

# **Tandem mass spectrometry analysis of prostaglandins and isoprostanes**

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1

## **Overview**

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

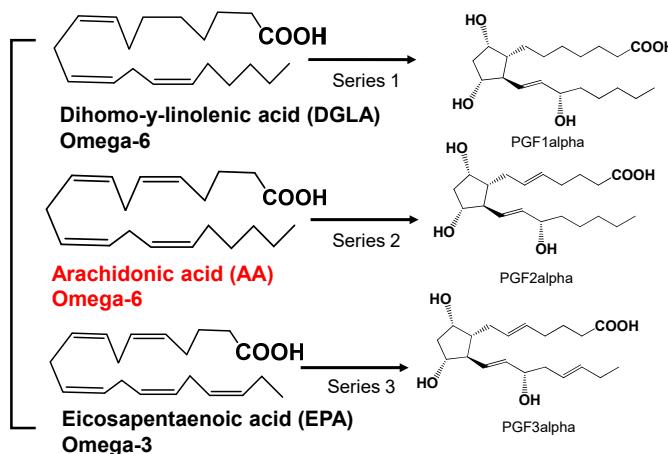
2

## 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 reproductive functions.
- The nematode *C. elegans* provides a platform for discovering roles of genes and mechanisms that would provide an ideal complement to mammalian systems.

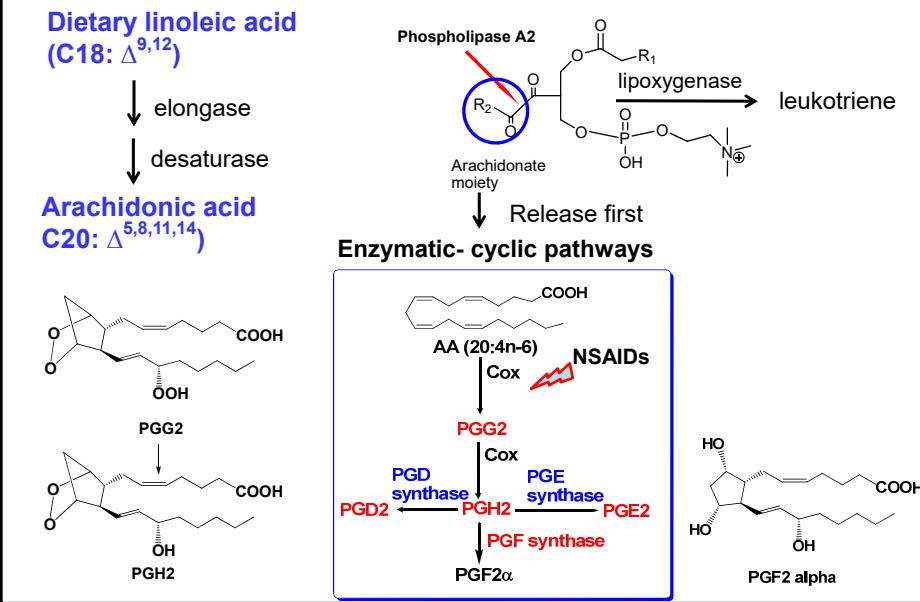
3

## Polyunsaturated fatty acids (PUFAs)- substrates for PGs



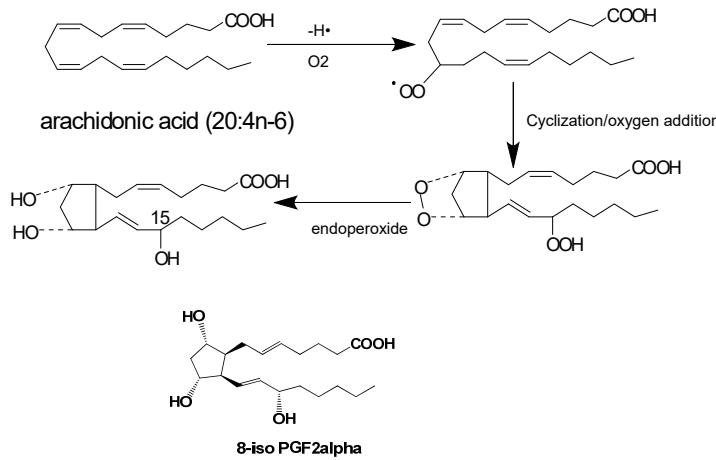
4

## Cox-dependent PGs synthesis



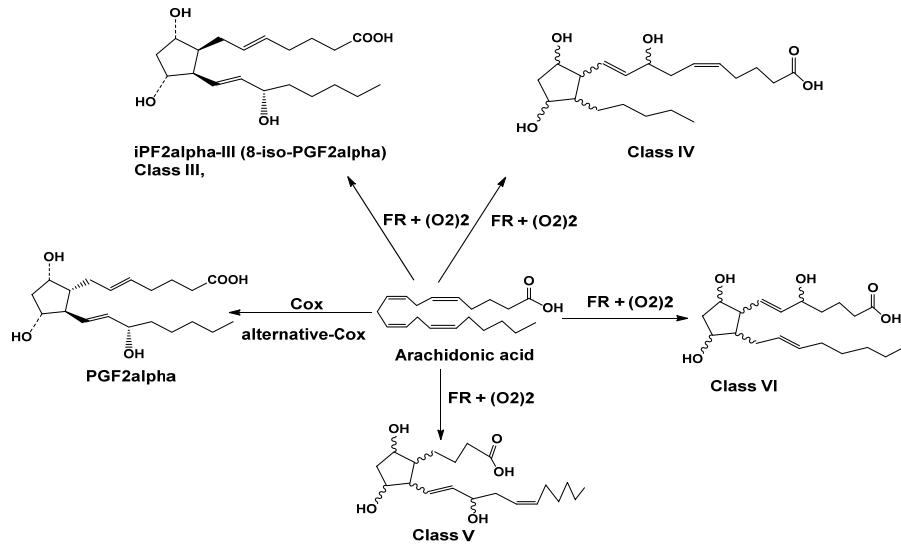
5

## Non-enzymatic F2-isoprostane (F2-IsoP) synthesis



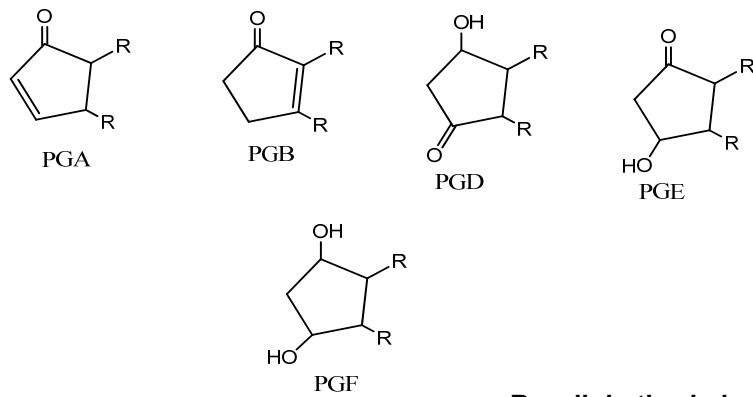
6

## Four classes of F2-isoprostanes from free-radical initiated reaction of arachidonic acid



7

## Structural representation PG based on ring features



8

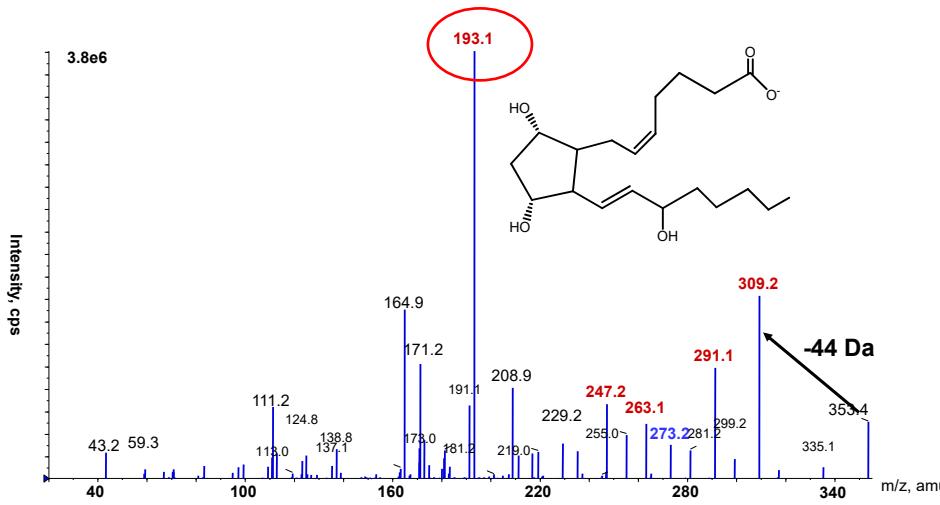
# Prostaglandin analysis

Concentration range nM-pM in biological samples

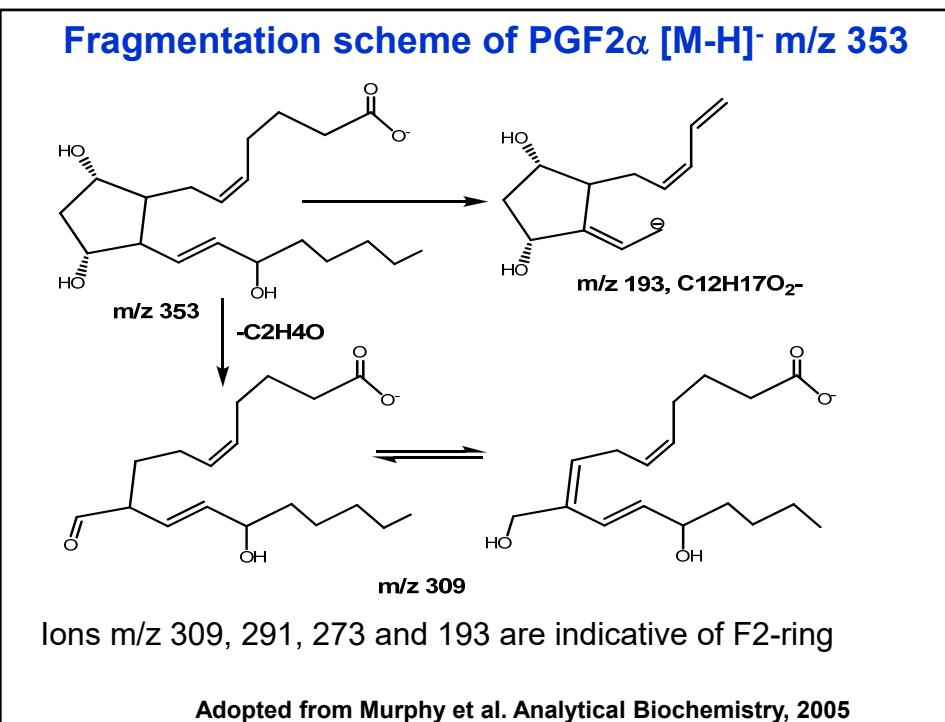
1. Immunoassay (poor specificity for isomeric PGs, and only one or a few compounds/assay)
1. GC-MS (derivatization needed)
1. LC-MS/MS

9

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

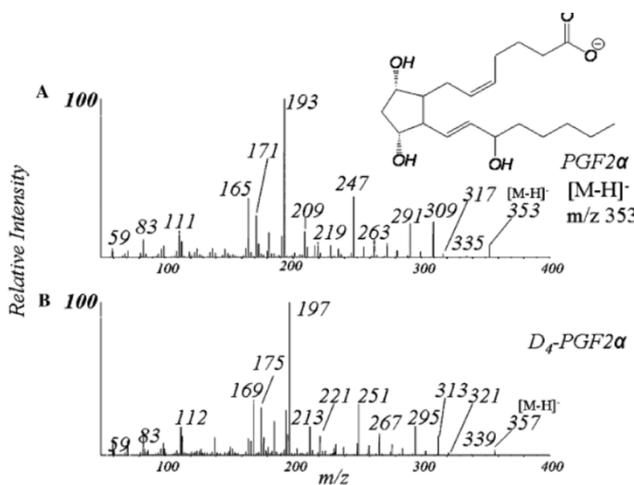


10



11

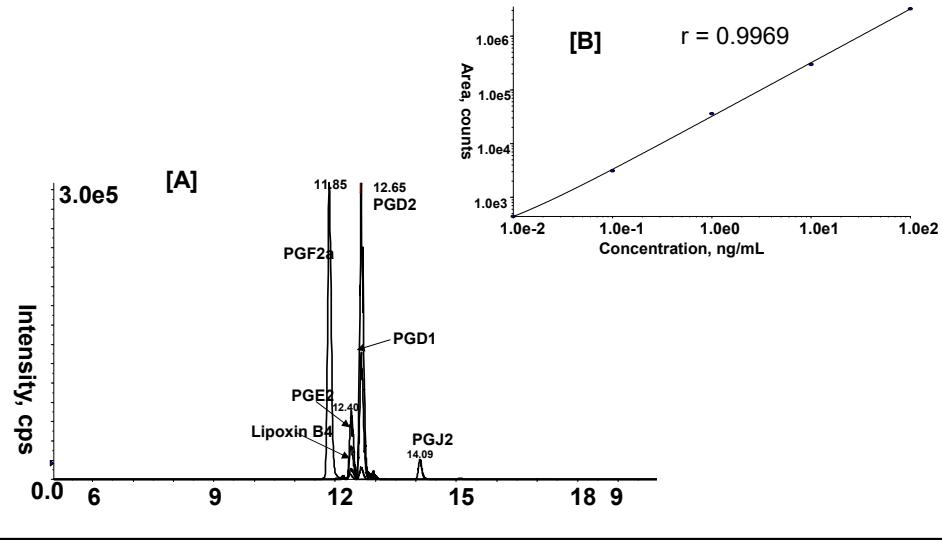
**What information does deuterium labeling at C-2 and C-3 of PGF2 provide us for structure elucidation of PG?**



Source: Murphy et al. Analytical Biochemistry, 2005

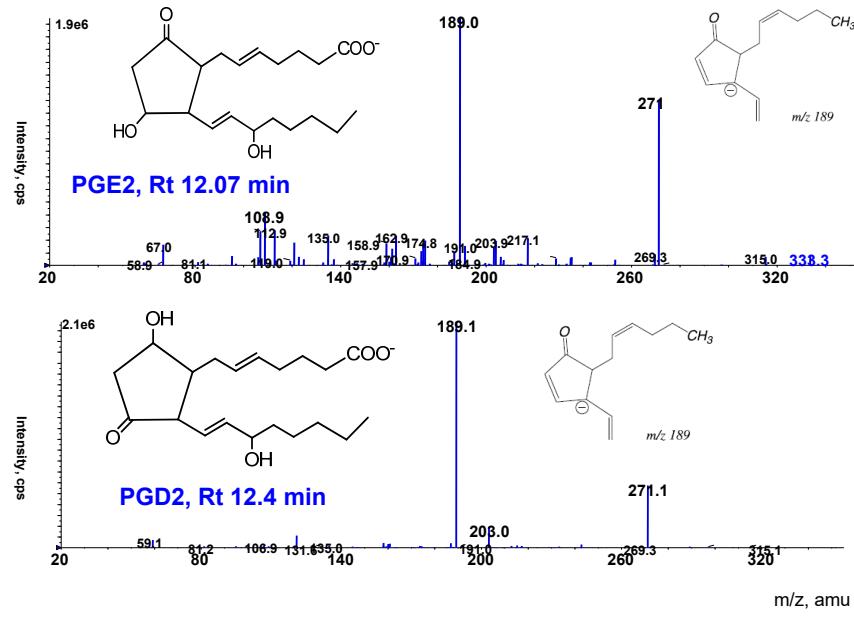
12

## Separation of PGs[A] and standard curve of PGF2alpha [B]



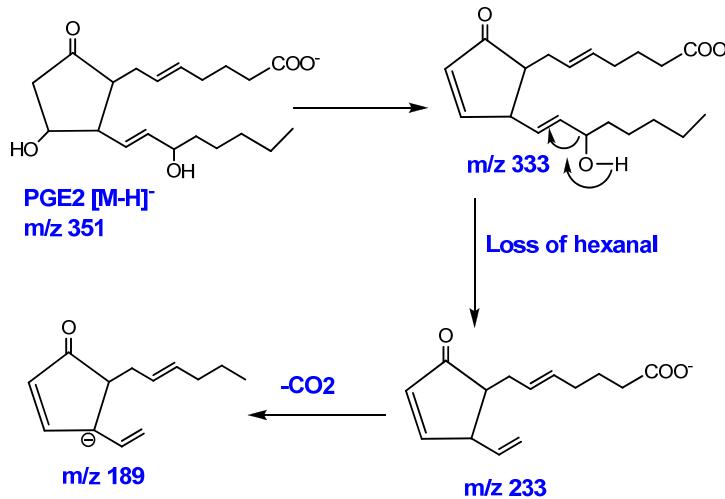
13

## MS/MS fragmentation of PGE2 and PGD2 m/z 351.00



14

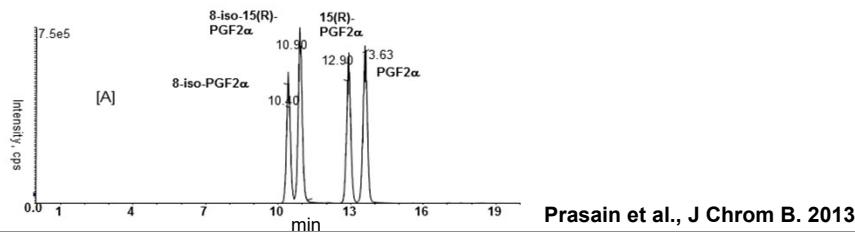
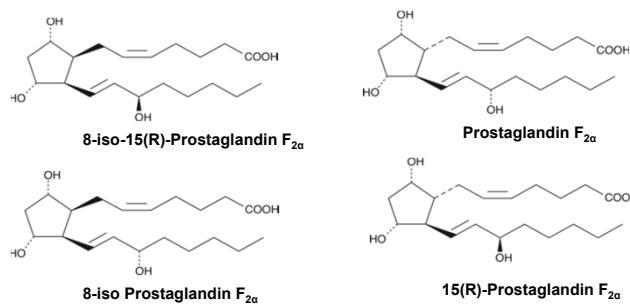
### MS/MS fragmentation of PGE<sub>2</sub> [M-H]<sup>-</sup> m/z 351



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

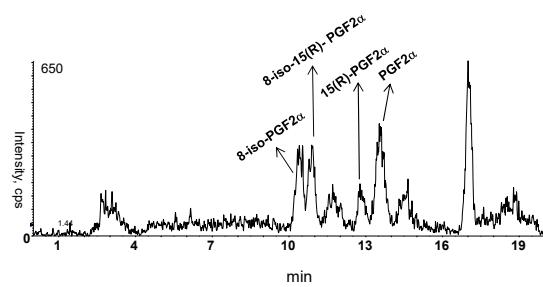
15

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



16

SRM chromatogram showing isoprostanes and PG in an AKI patient



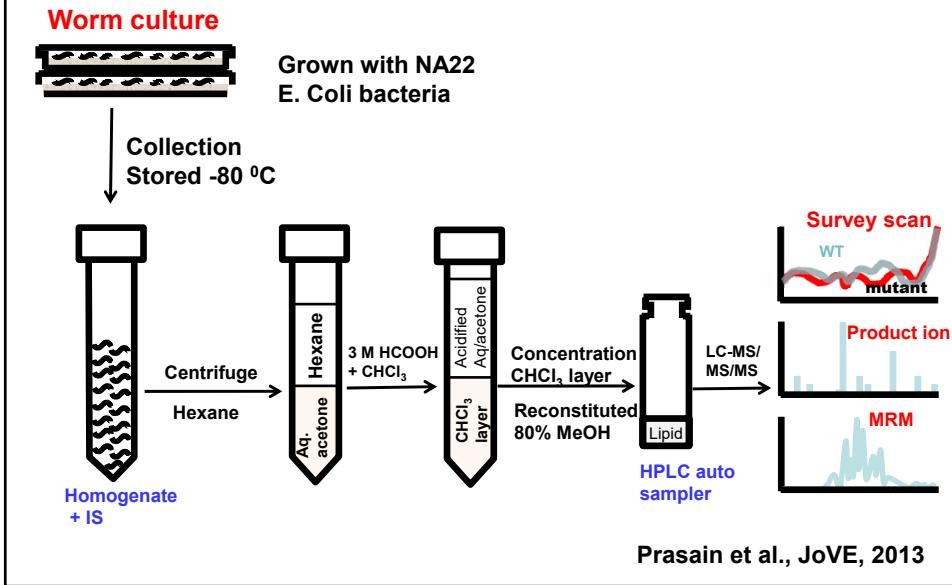
Prasain et al., J Chrom B. 2013

17

Cox-independent PGs

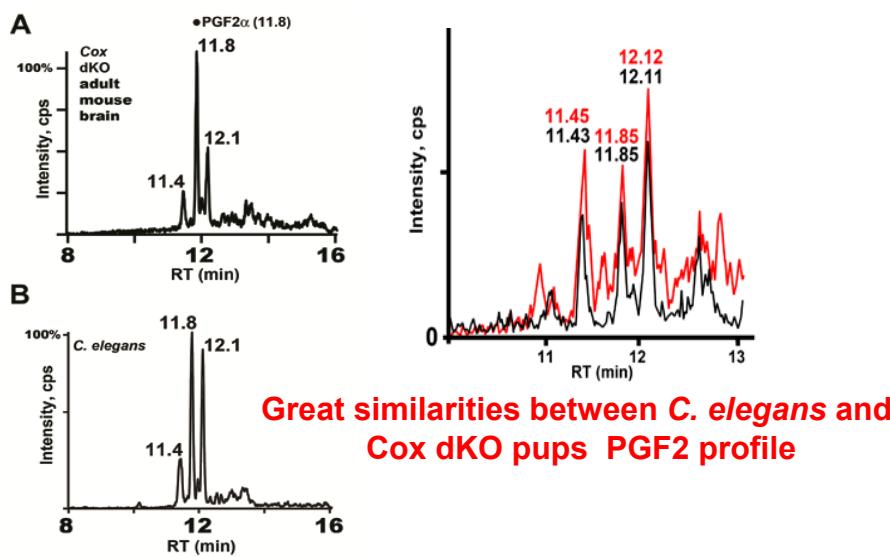
18

### C. elegans culture, lipid extraction and mass spectrometry analysis



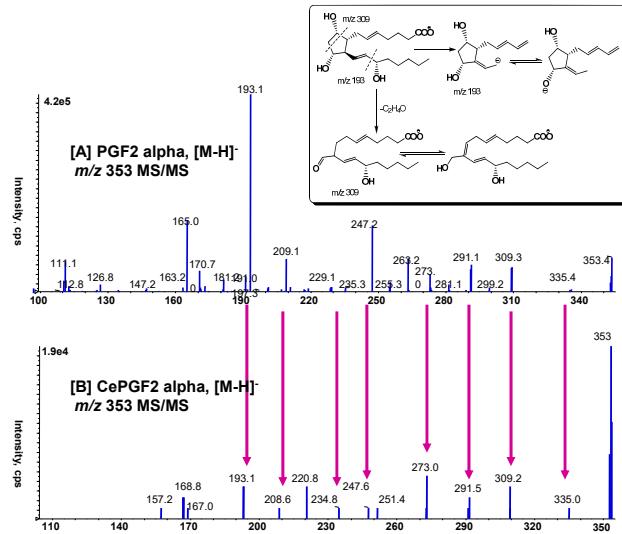
19

### Cox-independent PGs is widespread



20

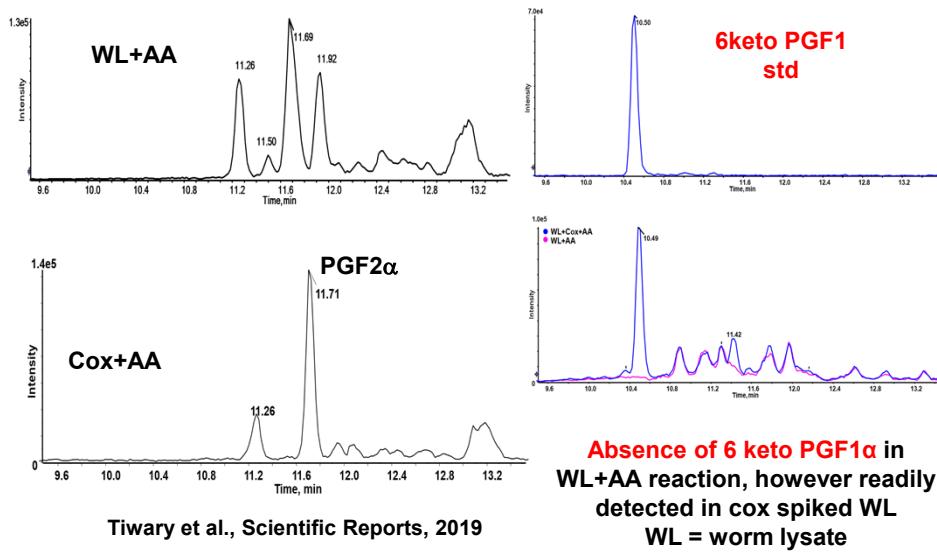
**LC-MS/MS of ion  $m/z$  353 [ $M-H^-$ ] from wild type *C. elegans* extract confirmed that CePGF<sub>2</sub> is a PGF<sub>2alpha</sub>-like PG**



Edmonds et al., Dev Cell. 2010

21

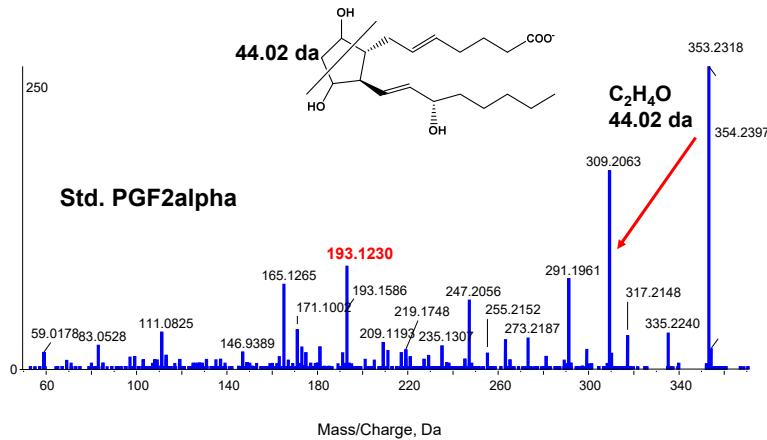
**Cox-independent F2-PGs with a signature profile, in vitro experiments**



Tiwary et al., Scientific Reports, 2019

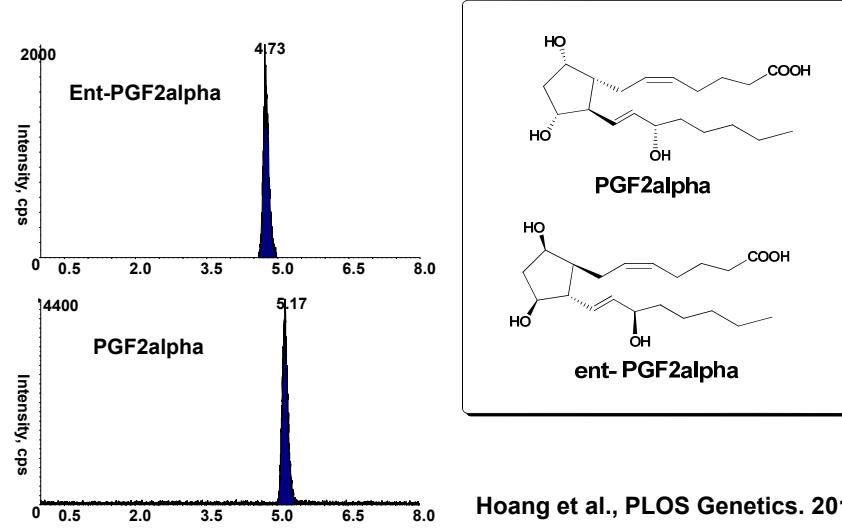
22

### High-resolution mass spectrometry analysis of PGF2alpha

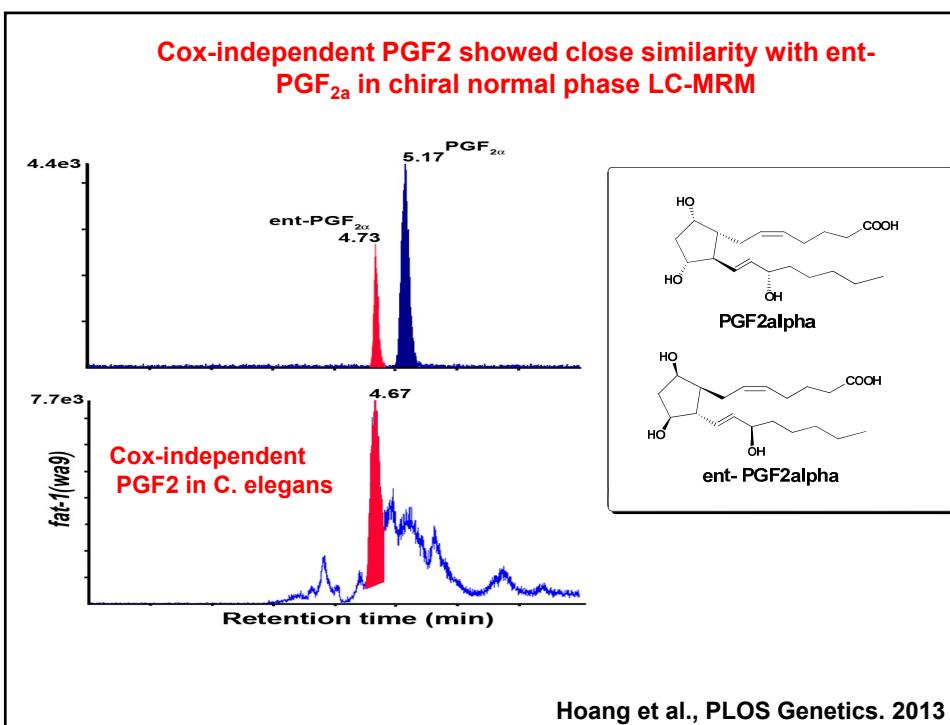


23

### 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



24



25

## Conclusions

- Based on liquid chromatography-tandem mass spectrometry (LC-MS/MS), genetic analyses, and bioactivity assays, *C. elegans* synthesizes Cox-independent 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.

26