Tandem mass spectrometry analysis of prostaglandins and isoprostanes

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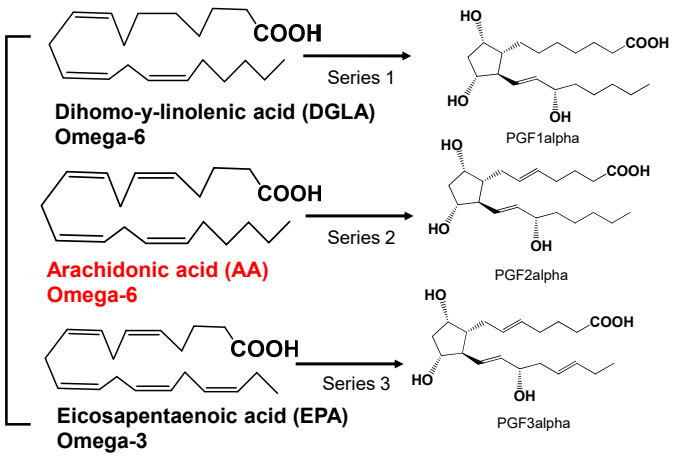
Overview

- **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.**

Polyunsaturated fatty acids (PUFAs)-substrates for PGs



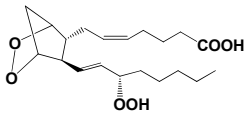
Cox-dependent PGs synthesis

Dietary linoleic acid
(C18: $\Delta^{9,12}$)

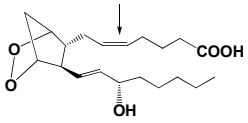
↓ elongase

↓ desaturase

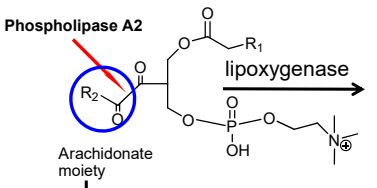
Arachidonic acid
C20: $\Delta^{5,8,11,14}$)



PGG2

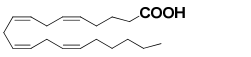


PGH2



↓ Release first

Enzymatic- cyclic pathways



AA (20:4n-6) NSAIDs

Cox

PGG2

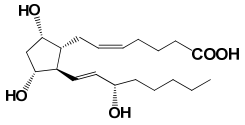
Cox

PGD synthase PGE synthase

PGD2 PGH2 PGE2

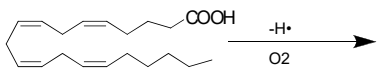
PGF synthase

PGF2α



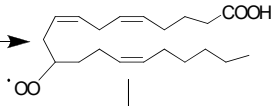
PGF2 alpha

Non-enzymatic isoprostane synthesis



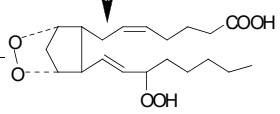
-H•

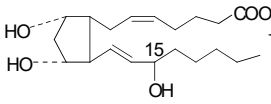
O2

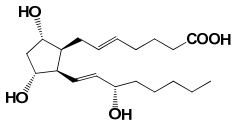


arachidonic acid (20:4n-6)

Cyclization

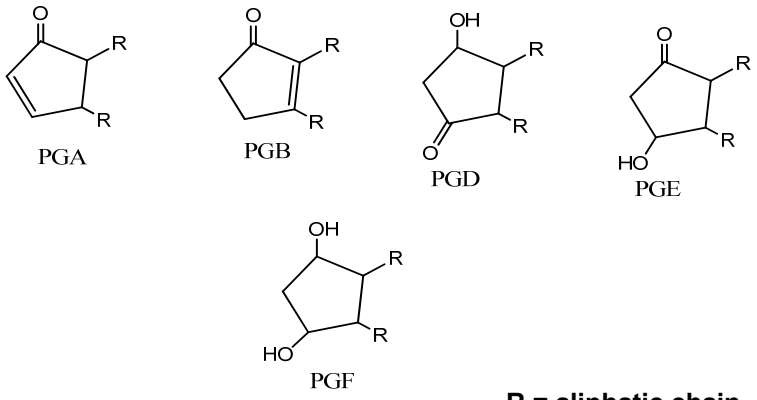






8-iso PGF2alpha

Structural representation PG based on ring features



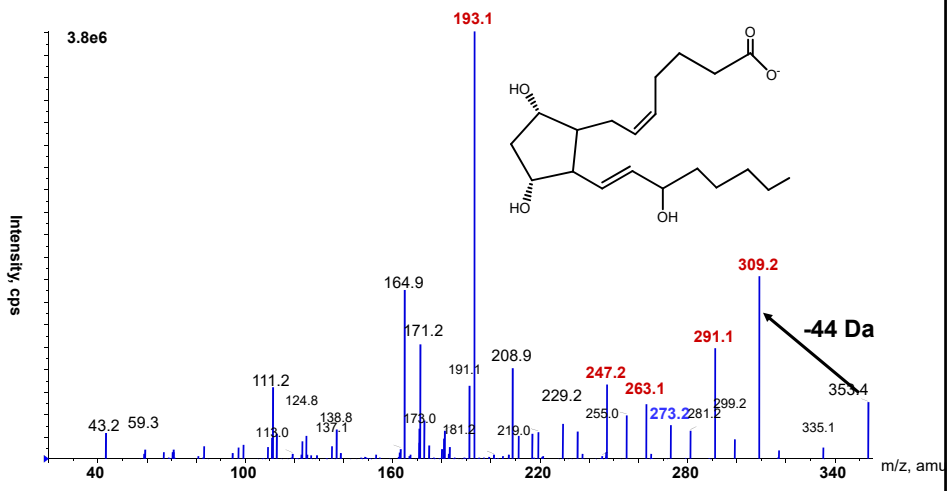
R = aliphatic chain

Prostaglandin analysis

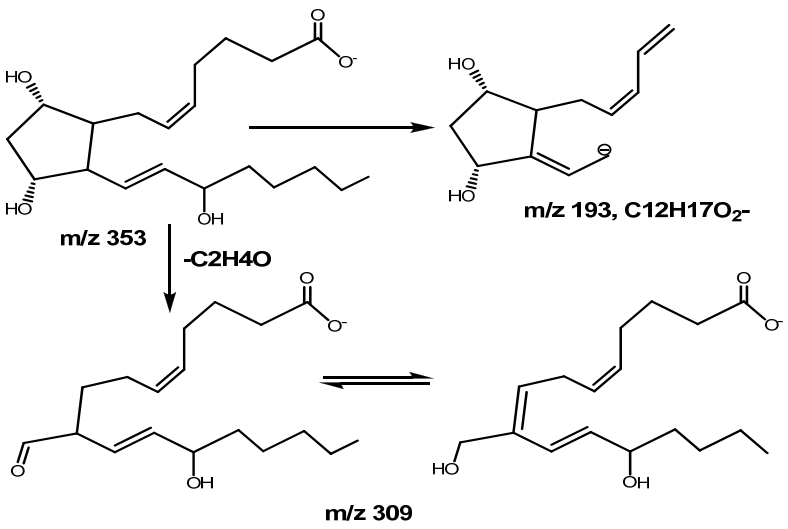
Concentration range nM-pM in biological samples

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

ESI-MS/MS of the [M-H]- from PGF2α m/z 353 using a quadrupole mass spectrometer



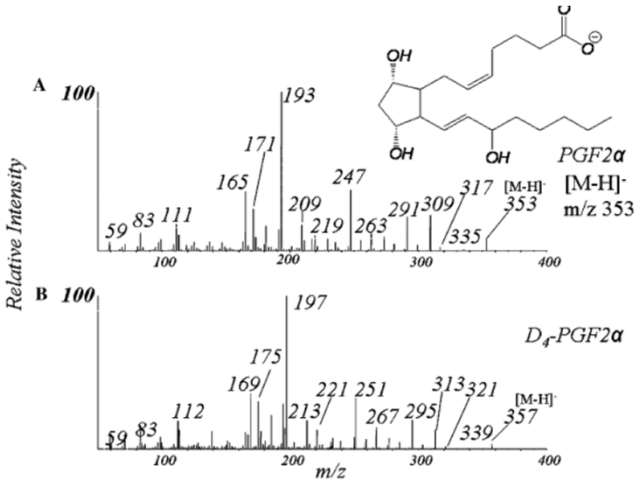
Fragmentation scheme of PGF2α [M-H]⁻ m/z 353



Ions m/z 309, 291, 273 and 193 are indicative of F2-ring

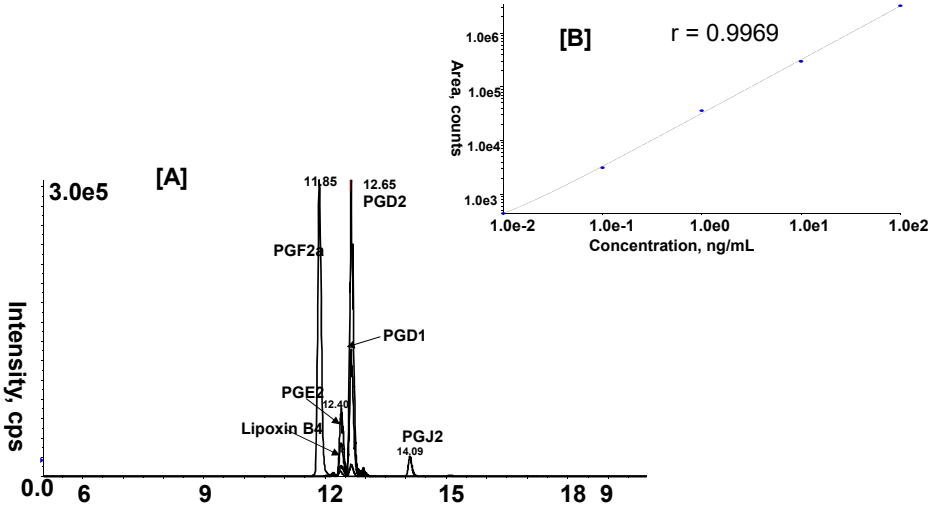
Adopted from Murphy et al. Analytical Biochemistry, 2005

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

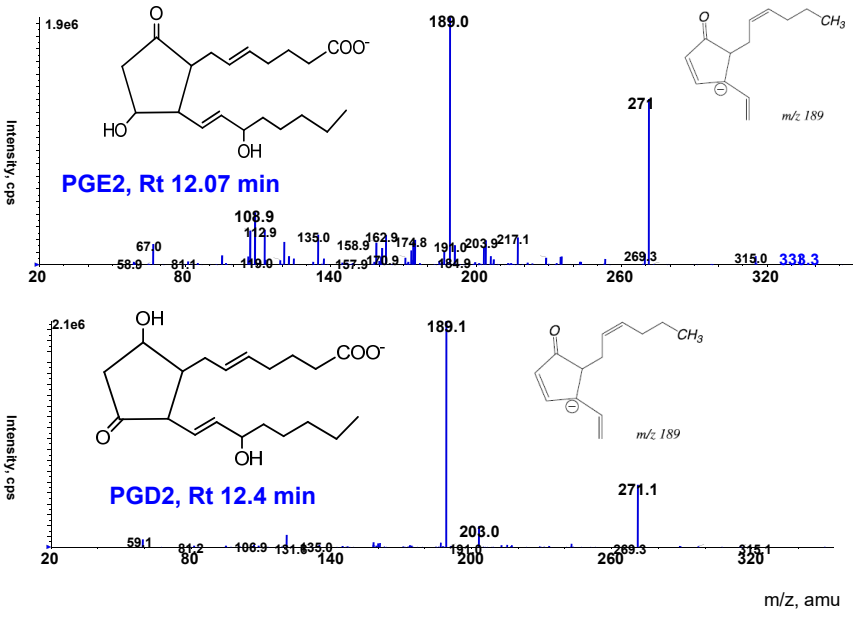


Source: Murphy et al. Analytical Biochemistry, 2005

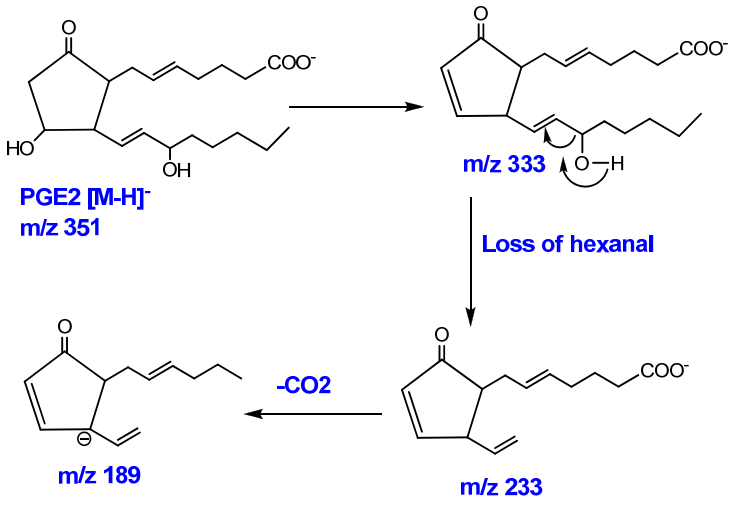
Separation of PGs[A] and standard curve of PGF2alpha [B]



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

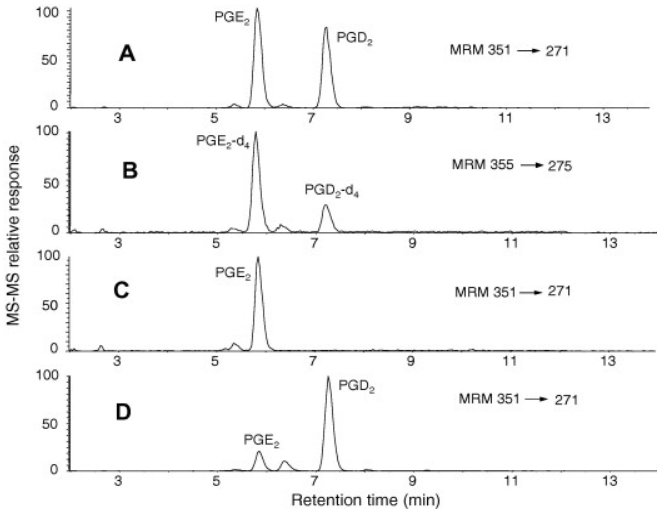


MS/MS fragmentation of PGE_2 $[M-H]^-$ *m/z* 351



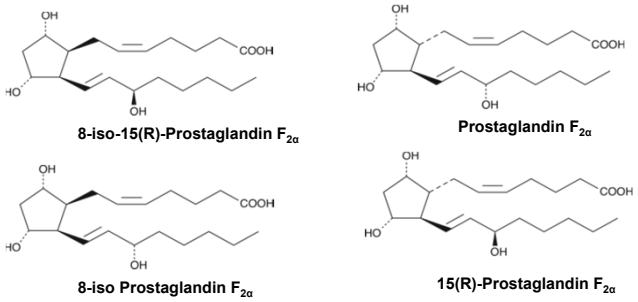
The first loss of water, *m/z* 189 and *m/z* 233 are characteristics of PGE_2/PGD_2

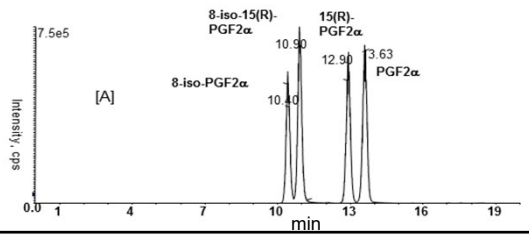
Deuterated PG standards are used for quantitative analysis of PGs in a extract



Source: Cao et al. Analytical Biochemistry, 2008

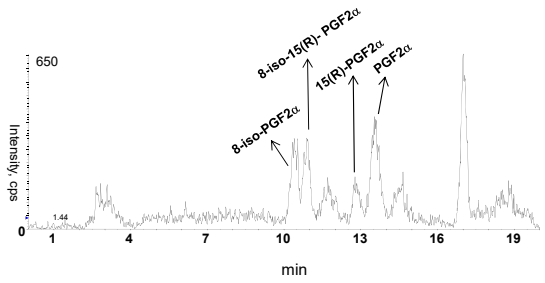
PGs and diastereoisomer isoprostanes can be distinguished based on retention time in LC-MS





Prasain et al., J Chrom B. 2013

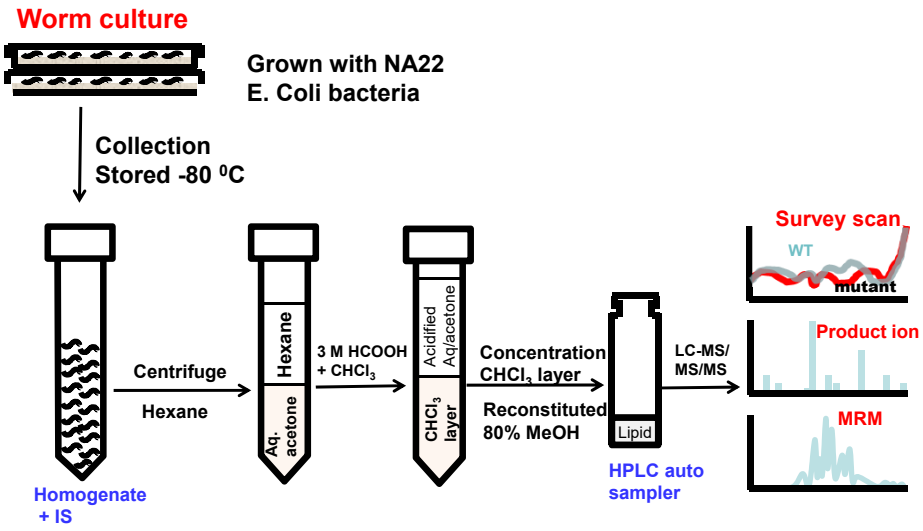
SRM chromatogram showing isoprostanes and PG in an AKI patience



Prasain et al., J Chrom B. 2013

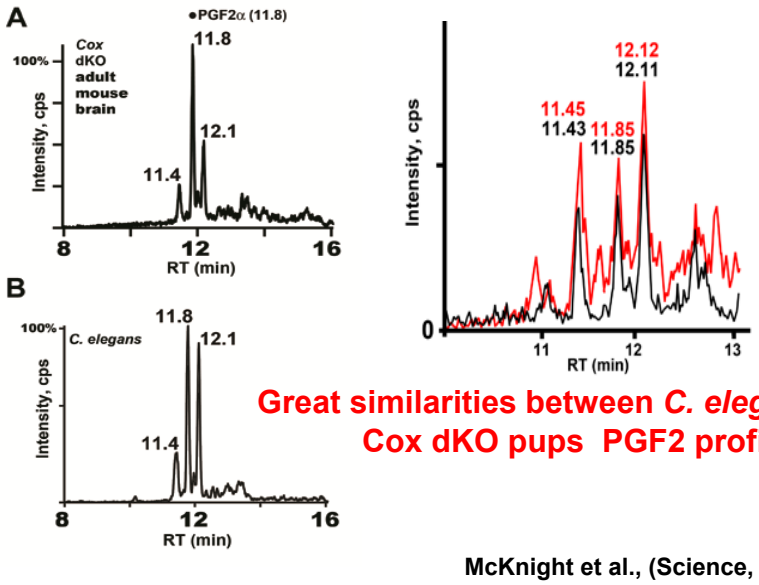
Cox-independent PGs

C. elegans culture, lipid extraction and mass spectrometry analysis



Prasain et al., JoVE, 2013

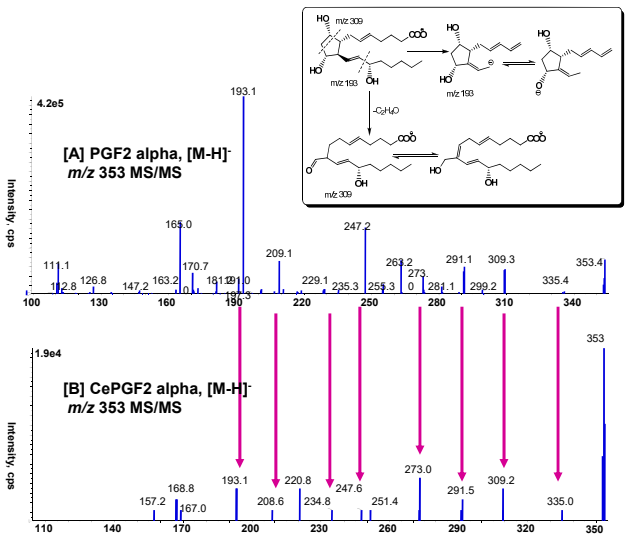
Cox-independent PGs is widespread



Great similarities between *C. elegans* and Cox dKO pups PGF2 profile

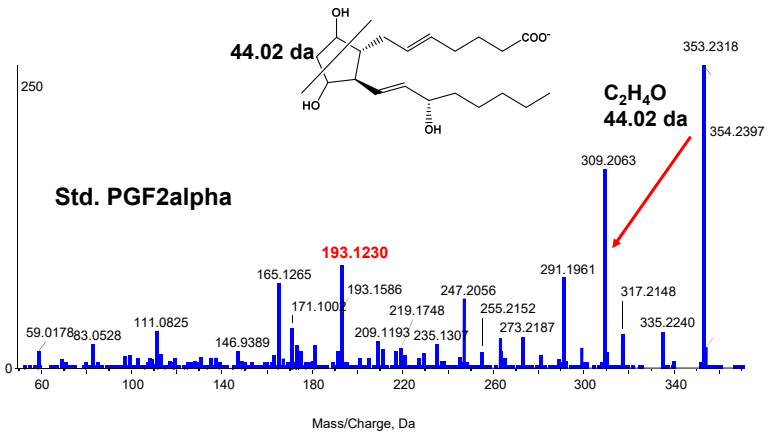
McKnight et al., (Science, 2014)

LC-MS/MS of ion *m/z* 353 $[M-H]^-$ from wild type *C. elegans* extract confirmed that $CePGF_2$ is a PGF_2alpha-like PG

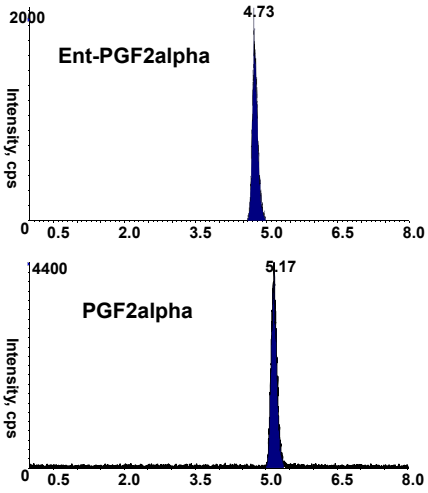


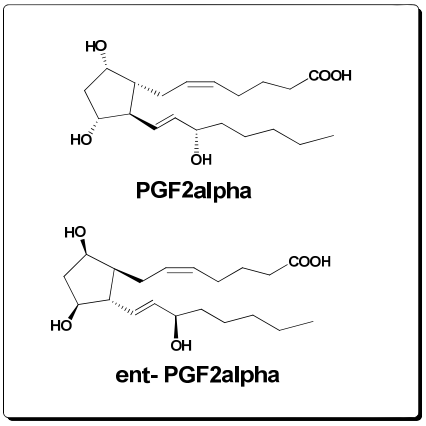
Edmonds et al., Dev Cell. 2010

High-resolution mass spectrometry analysis of PGF2alpha



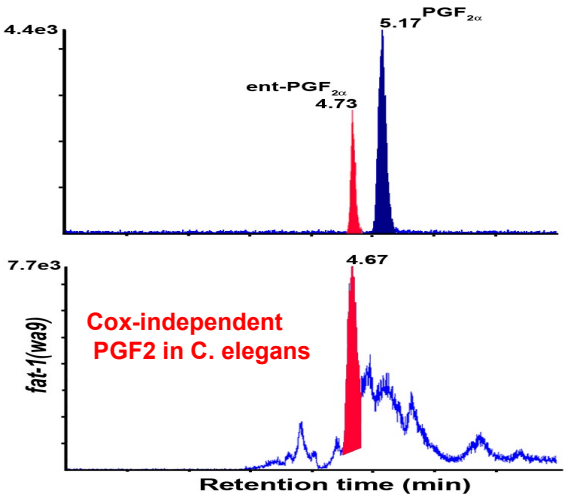
Separation of PGF_2alpha and its enantiomer only possible in chiral normal phase column (ChiralPak AD-H column) APCI –ve ion mode

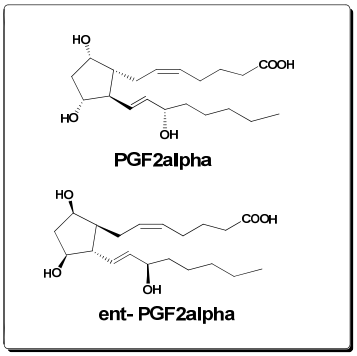




Hoang et al., PLOS Genetics. 2013

Cox-independent PGF2 showed close similarity with ent-PGF_{2a} in chiral normal phase LC-MRM





Hoang et al., PLOS Genetics. 2013

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.**