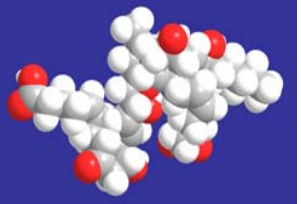
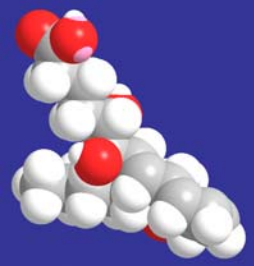
LIPID MAPS Lipidomics Workshop

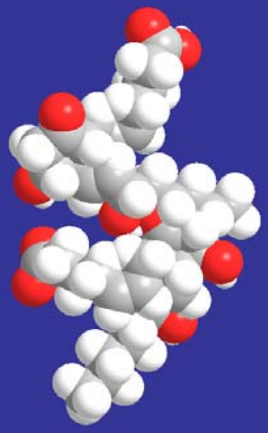
April 18, 2009

www.lipidmaps.org





Eicosanoids



Richard Harkewicz

Departments of Pharmacology, Chemistry and Biochemistry

The University of California, San Diego

La Jolla, CA

Other LIPID MAPS Eicosanoid Core Members:

Aaron Armando

Alexander Andreyev

Joshua Brooks

Matthew Buczynski

Darren Dumlao

Oswald Quehenberger

Edward A. Dennis
(Core Director)

Outline

A) Brief description of eicosanoids

B) Sample preparation/extraction

C) Analytical methodology: LC-MS

D) Library of eicosanoid standards

E) Chiral chromatography – *enzymatic or non-enzymatic?*

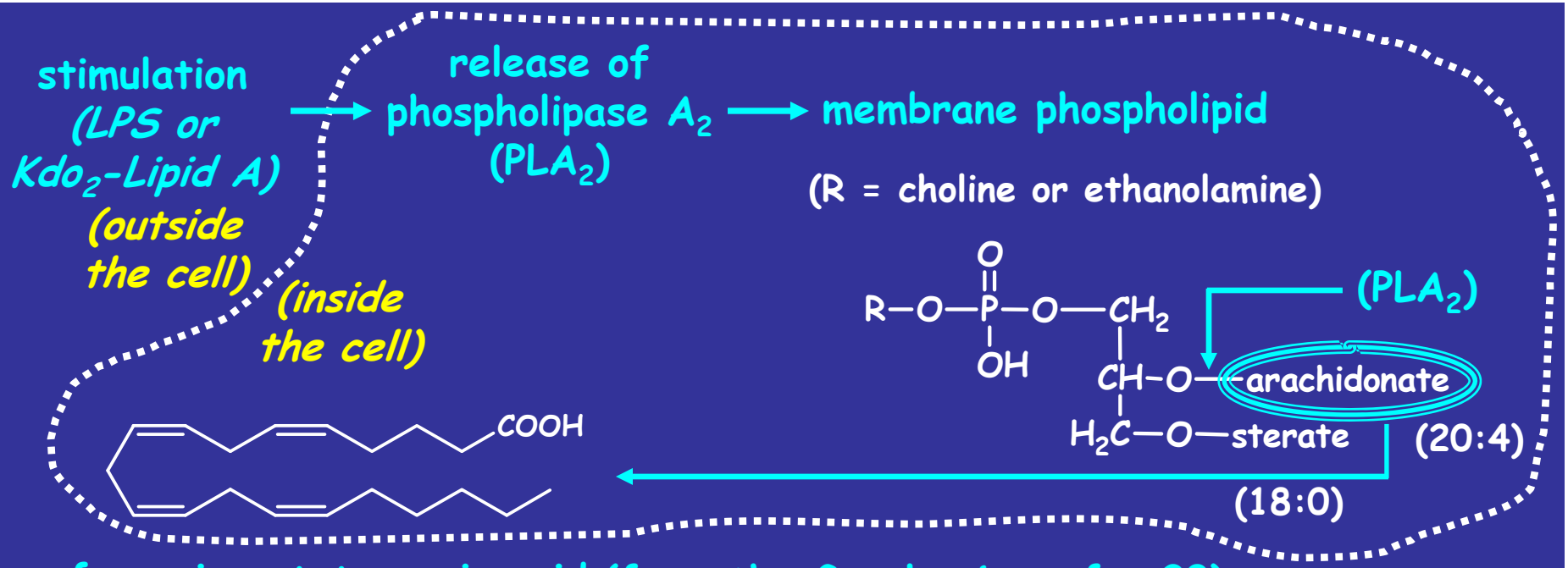
F) GC-MS methodology for free fatty acids

G) DIMPLES/MS: A stable isotope substrate labeling strategy enabling the search for novel eicosanoids

H) Comparison of LIPID MAPS eicosanoid approach with others in literature

I) Future plans

contact: rharkewicz@ucsd.edu



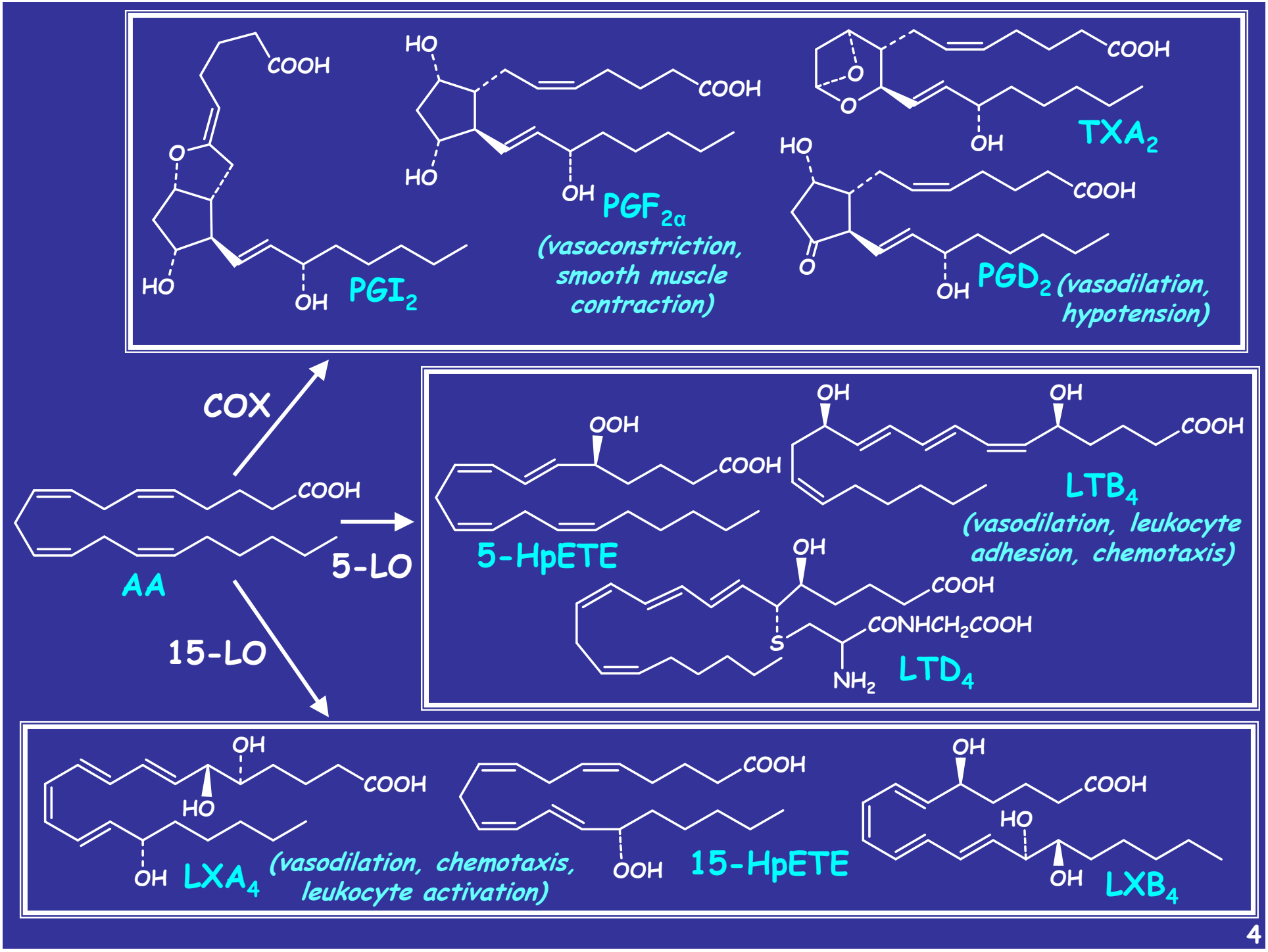
free eicosatetraenoic acid (from the Greek *eicosa* for 20)

AKA arachidonic acid (20:4, n=6)

Eicosanoids *– powerful mediators involved in inflammation that are derived from arachidonic acid and act in autocrine and paracrine fashion (signal at or immediately adjacent to their site of synthesis)*

- Once they are made, they are quickly secreted from the cell
- Transcription factor: Can also enter cell's nucleus, binding and activating nuclear receptors

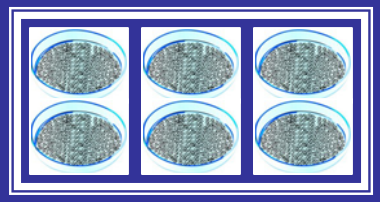
Sometimes referred to as *autocoids* or *local hormones*


COOH
HO
HO
OH
PGF2α
(vasoconstriction, smooth muscle contraction)
O
O
TXA2
HO
O
PGD2 (vasodilation, hypotension)
COX
AA
5-LO
15-LO
OOH
5-HpETE
LTB4
(vasodilation, leukocyte adhesion, chemotaxis)
CONHCH2COOH
S
NH2
LTD4
LXA4
(vasodilation, chemotaxis, leukocyte activation)
15-HpETE
LXB4

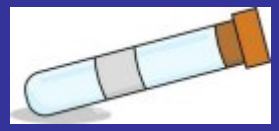
sample preparation and extraction

0 h → 24 h → 48 h → LC-MS analysis

- 0 h: Plate Cells
- 24 h: LPS *or* Kdo_2-Lipid A [100 ng/ml]
- 48 h: Collect Medium
- Solid phase extraction



1. Plate cells
   ↓ 24 h
2. Stimulate (LPS or Kdo)
   ↓ 24 h
3. Remove medium (≈2 ml per well) (≈2e6 cells per well)



1. Spike medium with deuterated internal standards (10 ng/100 µl)
2. Add EtOH to obtain 10% EtOH medium solution
3. Centrifuge 5 min @ 3000 rpm

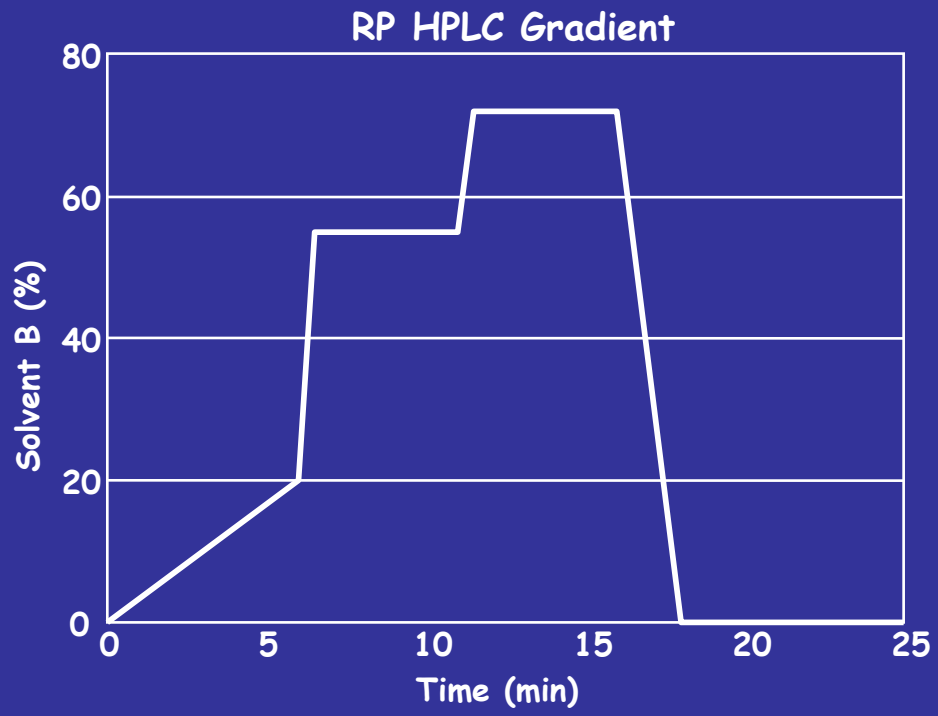
1. Solid phase extraction (Phenomenex® Strata-X 8B-S100-UBJ)
2. Prewash SPE column with 2 ml MeOH then 2 ml H_2O
3. Apply sample to column then wash with 2 ml 10% MeOH
4. Elute eicosanoids with 1 ml MeOH
5. Dry under vacuum and resuspend in 100 µl HPLC solvent A

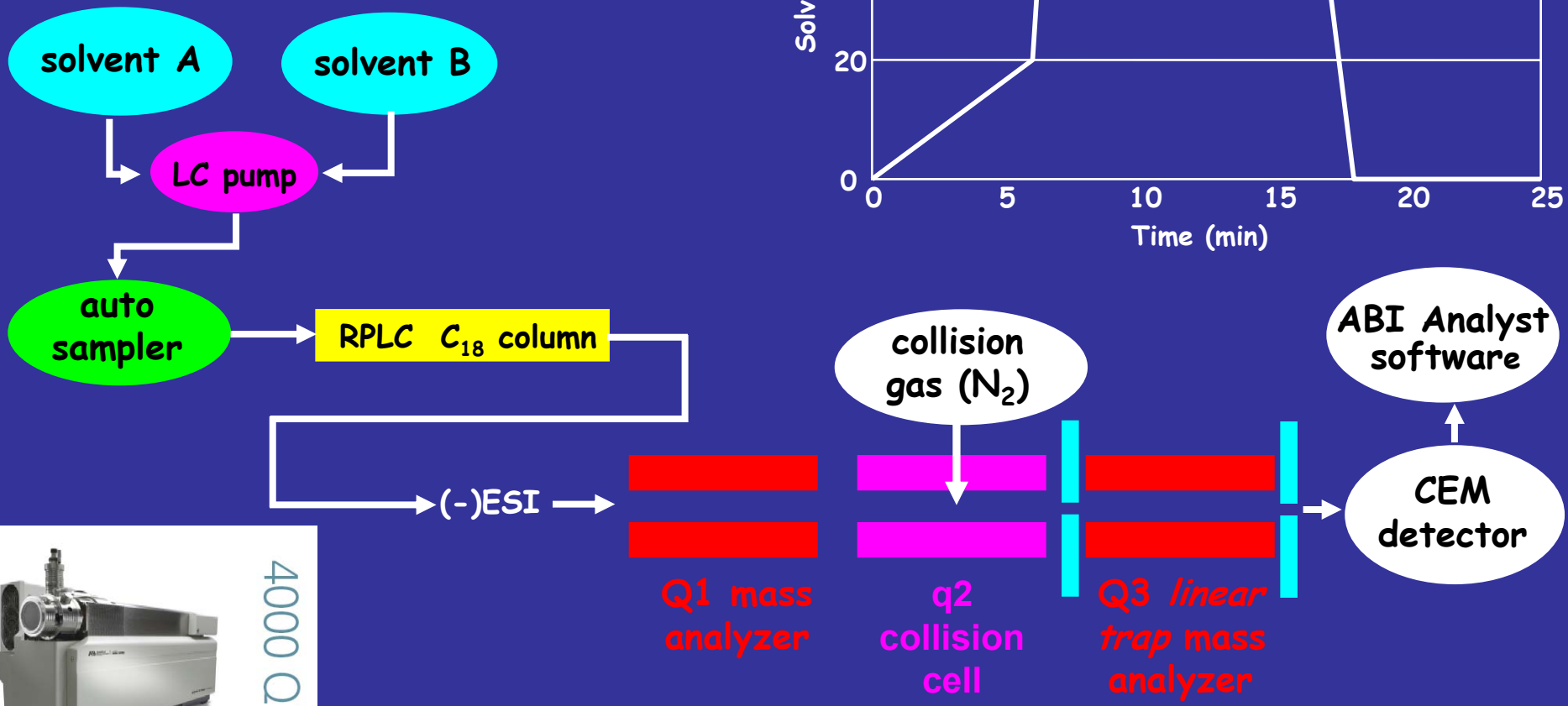
column
Vydac® 201TP52
2.1 mm X 250 mm

Solvent A
H_2O/ACN/formic acid:
63/37/0.02

Flow rate
300 µl/min

Solvent B
ACN/IPA:
50/50





Applied Biosystems® 4000 QTrap
Quadrupole mass spectrometer

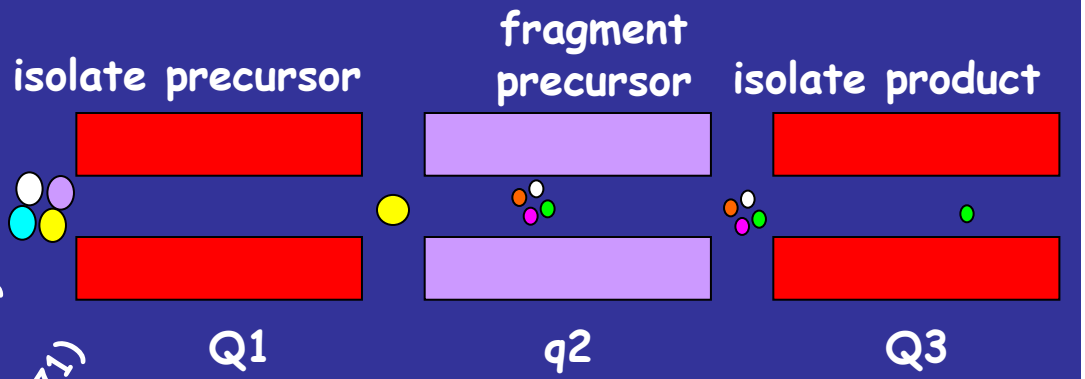
Eicosanoid Standard Mixture LC/MS - MRM

Multiple Reaction Monitoring

"specialized" form of MSMS

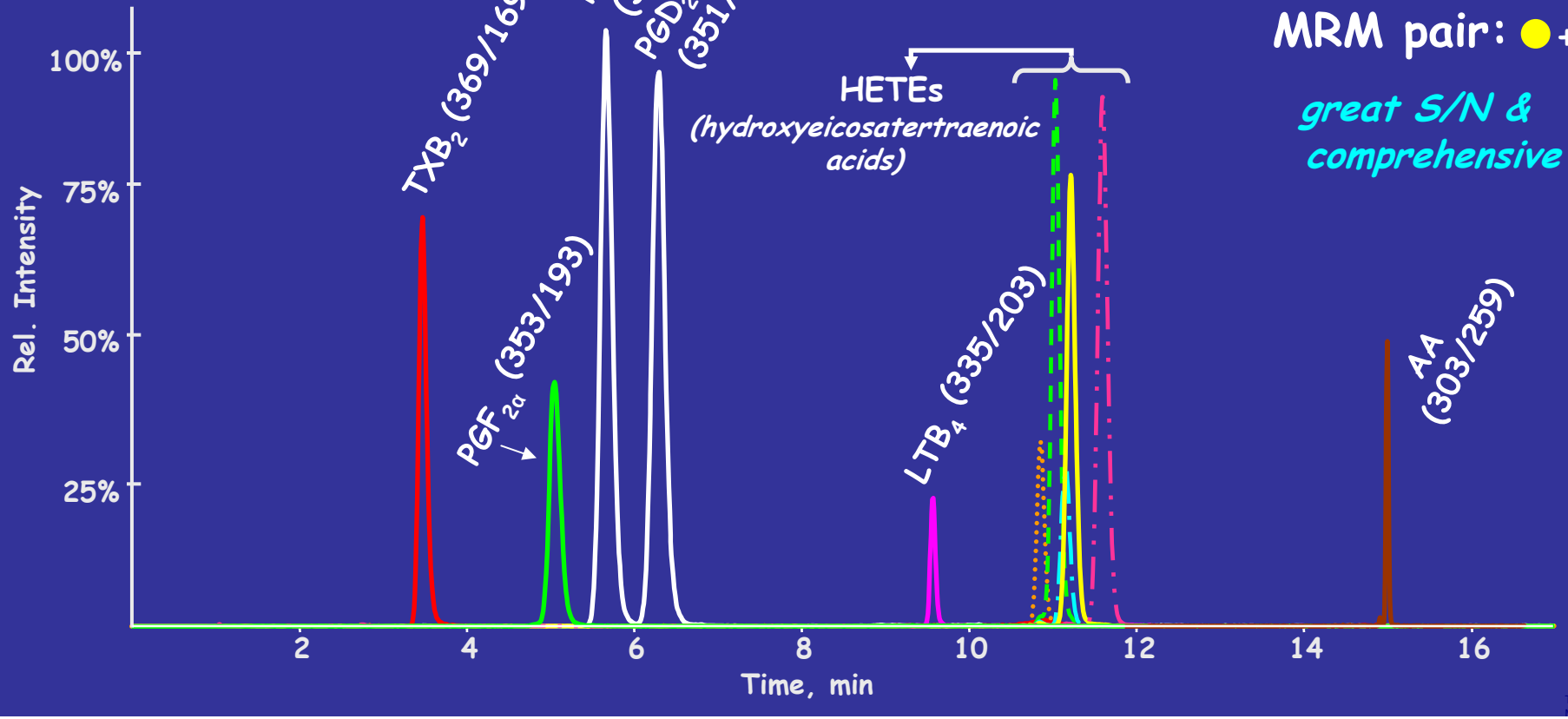
Applied Biosystems Analyst 1.5

"scheduled MRM" allowing >1000 MRM pairs

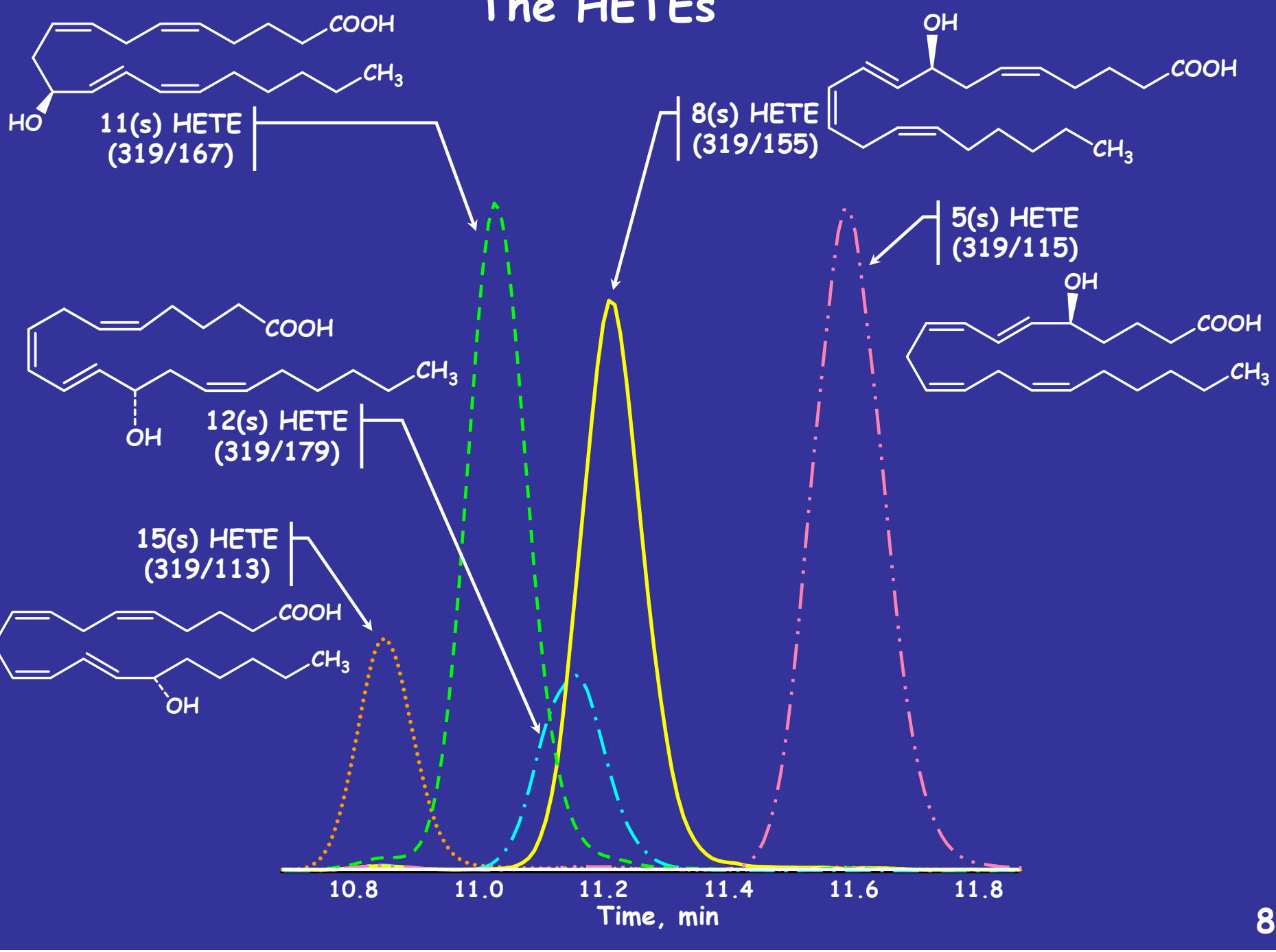


MRM pair: ● + ●

great S/N & comprehensive



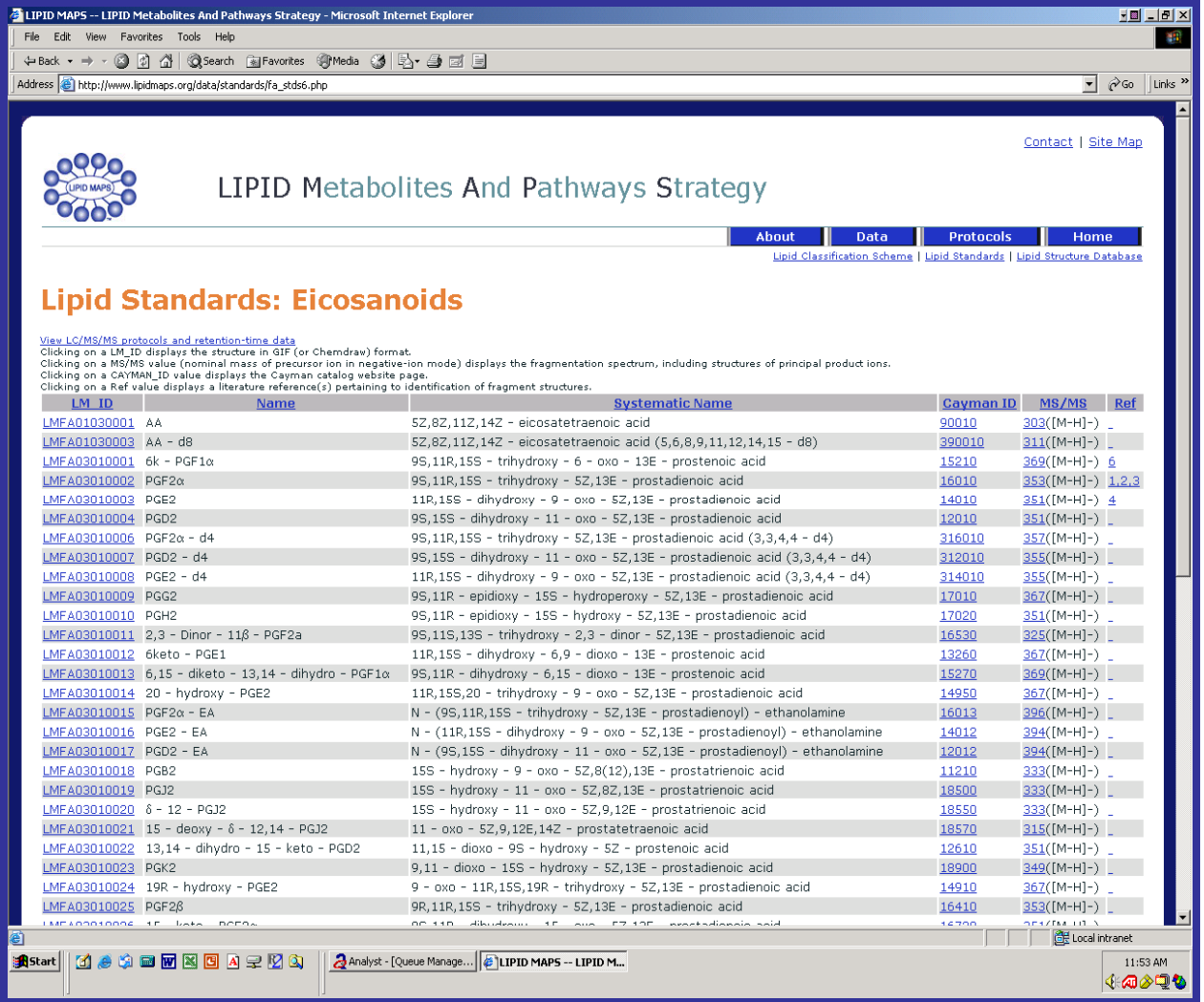
The HETEs


COOH
CH3
HO
11(s) HETE
(319/167)
OH
COOH
CH3
8(s) HETE
(319/155)
5(s) HETE
(319/115)
OH
COOH
CH3
COOH
CH3
OH
12(s) HETE
(319/179)
15(s) HETE
(319/113)
COOH
CH3
OH
10.8
11.0
11.2
11.4
11.6
11.8
Time, min

Library of Eicosanoid Standards (>200)
(http://www.lipidmaps.org)

Provides:

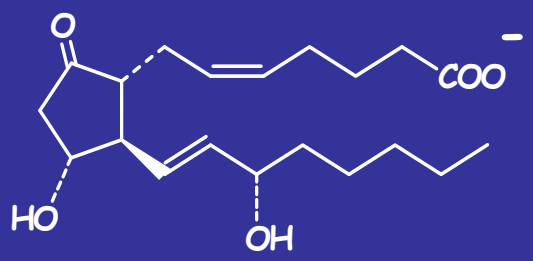
- Chemical structure in ChemDraw® format
- LC and MS protocols
- MSMS fragmentation spectra
- LC retention times for given set of conditions
- Web-link to Cayman Chemical for each eicosanoid



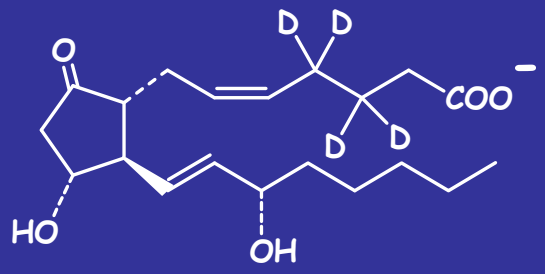
LM_ID	Name	Systematic Name	Cayman ID	MS/MS	Ref
LMFA01030001	AA	5Z,8Z,11Z,14Z - eicosatetraenoic acid	90010	303([M-H]-)	
LMFA01030003	AA - d8	5Z,8Z,11Z,14Z - eicosatetraenoic acid (5,6,8,9,11,12,14,15 - d8)	390010	311([M-H]-)	
LMFA03010001	6k - PGF1α	9S,11R,15S - trihydroxy - 6 - oxo - 13E - prostenoic acid	15210	369([M-H]-)	6
LMFA03010002	PGF2α	9S,11R,15S - trihydroxy - 5Z,13E - prostadienoic acid	16010	353([M-H]-)	1,2,3
LMFA03010003	PGE2	11R,15S - dihydroxy - 9 - oxo - 5Z,13E - prostadienoic acid	14010	351([M-H]-)	4
LMFA03010004	PGD2	9S,15S - dihydroxy - 11 - oxo - 5Z,13E - prostadienoic acid	12010	351([M-H]-)	
LMFA03010006	PGF2α - d4	9S,11R,15S - trihydroxy - 5Z,13E - prostadienoic acid (3,3,4,4 - d4)	316010	357([M-H]-)	
LMFA03010007	PGD2 - d4	9S,15S - dihydroxy - 11 - oxo - 5Z,13E - prostadienoic acid (3,3,4,4 - d4)	312010	355([M-H]-)	
LMFA03010008	PGE2 - d4	11R,15S - dihydroxy - 9 - oxo - 5Z,13E - prostadienoic acid (3,3,4,4 - d4)	314010	355([M-H]-)	
LMFA03010009	PGG2	9S,11R - epidioxy - 15S - hydroperoxy - 5Z,13E - prostadienoic acid	17010	367([M-H]-)	
LMFA03010010	PGH2	9S,11R - epidioxy - 15S - hydroxy - 5Z,13E - prostadienoic acid	17020	351([M-H]-)	
LMFA03010011	2,3 - Dinor - 11β - PGF2a	9S,11S,13S - trihydroxy - 2,3 - dinor - 5Z,13E - prostadienoic acid	16530	325([M-H]-)	
LMFA03010012	6keto - PGE1	11R,15S - dihydroxy - 6,9 - dioxo - 13E - prostenoic acid	13260	367([M-H]-)	
LMFA03010013	6,15 - diketo - 13,14 - dihydro - PGF1α	9S,11R - dihydroxy - 6,15 - dioxo - 13E - prostenoic acid	15270	369([M-H]-)	
LMFA03010014	20 - hydroxy - PGE2	11R,15S,20 - trihydroxy - 9 - oxo - 5Z,13E - prostadienoic acid	14950	367([M-H]-)	
LMFA03010015	PGF2α - EA	N - (9S,11R,15S - trihydroxy - 5Z,13E - prostadienoyl) - ethanolamine	16013	396([M-H]-)	
LMFA03010016	PGE2 - EA	N - (11R,15S - dihydroxy - 9 - oxo - 5Z,13E - prostadienoyl) - ethanolamine	14012	394([M-H]-)	
LMFA03010017	PGD2 - EA	N - (9S,15S - dihydroxy - 11 - oxo - 5Z,13E - prostadienoyl) - ethanolamine	12012	394([M-H]-)	
LMFA03010018	PGB2	15S - hydroxy - 9 - oxo - 5Z,8(12),13E - prostatrienoic acid	11210	333([M-H]-)	
LMFA03010019	PGJ2	15S - hydroxy - 11 - oxo - 5Z,8Z,13E - prostatrienoic acid	18500	333([M-H]-)	
LMFA03010020	δ - 12 - PGJ2	15S - hydroxy - 11 - oxo - 5Z,9,12E - prostatrienoic acid	18550	333([M-H]-)	
LMFA03010021	15 - deoxy - δ - 12,14 - PGJ2	11 - oxo - 5Z,9,12E,14Z - prostatetraenoic acid	18570	315([M-H]-)	
LMFA03010022	13,14 - dihydro - 15 - keto - PGD2	11,15 - dioxo - 9S - hydroxy - 5Z - prostenoic acid	12610	351([M-H]-)	
LMFA03010023	PGK2	9,11 - dioxo - 15S - hydroxy - 5Z,13E - prostadienoic acid	18900	349([M-H]-)	
LMFA03010024	19R - hydroxy - PGE2	9 - oxo - 11R,15S,19R - trihydroxy - 5Z,13E - prostadienoic acid	14910	367([M-H]-)	
LMFA03010025	PGF2β	9R,11R,15S - trihydroxy - 5Z,13E - prostadienoic acid	16410	353([M-H]-)	

The eicosanoid library provides information from which comprehensive methods can be created and used to survey eicosanoid release from stimulated cells

use of deuterium labeled internal standards allows absolute quantitation for a number of eicosanoids

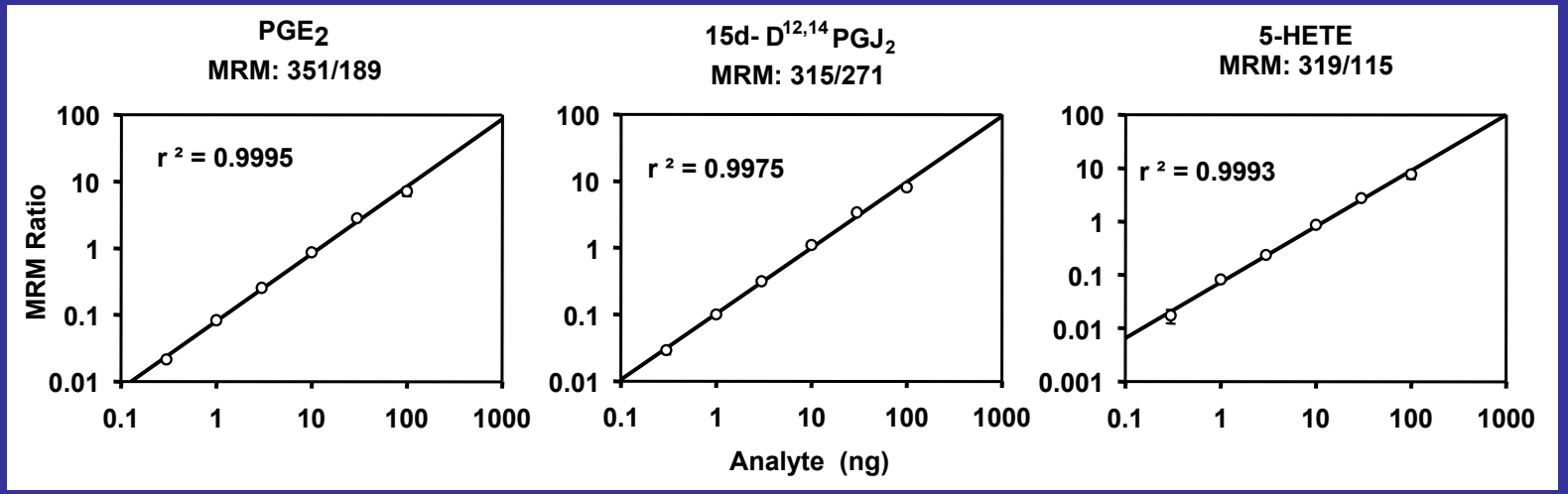


PGE_2 $[M-H]^-$ = 351



PGE_2 - d4 $[M-H]^-$ = 355

example of internal standard calibration curves used for quantitation



chiral chromatography aids in determining enzymatic vs. nonenzymatic origin

atmospheric pressure chemical ionization (APCI) used

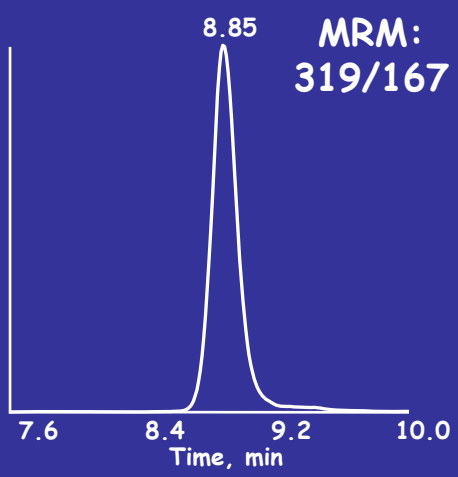
column
Chiralpak® AD-H
4.6 mm X 250 mm

Flow rate
500 µl/min

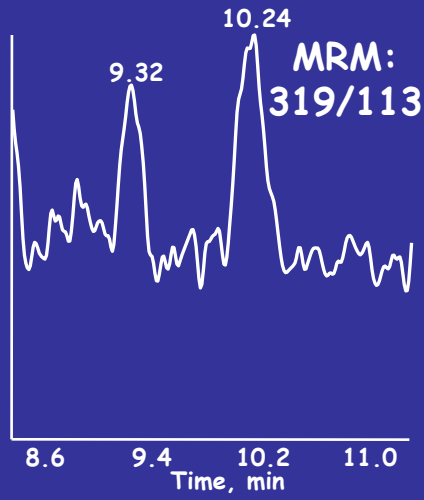
Solvent A
hexane/EtOH/H_2O/formic acid:
96/4/0.08/0.02

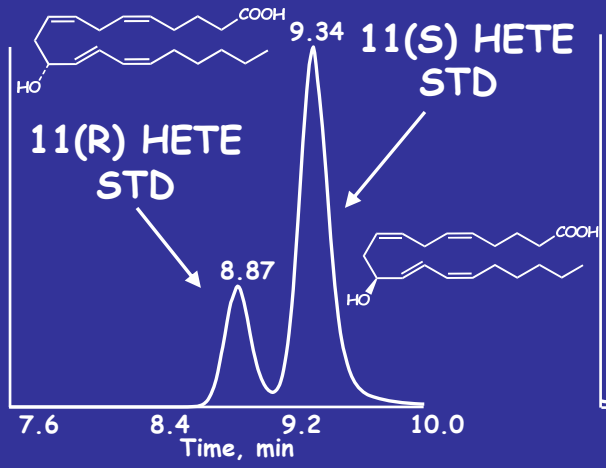
Solvent B
EtOH : 100

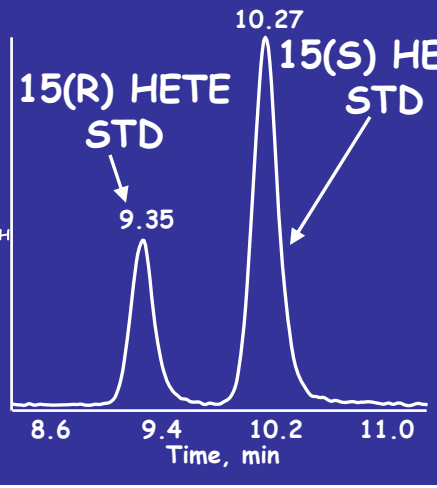
Kdo_2-Lipid A stimulated RAW cells



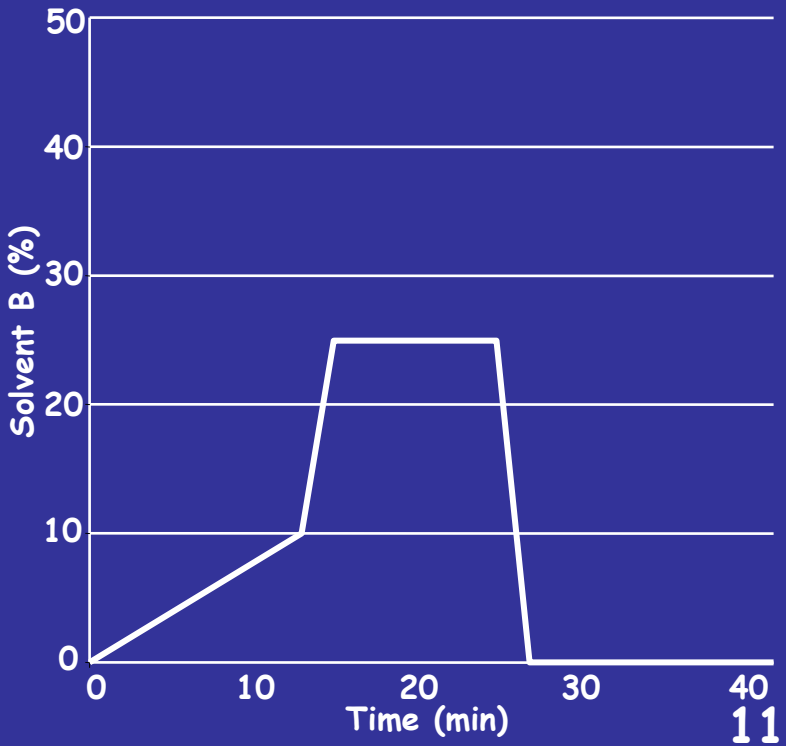
Kdo_2-Lipid A stimulated RAW cells







NP HPLC Gradient



GC-MS analysis of free fatty acids

- Pentafluorobenzyl (PFB) derivatives of the free fatty acids are formed and negative chemical ionization employed. *Produces intact molecular anions - optimal for quantitation (using deuterium labeled standards).*
- Agilent 6890N gas chromatograph
- Agilent 5973 single quadrupole mass analyzer
- Selected ion monitoring (SIM) mode monitoring the $[M-H]^-$ anions.

analysis of essential fatty acids in macrophages

Quehenberger et al. (2008) Prostaglandins Leukot. Essent. Fatty Acids 79 pp. 123-129

Lauric acid (12:0)
Myristic acid (14:0)
Pentadecanoic acid (15:0)
Palmitic acid (16:0)
Palmitoleic acid (16:1)
Heptadecanoic acid (17:0)
Heptadecaenoic acid (17:1)
Stearic acid (18:0)
Oleic acid (18:1)
Linoleic acid (18:2)
α-Linolenic acid (18:3)
γ-Linolenic acid (18:3)
Stearidonic acid (18:4)
Arachidic acid (20:0)
Eicosadienoic acid (20:2)
11,14,17-Eicosatrienoic acid (20:3
Bishomo-γ-linolenic acid (20:3)
5,8,11-Eicosatrienoic acid (20:3
Arachidonic acid (20:4)
Eicosapentaenoic acid (20:5)
Behenic acid (22:0)
cis-Erucic acid (22:1)
Docosadienoic acid (22:2)
Docosatrienoic acid (22:3)
Docosatetraenoic acid (22:4)
Docosapentaenoic acid (22:5)
Docosahexaenoic acid (22:6)
Tricosanoic acid (23:0)
Lignoceric acid (24:0)
cis-Selacholeic acid (24:1)
Cerotic acid (26:0)

Are biologically significant eicosanoids being overlooked?

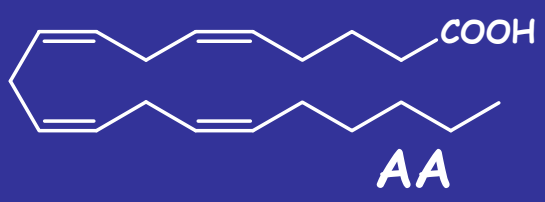
Are there species for which we have no prior knowledge of or expectation of their presence and, hence, no available MRM pairs required for their detection?

To address these concerns a mass spectral based stable isotope labeling strategy has been developed

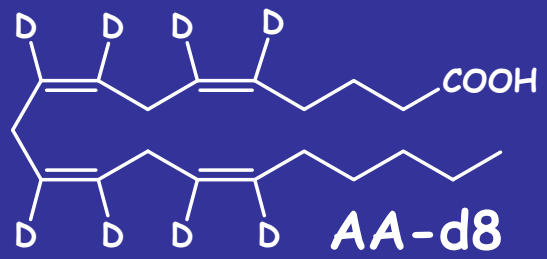
DIMPLES/MS: Diverse Isotope Metabolic Profiling of Labeled Exogenous Substrates using Mass Spectrometry

Harkewicz et al. (2007) J. Biol. Chem. 282 pp. 2899-2910

Incubation of cells in medium supplemented with deuterium-labeled arachidonic acid (AA-d8)

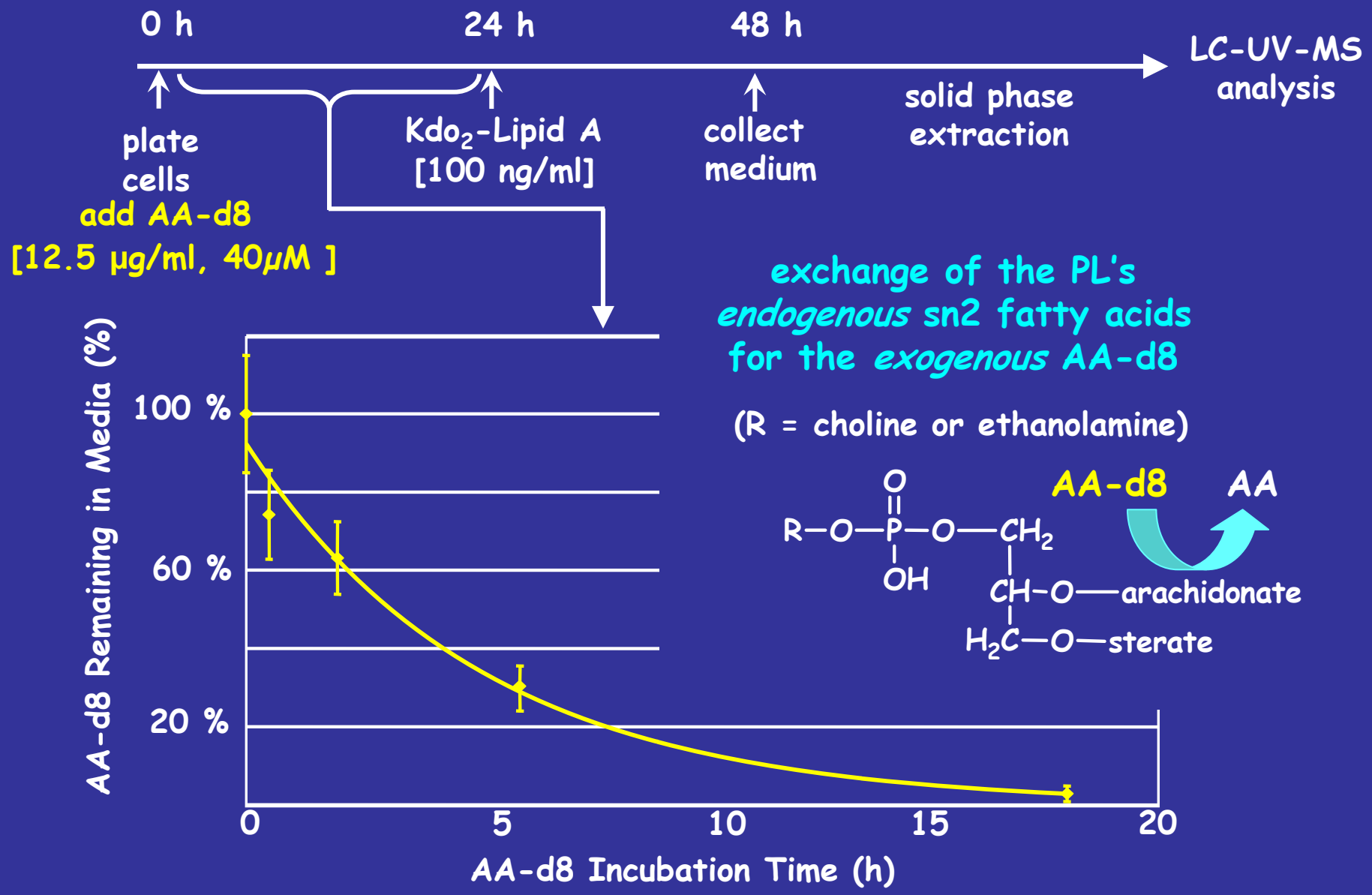


AA



AA-d8

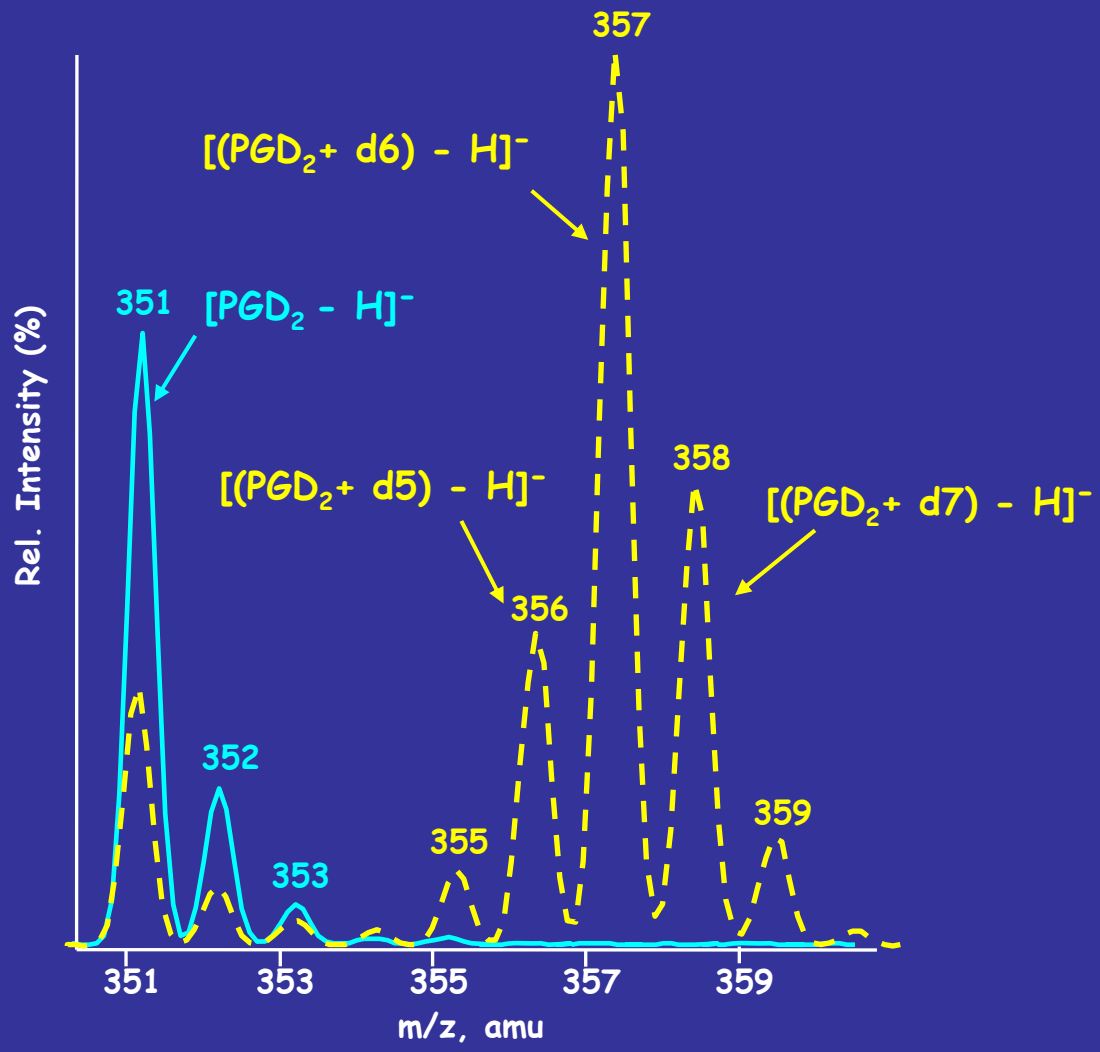
uptake and incorporation of AA-d8 by cells

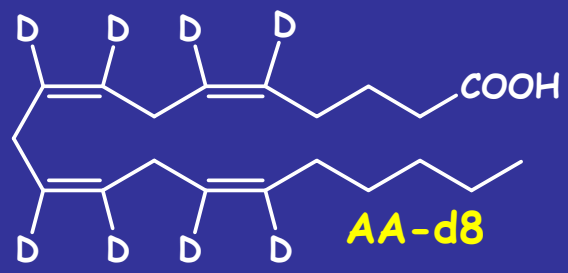


so as not to be a targeted analysis
full-scan MS

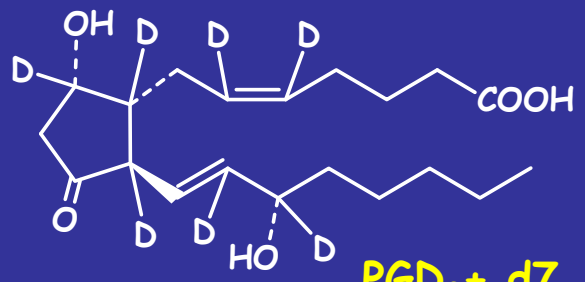
AA/AA-d8 mass spectral doublet indicative of eicosanoid:
(1) AA origin
2) upregulated by Kdo

10% serum (fetal calf)
AA-d8 supplemented





COX
+
PGD-synthase



observed production of a class of dihomoprostaglandins

Harkewicz et al. (2007) J. Biol. Chem. 282 pp. 2899-2910

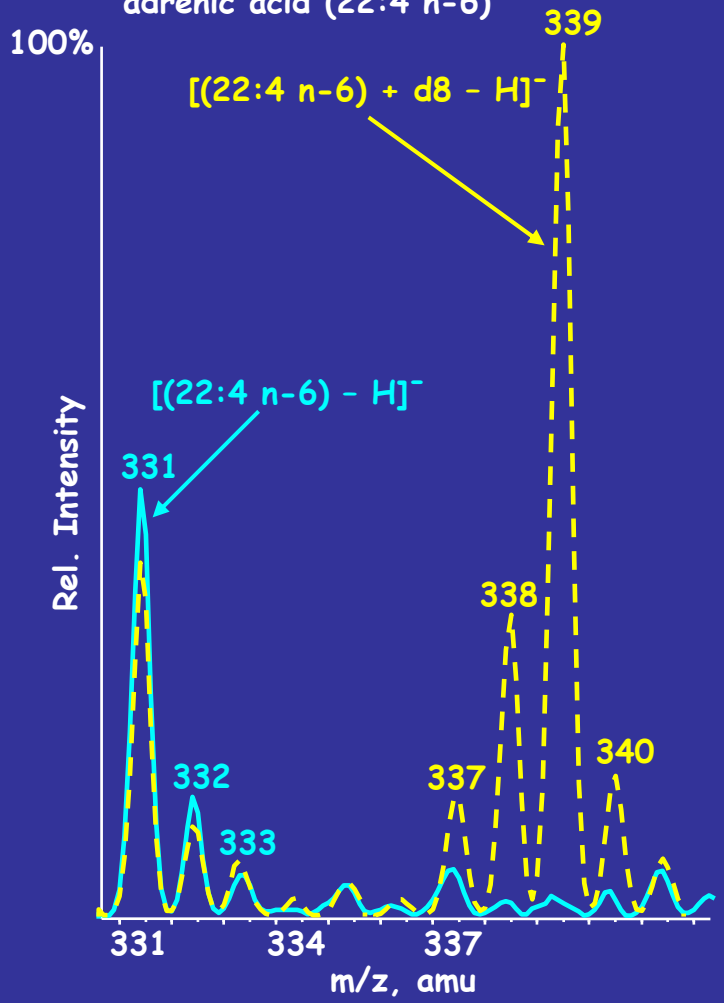
Kdo_2 – Lipid A stimulated RAW cells

Control (NO AA-d8)

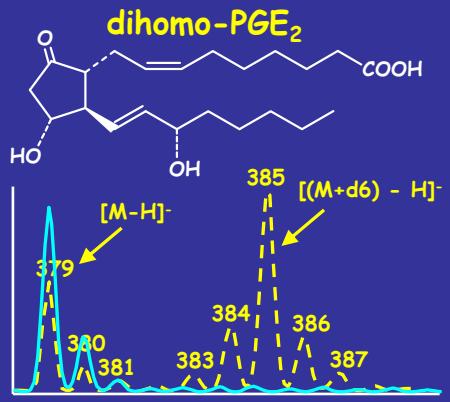
AA-d8 supplemented

m/z = 331

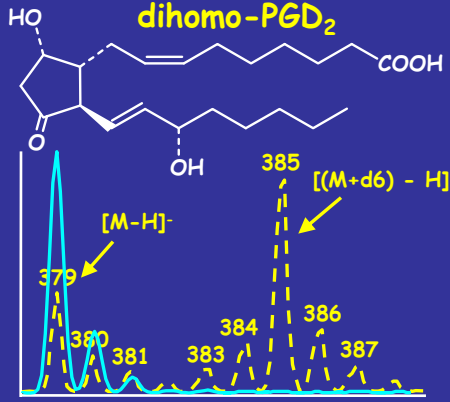
adrenic acid (22:4 n-6)



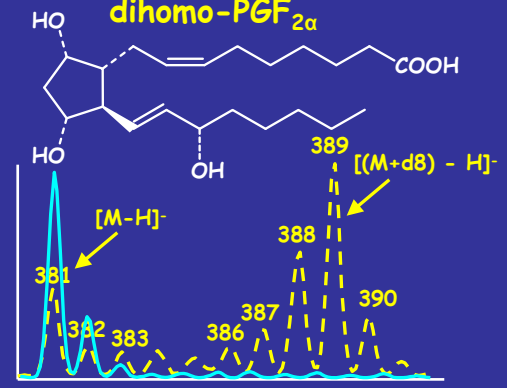
dihomo-PGE_2



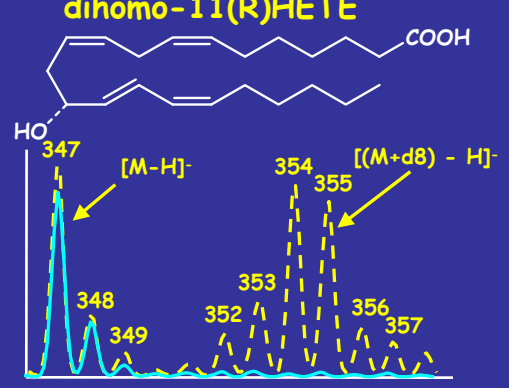
dihomo-PGD_2



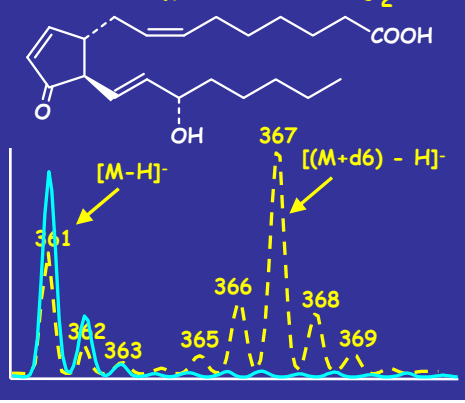
dihomo-$PGF_{2\alpha}$



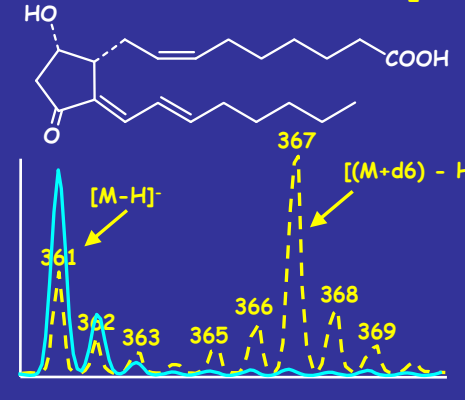
dihomo-11(R)HETE



dihomo-$\Delta^{12,14}$-PGJ_2



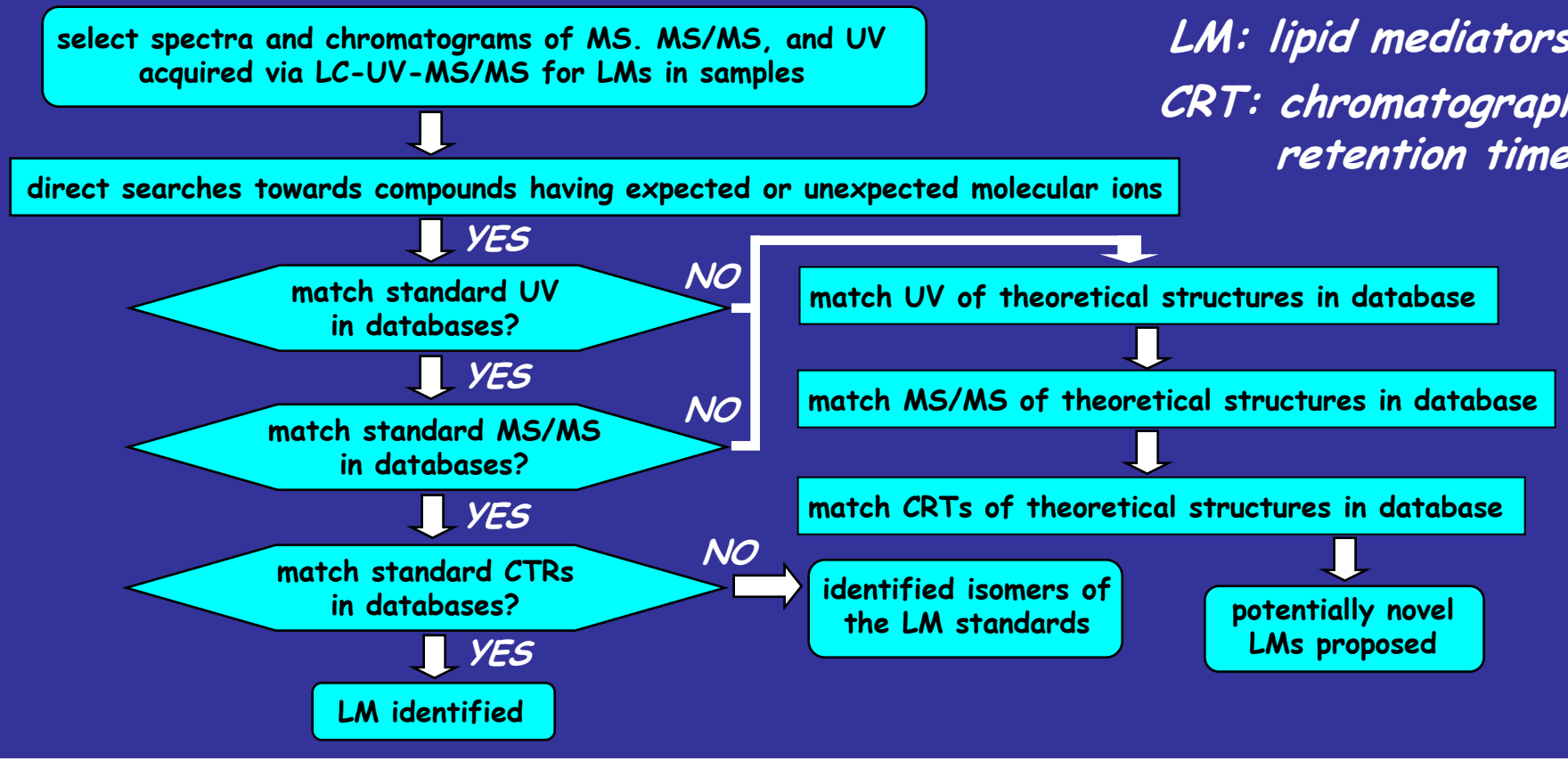
dihomo-15d-$\Delta^{12,14}$-PGD_2



another approach to eicosanoid lipidomics and the search for novel eicosanoids

Serhan Lab developing theoretical databases and algorithms based on virtual UV absorption, tandem mass spectrometry and LC retention times for identifying potential eicosanoids .

Y. Lu et al. (2005) J. Lipid Res. 46 pp. 790-802

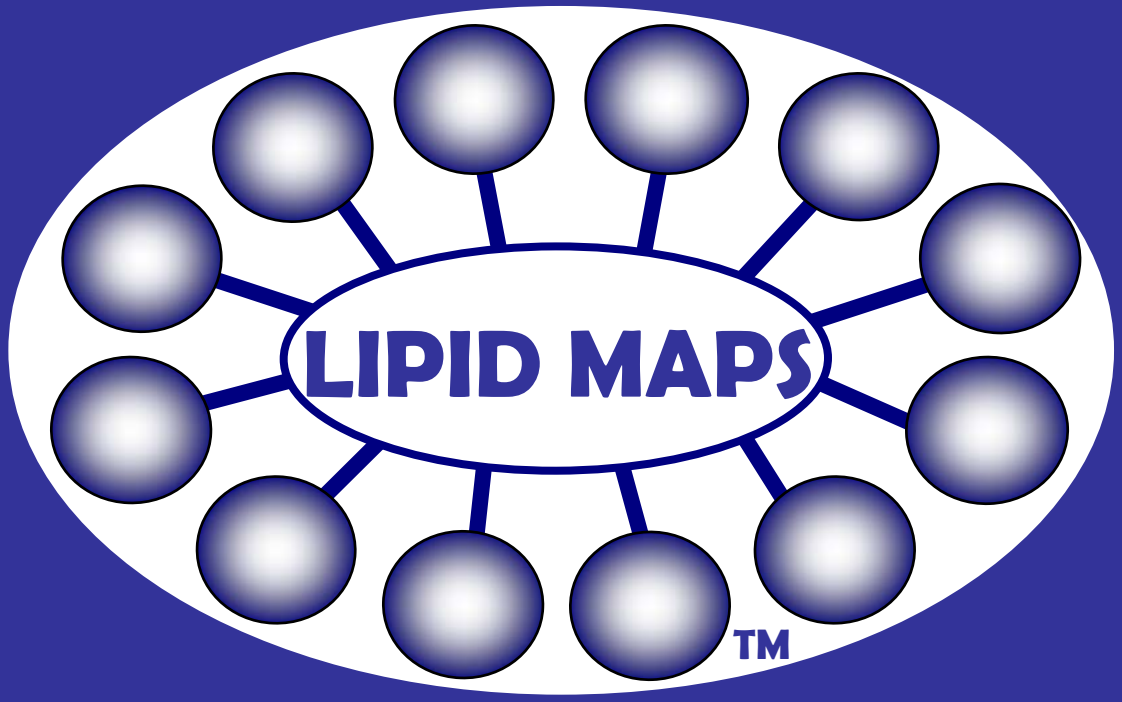


LM: lipid mediators

CRT: chromatographic retention time

Future Plans

- Continue to expand eicosanoid library
- Incorporate UV detection and analyses into eicosanoid surveys
- Expand search for novel eicosanoids
- Similar studies with Ω-3 fatty acid supplementation
  EPA (20:5 n-3) and DHA (22:6 n-3)



www.lipidmaps.org

Thank You