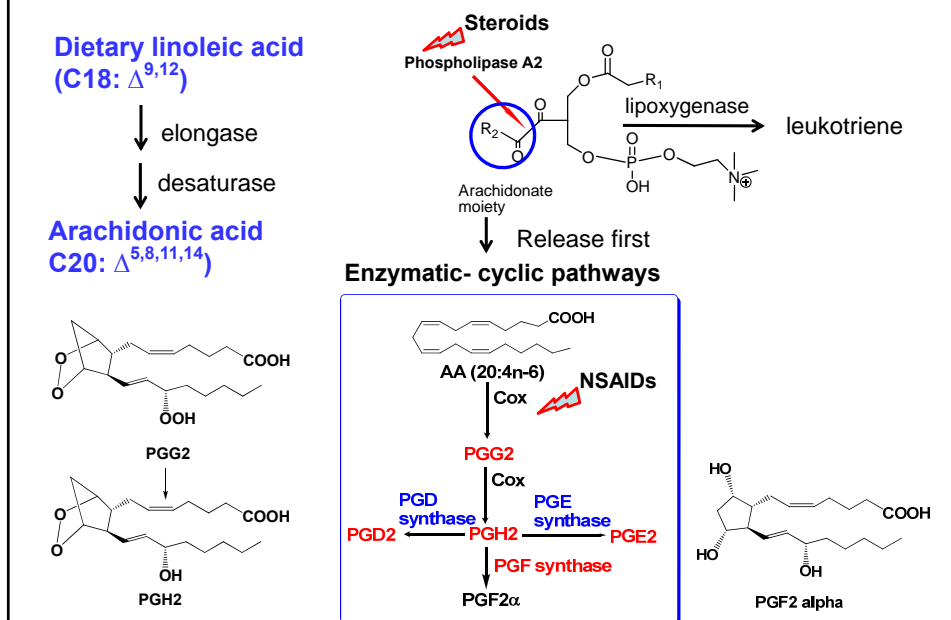
## Overview

- Introduction to PGs and their synthesis
- Mass spectrometry characterization of PGs and isoprostanes
- PGs in Cox-dKO pups and *C. elegans*

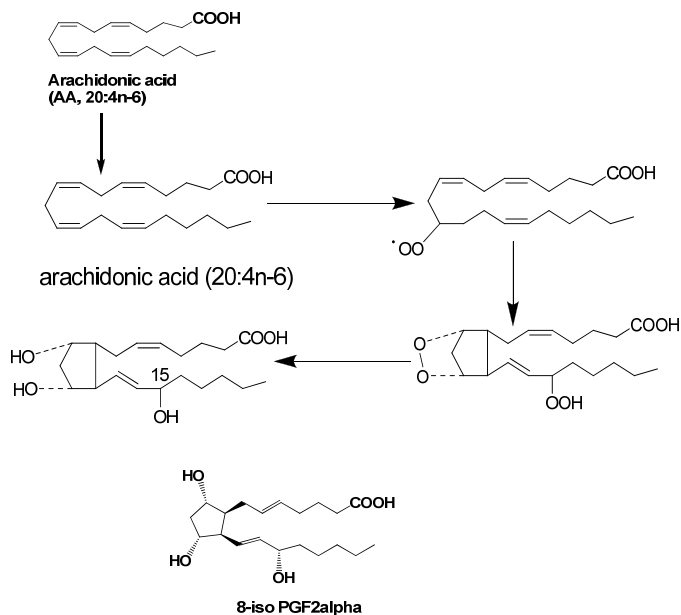
## Prostaglandins

- Derived from 20 carbon PUFA, have short half-lives and act as local hormones
- Bind to specific cell surface G-protein coupled receptors and implicated in a number of physiological processes including reproductive function.
- NSAIDs acts through inhibiting Cox and hence PGs and exert various effects, including infertility. However, the genetics of prostaglandin synthesis and action have largely been unexplored *in vivo*.
- Mammalian systems are not well suited for discovering new genes and molecular mechanisms involved in PG action.
- The nematode *C. elegans* provides a platform for discovering roles of genes and mechanisms that would provide an ideal complement to mammalian systems.

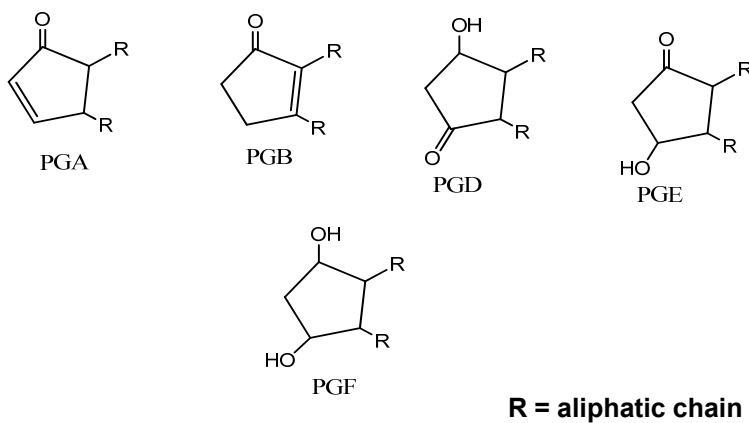
## Cox-dependent PGs synthesis



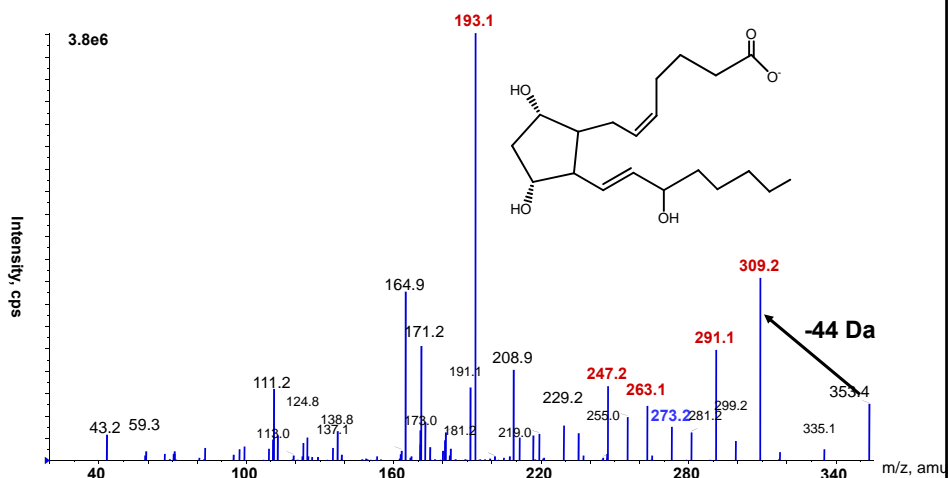
## Non-enzymatic isoprostane synthesis



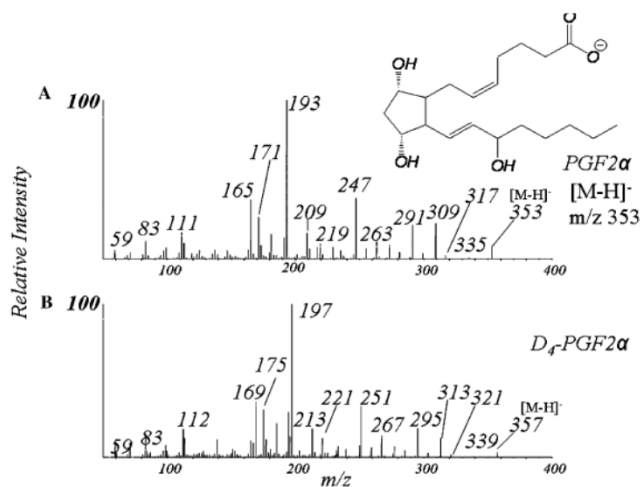
## Structural representation PG based on ring features



## ESI-MS/MS of the [M-H]<sup>-</sup> from PGF<sub>2</sub>α m/z 353 using a quadrupole mass spectrometer

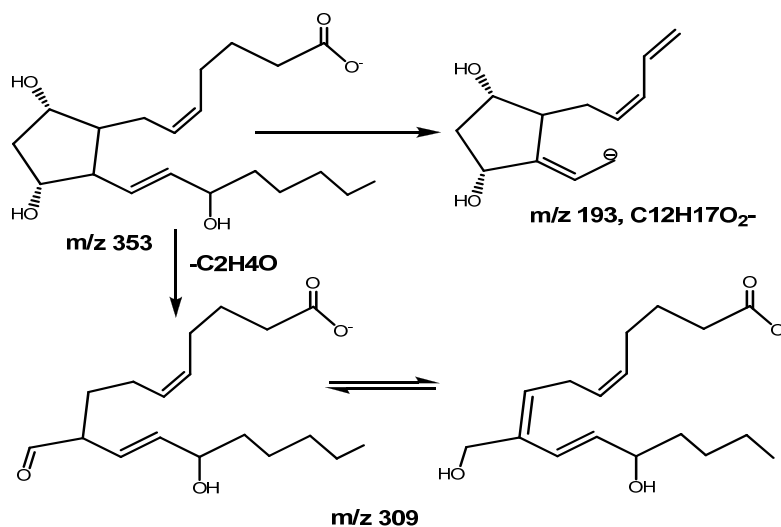


## What information does deuterium labeling at C-2 and C-3 of PGF<sub>2</sub> provide us for structure elucidation of PG?



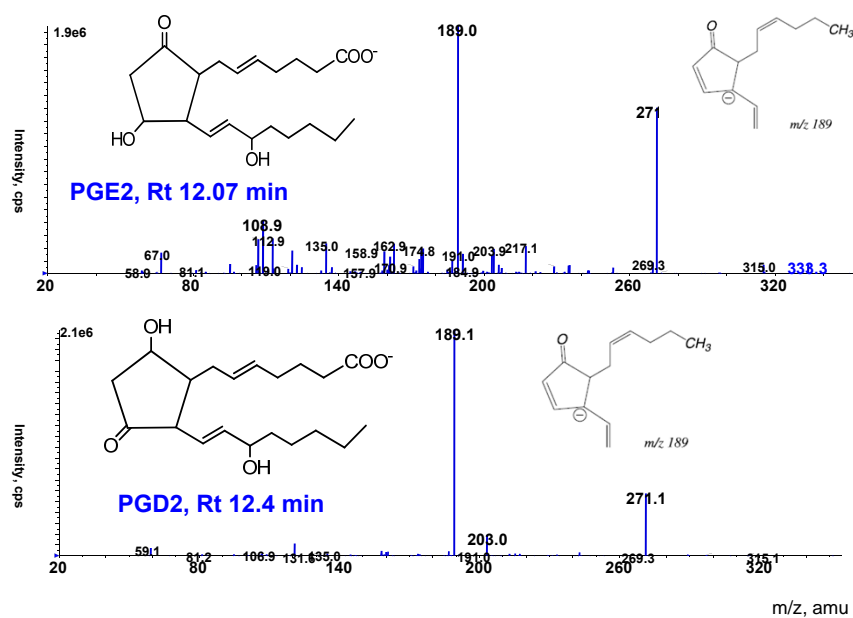
Source: Murphy et al. Analytical Biochemistry, 2005

### Fragmentation scheme of PGF<sub>2</sub>α [M-H]<sup>-</sup> m/z 353

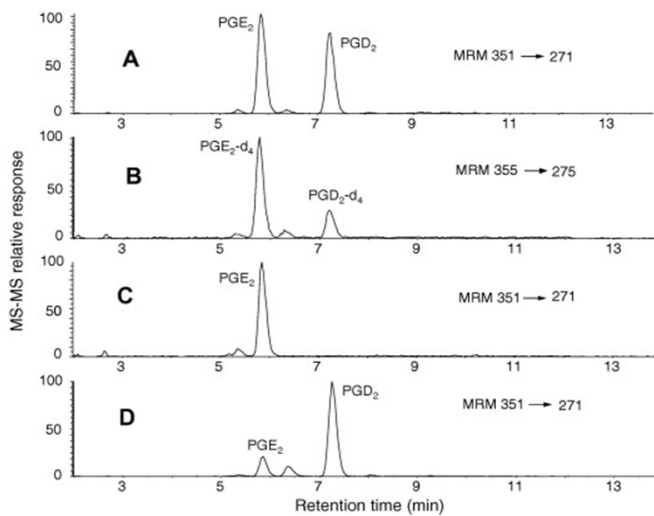


Ions  $m/z$  309, 291, 273 and 193 are indicative of F<sub>2</sub>-ring

### MS/MS fragmentation of PGE<sub>2</sub> and PGD<sub>2</sub> $m/z$ 351.00

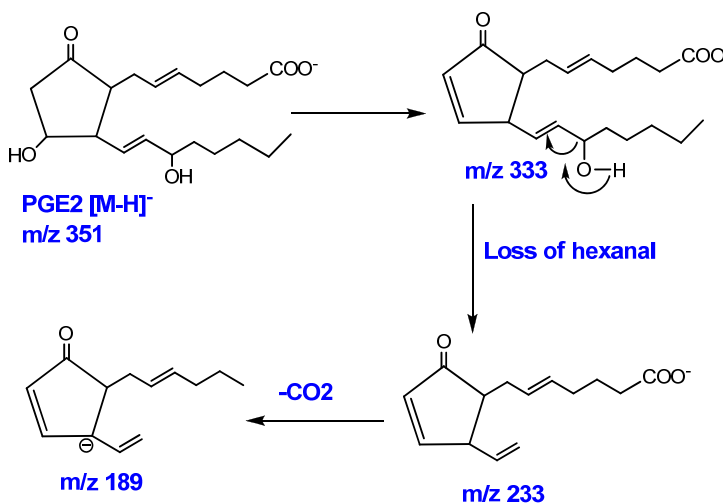


## Deuterated PG standards are used for quantitative analysis of PGs in a extract



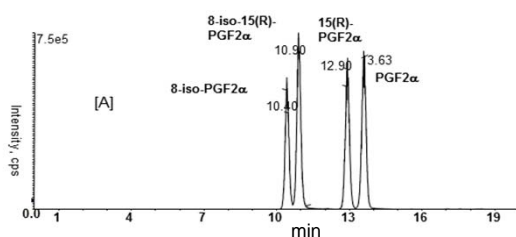
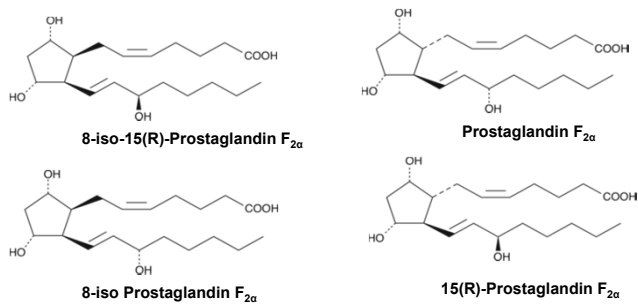
Source: Cao et al. Analytical Biochemistry, 2008

## MS/MS fragmentation of PGE<sub>2</sub> [M-H]<sup>-</sup> m/z 351



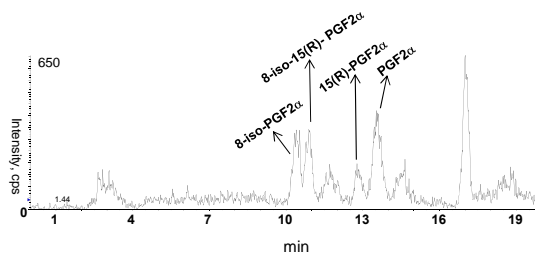
The first loss of water, m/z 189 and m/z 233 are characteristics of PGE<sub>2</sub>/PGD<sub>2</sub>

**PGs and diastereoisomer isoprostanes can be distinguished based on retention time in LC-MS**



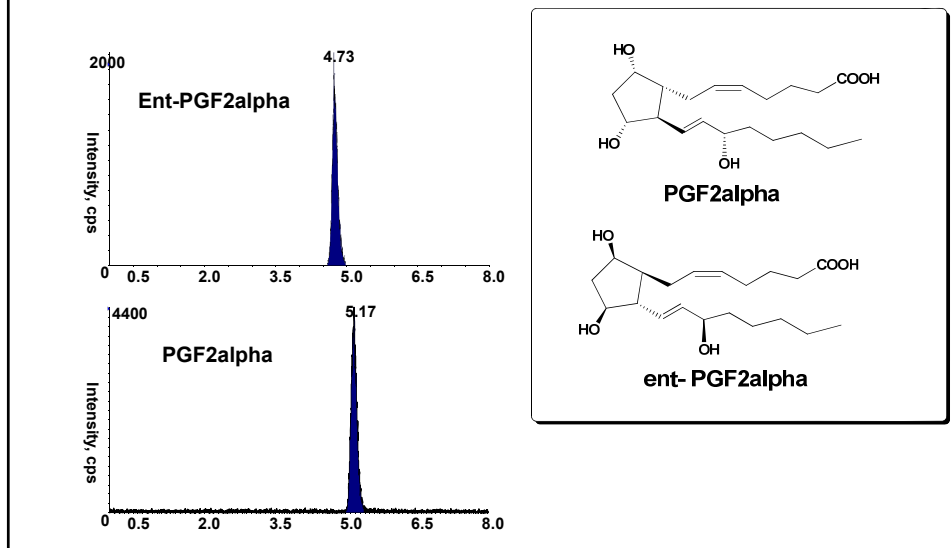
Prasain et al., J Chrom B. 2013

**SRM chromatogram showing isoprostanes and PG in an AKI patient**

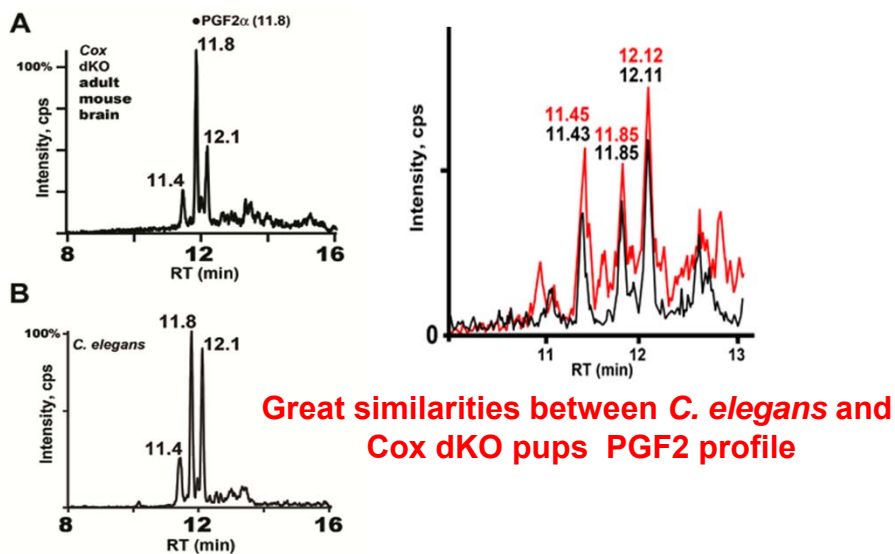


Prasain et al., J Chrom B. 2013

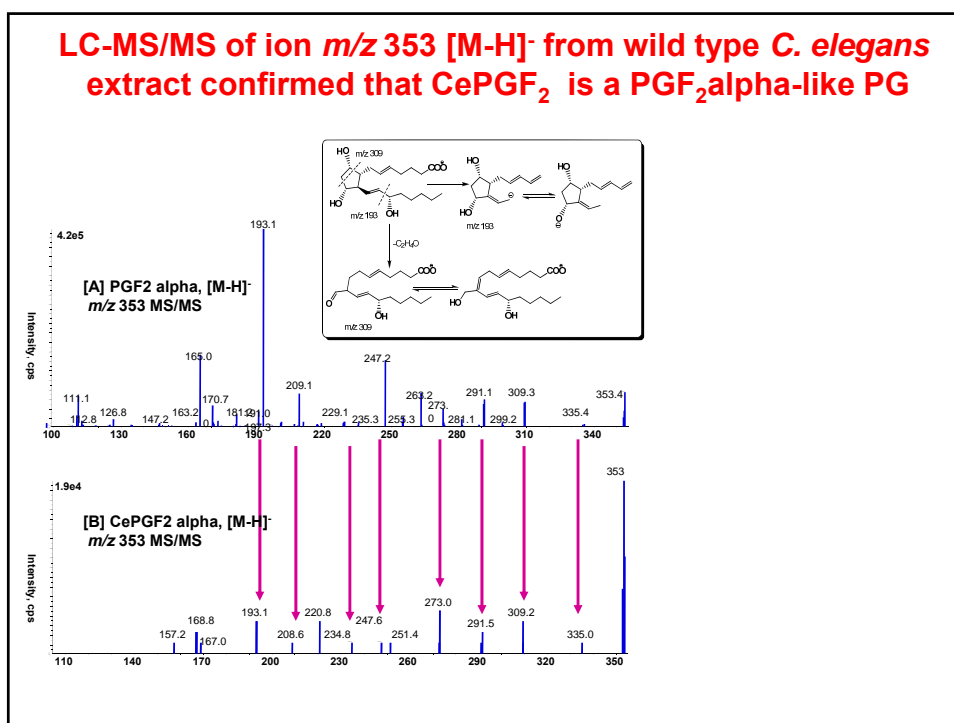
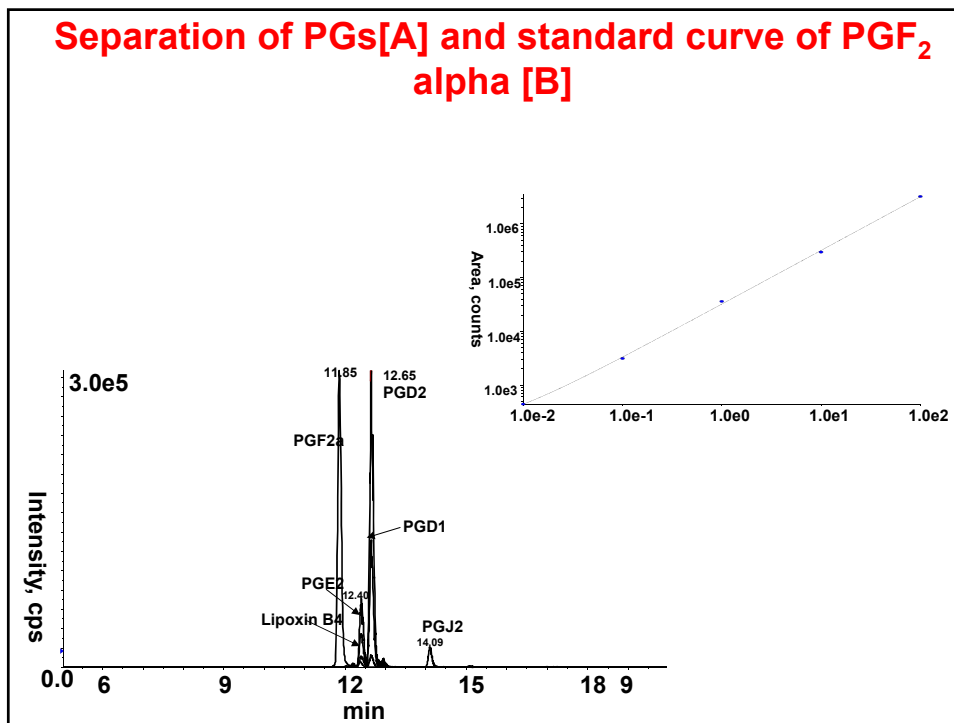
**Separation of PGF<sub>2</sub>alpha and its enantiomer only possible in chiral normal phase column (ChiralPak AD-H column) APCI -ve ion mode**

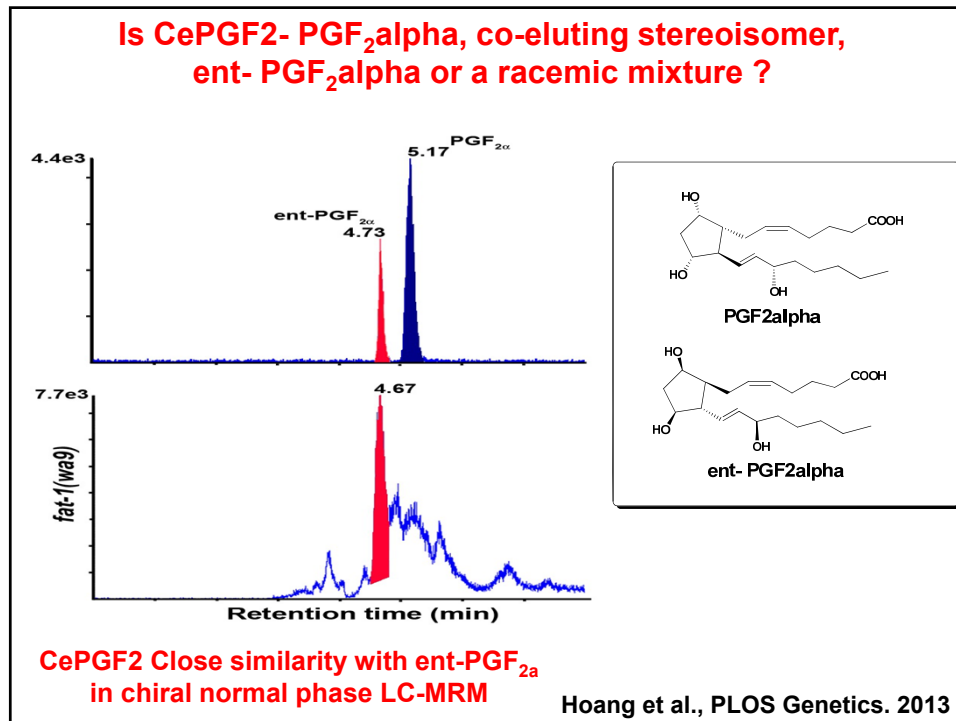


**Cox-independent PGs is widespread**









## Conclusions

- Based on liquid chromatography-tandem mass spectrometry (LC-MS/MS), genetic analyses, and bioactivity assays, *C. elegans* synthesizes Cox-independent sperm guiding F-series PGs from PUFA precursors.
- F-series PGs are synthesized in Cox-deficient mice, indicating the possible existence of similar mechanisms in other animals.