

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

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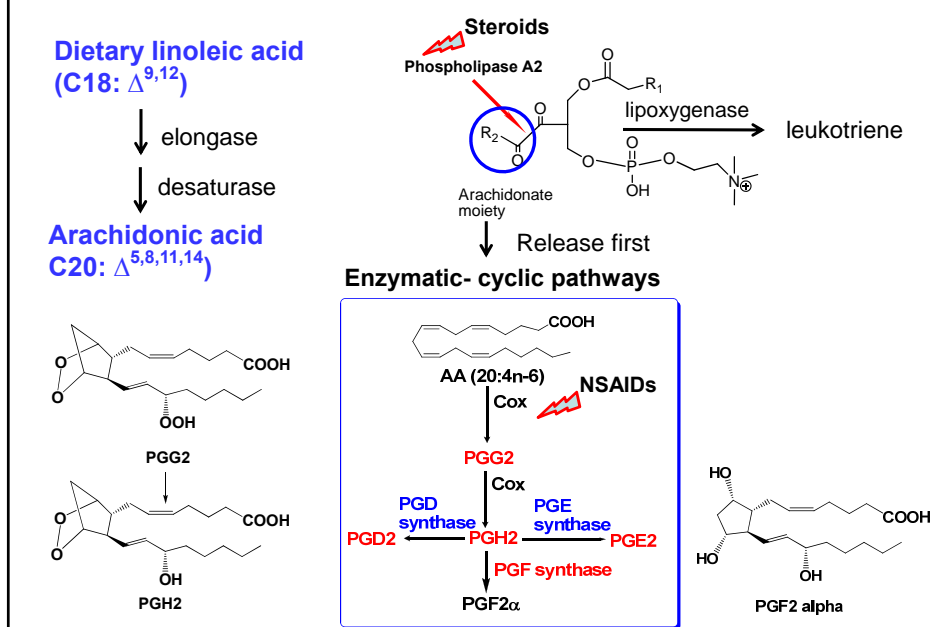
## **Overview**

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

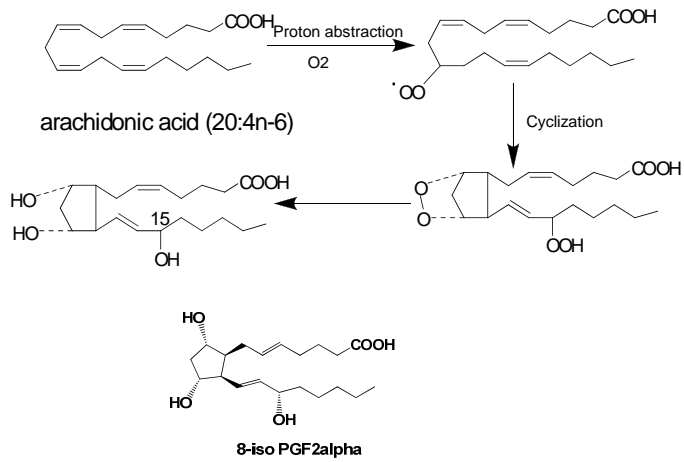
## Prostaglandins

- 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.

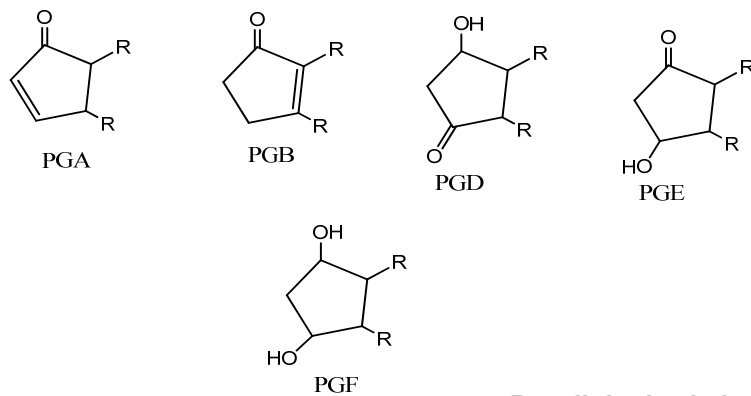
## Cox-dependent PGs synthesis



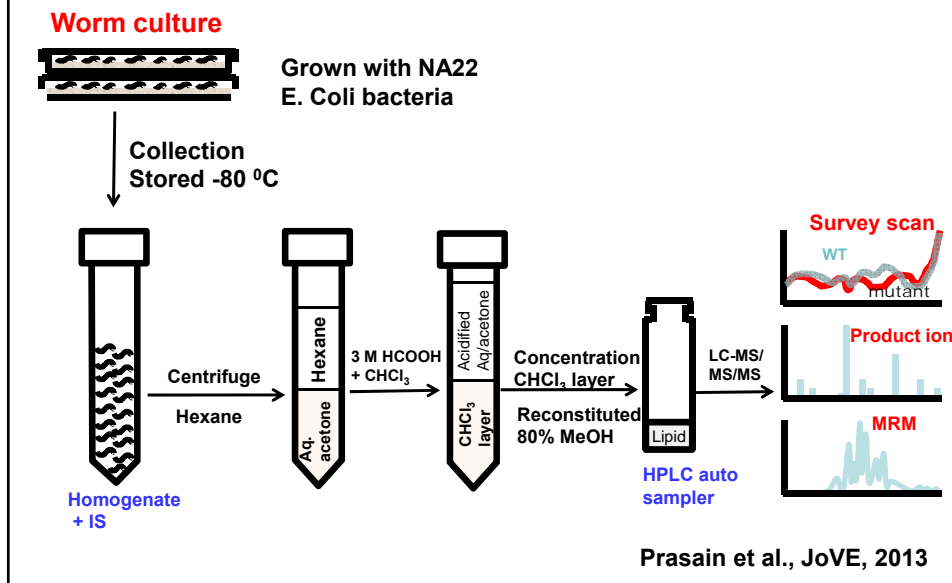
## Non-enzymatic isoprostane synthesis



## Structural representation PG based on ring features



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



## LC-MS/MS data summary for chemically synthesized F-series PG standards.

Name	RT (min)*	[M-H] <sup>-</sup> m/z	Key product ions in CID (MS/MS)
PGD <sub>2</sub>	12.56	351	315, 271, 233, 203, 189
PGE <sub>2</sub>	12.23	351	333, 315, 271, 235, 189, 175, 109
PGH <sub>2</sub>	12.23	351	333, 315, 271, 235, 217, 189, 175, 113, 109
PGF <sub>1α</sub>	11.83*	355	337, 319, 311, 301, 293, 275, 265, 249, 237, 211, 195
8-iso PGF <sub>1α</sub>	11.34	355	337, 319, 311, 293, 275, 265, 249, 237, 219, 211, 183
9β-PGF <sub>1α</sub>	11.36	355	337, 319, 311, 301, 293, 275, 265, 237, 211, 183, 167
8-iso 9β-PGF <sub>1α</sub>	11.44	355	337, 319, 311, 293, 275, 265, 219, 211, 183
9β, 11β-PGF <sub>1α</sub>	11.99	355	337, 319, 311, 301, 293, 275, 265, 237, 219, 211, 183
PGF <sub>2α</sub>	11.73*	353	335, 317, 309, 291, 273, 263, 247, 209, 193, 171, 165
ent-PGF <sub>2α</sub>	11.71	353	335, 317, 309, 291, 273, 247, 209, 193, 191, 171, 165
11β PGF <sub>2α</sub>	11.48	353	335, 317, 309, 291, 273, 247, 209, 193, 173, 165, 111
15(R)-PGF <sub>2α</sub>	11.89	353	335, 317, 309, 291, 273, 247, 209, 193, 191, 171, 165
8-iso PGF <sub>2α</sub>	11.31	353	335, 307, 309, 291, 273, 247, 209, 193, 181, 171, 165
5-trans PGF <sub>2α</sub>	11.60	353	335, 317, 309, 291, 273, 247, 209, 193, 171, 165, 111
8-iso 15(R)-PGF <sub>2α</sub>	11.39	353	335, 317, 309, 291, 273, 263, 247, 209, 193, 171, 165
9β-PGF <sub>2α</sub>	11.22	353	335, 317, 309, 291, 273, 255, 247, 193, 173, 171, 165
PGF <sub>3α</sub>	11.26	351	333, 315, 307, 289, 271, 245, 219, 209, 193, 191, 165
8-iso PGF <sub>3α</sub>	10.83	351	333, 315, 307, 289, 271, 245, 219, 209, 193, 191, 171
2,3-Dinor-11β-PGF <sub>2α</sub>	10.67	325	261, 245, 227, 219, 173, 163, 153, 145, 137, 113, 107
19(R)-hydroxy PGF <sub>2α</sub>	9.19	369	351, 333, 325, 315, 307, 263, 235, 209, 193, 171, 165
20-hydroxy PGF <sub>2α</sub>	9.13	369	351, 333, 325, 315, 307, 263, 209, 193, 181, 171, 165

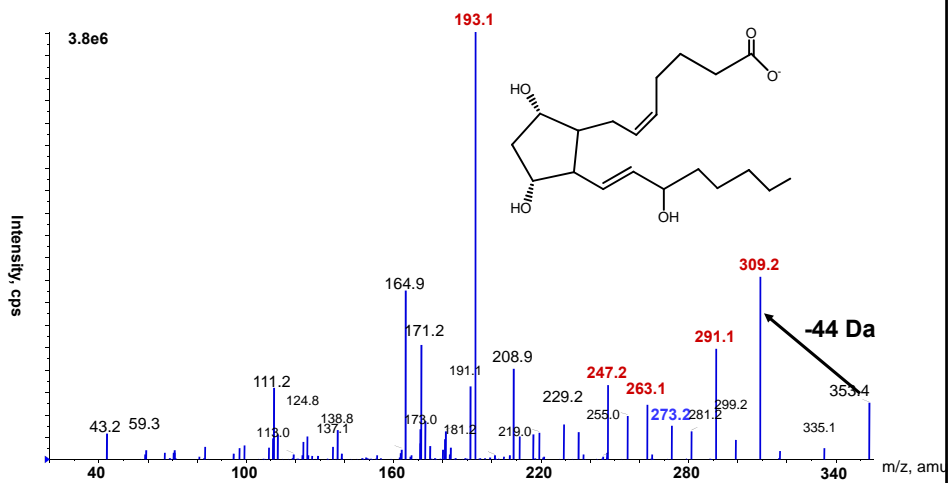
Retention time (RT), parent ion mass ([M-H]<sup>-</sup>), and key product ion masses are shown for prostaglandin (PG) standards.

\*Isomers within each prostaglandin class (i.e. PGF<sub>2α</sub> isomers) were run together and RTs are directly comparable. PGF<sub>1α</sub> and PGF<sub>2α</sub> classes were run on different days and a slight RT shift is observed. For example, the RTs for PGF<sub>1α</sub> and PGF<sub>2α</sub> are indistinguishable when run together.

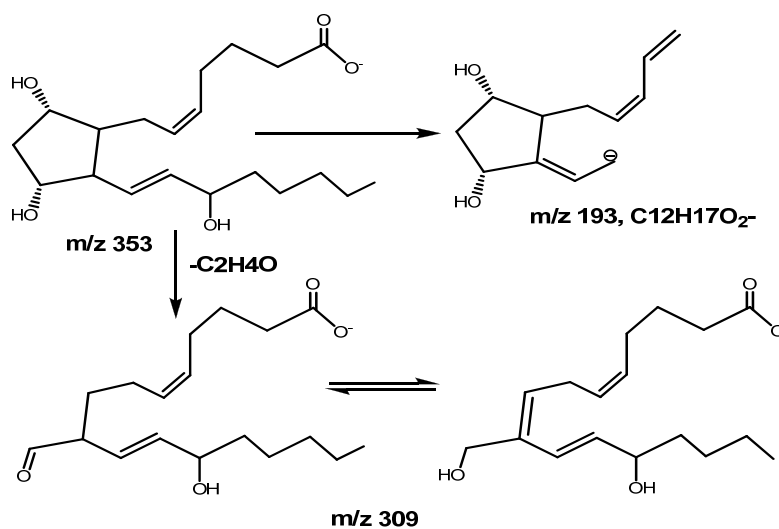
doi:10.1371/journal.pgen.1003271.t002

Hoang et al., 2013

## ESI-MS/MS of the $[M-H]^-$ from PGF $_{2\alpha}$ m/z 353 using a quadrupole mass spectrometer

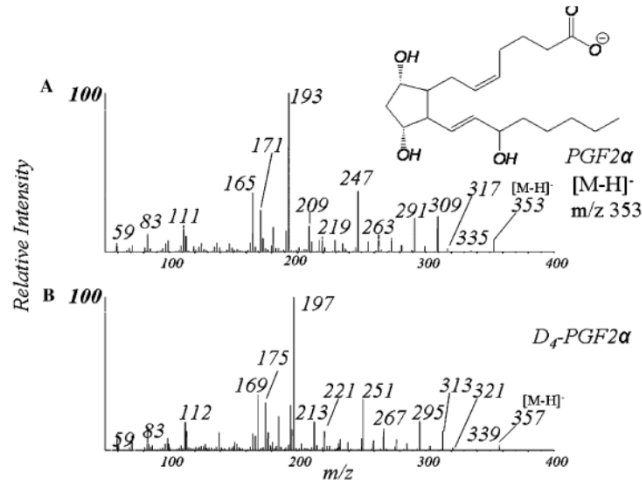


## Fragmentation scheme of PGF $_{2\alpha}$ $[M-H]^-$ m/z 353



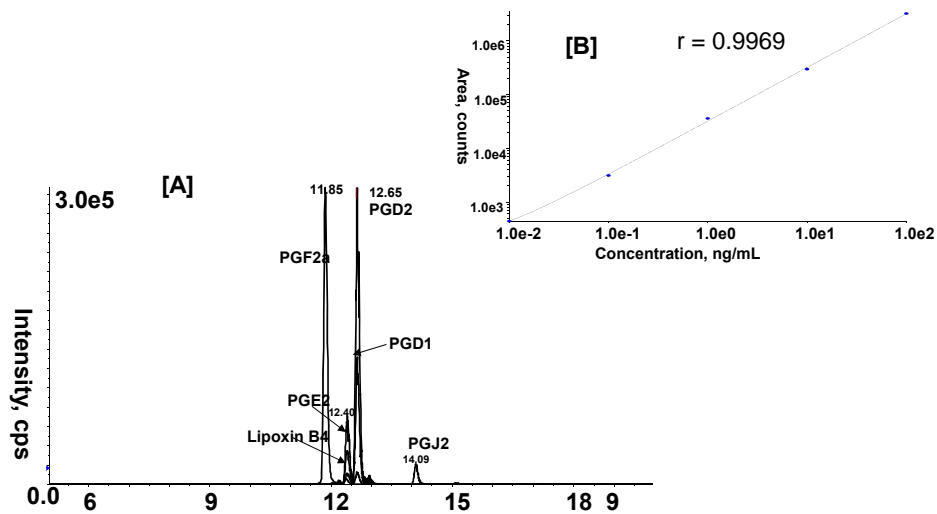
Ions m/z 309, 291, 273 and 193 are indicative of F<sub>2</sub>-ring

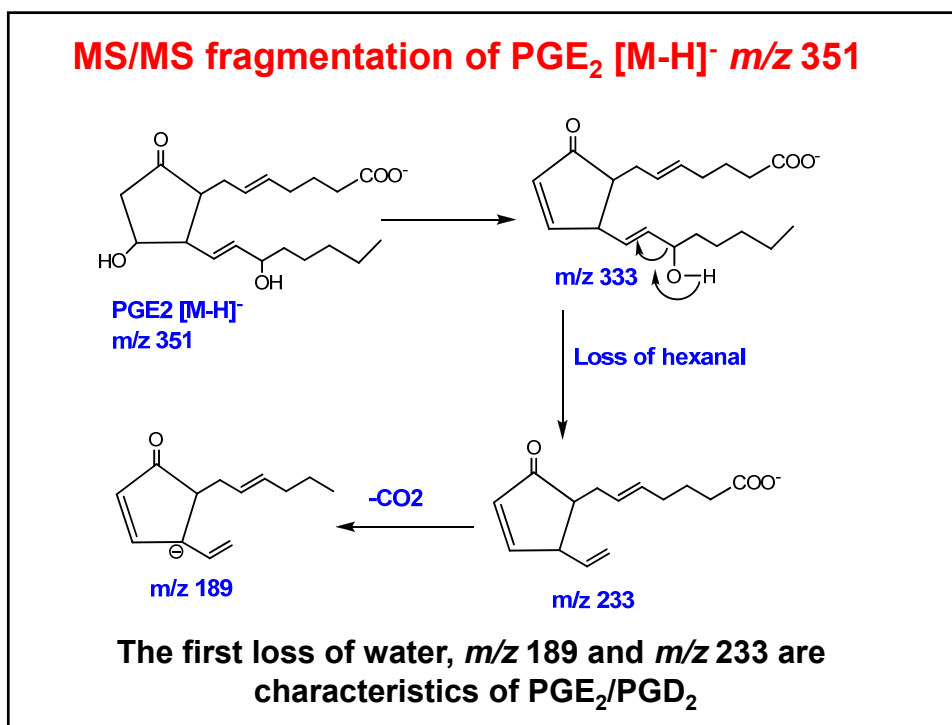
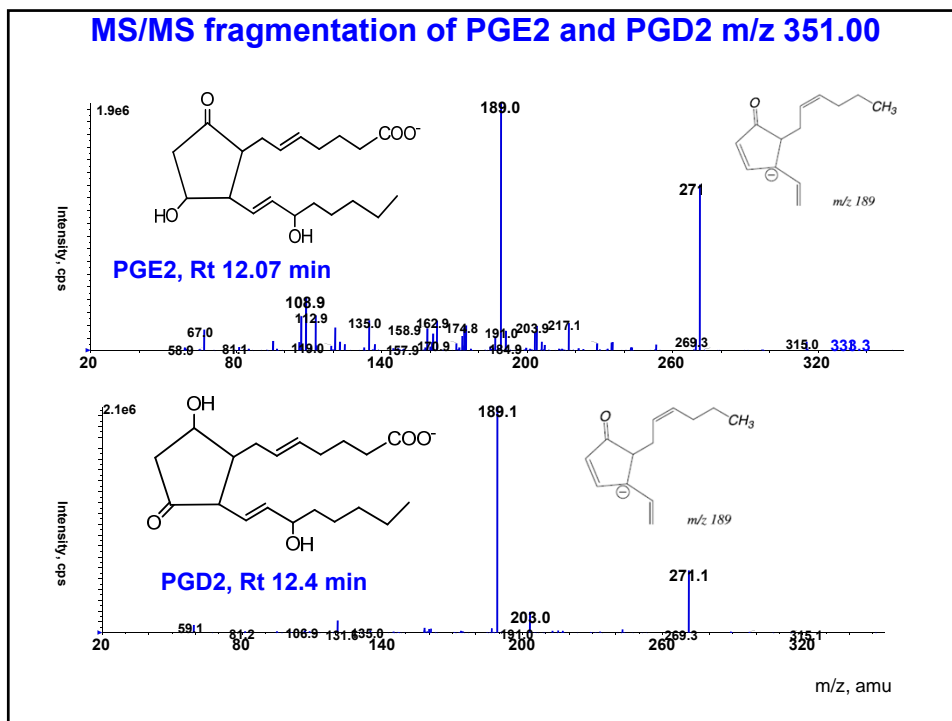
**What information does deuterium labeling at C-2 and C-3 of PGF2 provide us for structure elucidation of PG?**



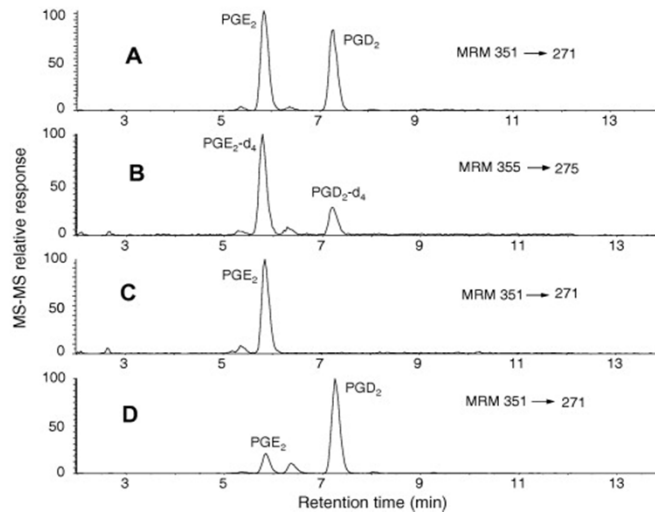
Source: Murphy et al. Analytical Biochemistry, 2005

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



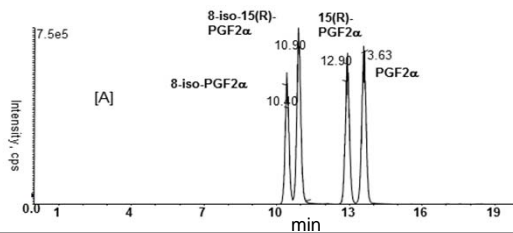
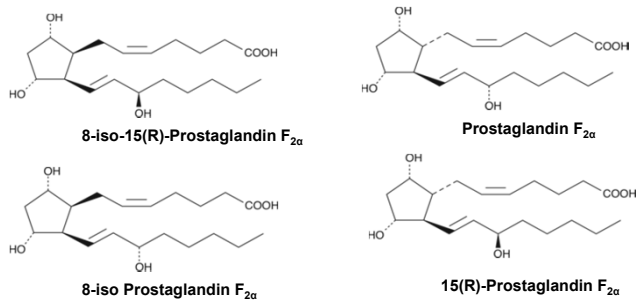


## Deuterated PG standards are used for quantitative analysis of PGs in a extract



Source: Cao et al. Analytical Biochemistry, 2008

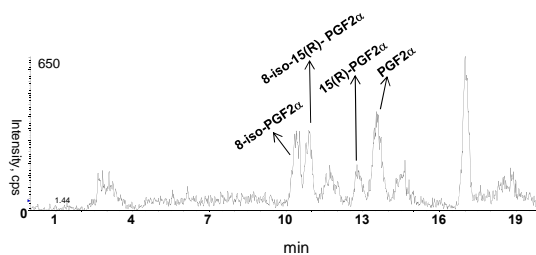
## PGs and diastereoisomer isoprostanes can be distinguished based on retention time in LC-MS



Prasain et al., J Chrom B. 2013

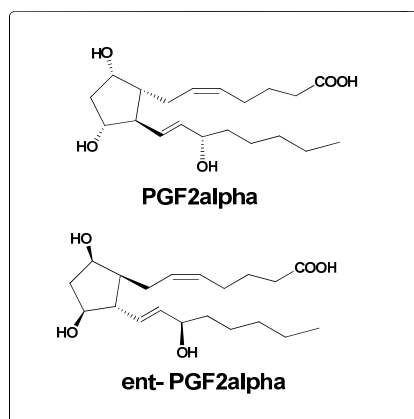
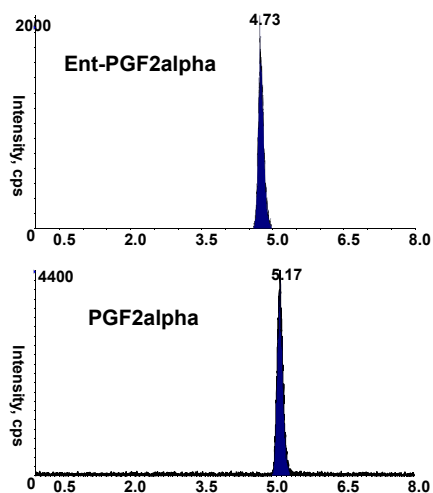


## SRM chromatogram showing isoprostanes and PG in an AKI patient

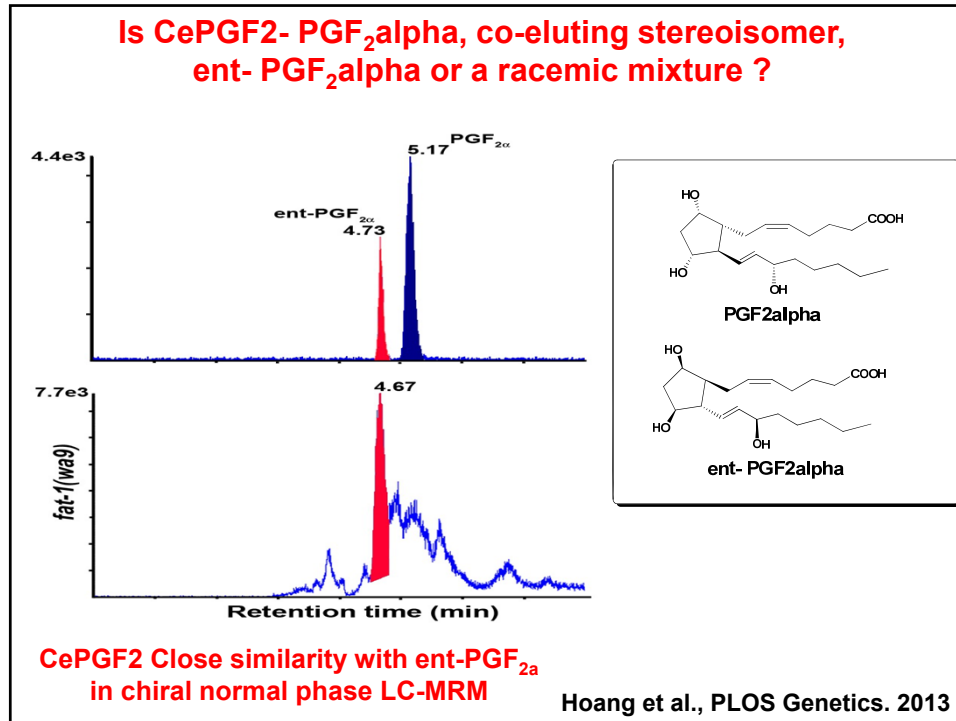


Prasain et al., J Chrom B. 2013

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







## 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.