HARVARD MEDICAL SCHOOL

BRIGHAM AND WOMEN'S HOSPITAL

CETRI
Center for Experimental Therapeutics and Reperfusion Injury
CNSerhanlab

Targeted Lipid Mediator Metabololipidomics LC-MS-MS

Eicosanoids and SPM workup

Center for Experimental Therapeutics and Reperfusion Injury,
Brigham and Women's Hospital, and
Harvard Medical School

Correspondence should be addressed to:
Prof. Charles N. Serhan
Hale Building for Transformative Medicine
60 Fenwood Road
Boston, MA 02115

Email: cnserhan@zeus.bwh.harvard.edu; Tel: 617-525-5001; Fax: 617-525-5017

We acknowledge the support of the NIH Grant P01GM095467

Prof. Charles N. SERHAN Laboratory
Brigham & Women's Hospital
Center for Experimental Therapeutics
and Reperfusion Injury
Harvard Medical School

Lipid Mediator Metabololipidomics LC-MS-MS workup

Commercially Available Standards	**Cayman Catalog Numbers**
<u>Specialized Pro-Resolving Mediators</u>	
Lipoxin A_4	(90410)
Lipoxin B_4	(90420)
5S,15S-diHETE	(35280)
15epi-Lipoxin A_4	(90415)
Resolvin D1	(10012554)
17epi-Resolvin D1	(13060)
Resolvin D2	(10007279)
Resolvin D3	(13834)
Resolvin D5	(10007280)
10(S),17(S)-DiHDHA (PDX)	(10008128)
Maresin 1	(10878)
Lipoxin A_5	(10011453)
<u>Biosynthesis Pathway Markers</u>	
AA	(90010)
5-HETE	(34210)
15(S)-HpETE	(44720)
5(S)-HpETE	(44230)
12-HETE	(34550)
15-HETE	(34700)
DHA	(90310)
17(S)-HpDHA	(13185)
14(S)-HDHA	(15253)
4-HDHA	(33200)
7-HDHA	(33300)
17-HDHA	(33650
EPA	(90110)
5-HEPE	(32200)
12-HEPE	(32540)
15-HEPE	(32700)
18-HEPE	(32840)
<u>AA bioactive Metabolome</u>	
PGE_2	(14010)
PGD_2	(12010)
$PGF_{2\alpha}$	(16010)
20-OH-LTB_4	(20190)
LTB_4	(20110)
LTC_4	(20210)
LTD_4	(20310)
LTE_4	(20410)
TXB_2	(19030)

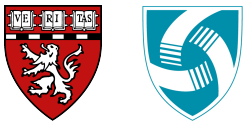
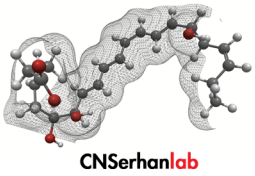


Table of Contents

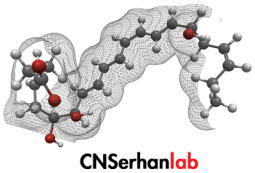


CETRI
Center for Experimental Therapeutics
and Reperfusion Injury

CNSerhanlab

Lipid Mediator Structures & Stereochemistry

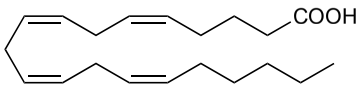
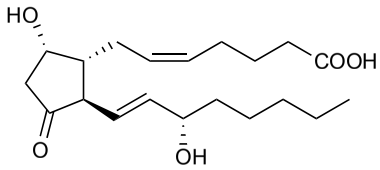
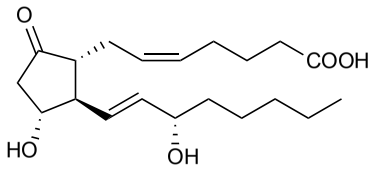
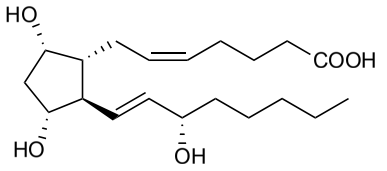
Prof. Charles N. SERHAN Laboratory
Brigham & Women's Hospital
Center for Experimental Therapeutics
and Reperfusion Injury
Harvard Medical School

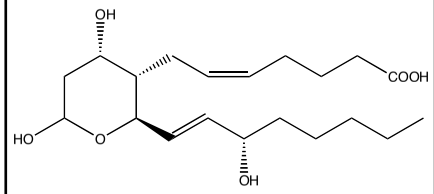
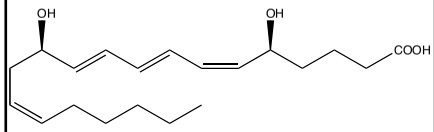
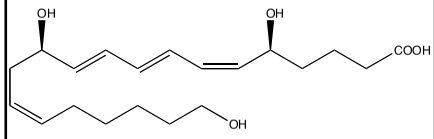
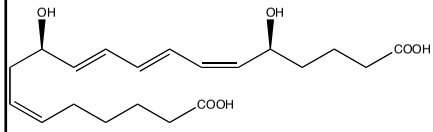


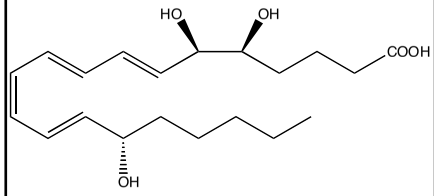
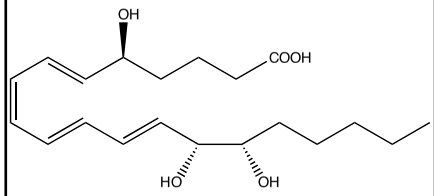
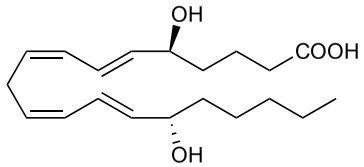
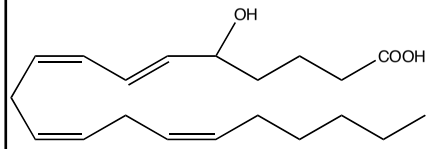
CETRI
Center for Experimental Therapeutics
and Reperfusion Injury

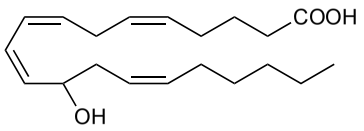
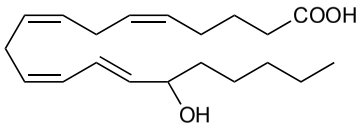
CNSerhanlab

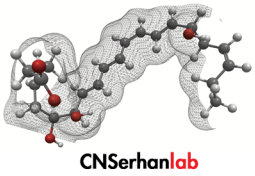
Arachidonic Acid Metabolome

Abbreviation	Structure	Formula	Chemical Name	Trivial Name	Complete Stereochemistry
AA		$C_{20}H_{32}O_2$	Eicosatetraenoic acid	Arachidonic acid	5*Z*,8*Z*,11*Z*,14*Z*-Eicosatetraenoic acid
PGD_2		$C_{20}H_{32}O_5$	11-dehydro-prostaglandin $F_{2\alpha}$	Prostaglandin D_2	11-oxo-9α,15*S*-dihydroxy-5*Z*,13*E*-prostadienoic acid
PGE_2		$C_{20}H_{32}O_5$	9-dehydro-prostaglandin $F_{2\alpha}$	Prostaglandin E_2	9-oxo-11α,15*S*-dihydroxy-5*Z*,13*E*-prostadienoic acid
$PGF_{2\alpha}$		$C_{20}H_{34}O_5$	9α,11α,15*S*-trihydroxy-prostaglandin $F_{2\alpha}$	Prostaglandin $F_{2\alpha}$	9*α*,11α,15*S*-trihydroxy-5*Z*,13*E*-prostadienoic acid

Abbreviation	Structure	Formula	Chemical Name	Trivial Name	Complete Stereochemistry
TxB_2		$C_{20}H_{34}O_6$	9α,11,15*S*-trihydroxy-thromboxane B_2	Thromboxane B_2	9α,11,15*S*-trihydroxy-5*Z*,13*E*-thrombadienoic acid
LTB_4		$C_{20}H_{32}O_4$	5*S*,12*R*-dihydroxy-LTB_4	Leukotriene B_4	5*S*,12*R*-dihydroxy-6*Z*,8*E*,10*E*,14*Z*-eicosatetraenoic acid
20-OH-LTB_4		$C_{20}H_{32}O_5$	5*S*,12*R*,20-trihydroxy-LTB_4	20-hydroxy-Leukotriene B_4	5*S*,12*R*,20-trihydroxy-6*Z*,8*E*,10*E*,14*Z*-eicosatetraenoic acid
20-COOH-LTB_4		$C_{20}H_{30}O_6$	5*S*,12*R*-dihydroxy-20-carboxy-LTB_4	20-carboxy-Leukotriene B_4	5*S*,12*R*-dihydroxy-6*Z*,8*E*,10*E*,14*Z*-20-carboxy-eicosatetraenedioic acid

Abbreviation	Structure	Formula	Chemical Name	Trivial Name	Complete Stereochemistry
LXA_4		$C_{20}H_{32}O_5$	5*S*,6*R*,15*S*-trihydroxy-LXA_4	Lipoxin A_4	5*S*,6*R*,15*S*-trihydroxy-7*E*,9*E*,11*Z*,13*E*-eicosatetraenoic acid
LXB_4		$C_{20}H_{32}O_5$	5*S*,14*R*,15*S*-trihydroxy-LXB_4	Lipoxin B_4	5*S*,14*R*,15*S*-trihydroxy-6*E*,8*Z*,10*E*,12*E*-eicosatetraenoic acid
5S,15S-diHETE		$C_{20}H_{32}O_4$	5*S*,15*S*-dihydroxy-ETE	5*S*,15*S*-dihydroxy-eicosatetraenoic acid	5*S*,15*S*-dihydroxy-6*E*,8*Z*,11*Z*,13*E*-eicosatetraenoic acid
5-HETE		$C_{20}H_{32}O_3$	5-hydroxy-ETE	5-hydroxy-eicosatetraenoic acid	5-hydroxy-6*E*,8*Z*,11*Z*,14*Z*-eicosatetraenoic acid

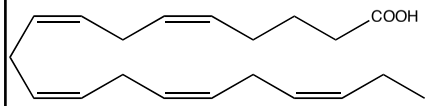
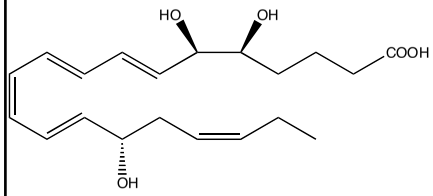
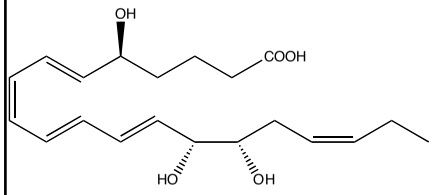
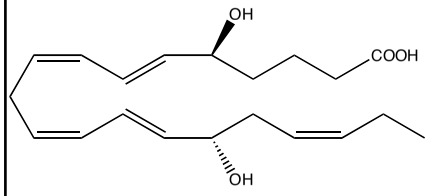
Abbreviation	Structure	Formula	Chemical Name	Trivial Name	Complete Stereochemistry
12-HETE		$C_{20}H_{32}O_3$	12-hydroxy-ETE	12-hydroxy-eicosatetraenoic acid	12-hydroxy-5*Z*,8*Z*,10*E*,14*Z*-eicosatetraenoic acid
15-HETE		$C_{20}H_{32}O_3$	15-hydroxy-ETE	15-hydroxy-eicosatetraenoic acid	15-hydroxy-5*Z*,8*Z*,11*Z*,13*E*-eicosatetraenoic acid

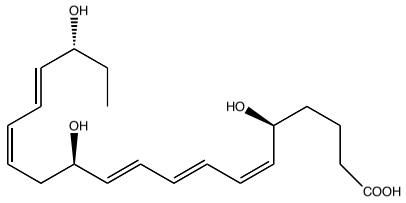
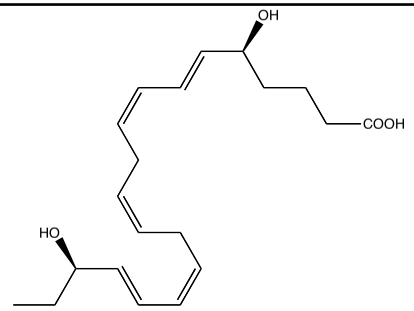
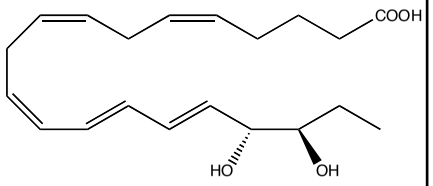
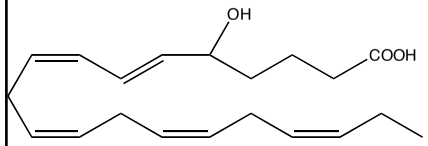


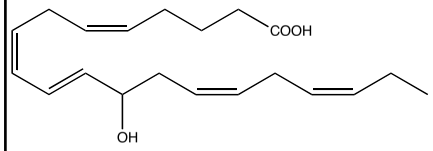
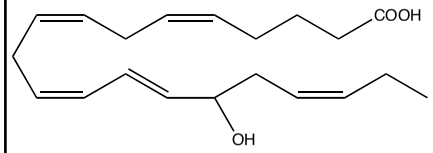
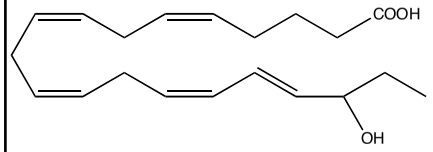
CETRI
Center for Experimental Therapeutics
and Reperfusion Injury

CNSerhanlab

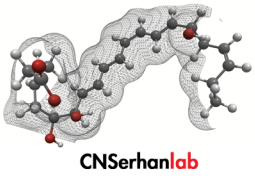
Eicosapentaenoic Acid Metabolome

Abbreviation	Structure	Formula	Chemical Name	Trivial Name	Complete Stereochemistry
EPA		$C_{20}H_{30}O_2$	Eicosapentaenoic acid	Eicosapentaenoic acid	5*Z*,8*Z*,11*Z*,14*Z*,17*Z*-eicosapentaenoic acid
LXA_5		$C_{20}H_{30}O_5$	5*S*,6*R*,15*S*-trihydroxy-LXA_5	Lipoxin A_5	5*S*,6*R*,15*S*-trihydroxy-7*E*,9*E*,11*Z*,13*E*,17*Z*-eicosapentaenoic acid
LXB_5		$C_{20}H_{30}O_5$	5*S*,14*R*,15*S*-trihydroxy-LXB_5	Lipoxin B_5	5*S*,14*R*,15*S*-trihydroxy-6*E*,8*Z*,10*E*,12*E*,17*Z*-eicosapentaenoic acid
5S,15S-diHEPE		$C_{20}H_{30}O_4$	5*S*,15*S*-dihydroxy-EPE	5*S*,15*S*-dihydroxy-eicosapentaenoic acid	5*S*,15*S*-dihydroxy-6*E*,8*Z*,11*Z*,13*E*,17*Z*-eicosapentaenoic acid

Abbreviation	Structure	Formula	Chemical Name	Trivial Name	Complete Stereochemistry
RvE1		$C_{20}H_{30}O_5$	5*S*,12*R*,18*R*-trihydroxy-EPE	Resolvin E1	5*S*,12*R*,18*R*-trihydroxy-6*Z*,8*E*,10*E*,14*Z*,16*E*-eicosapentaenoic acid
RvE2		$C_{20}H_{30}O_4$	5*S*,18*R*-dihydroxy-EPE	Resolvin E2	5*S*,18*R*-dihydroxy-6*E*,8*Z*,11*Z*,14*Z*,16*E*-eicosapentaenoic acid
RvE3		$C_{20}H_{30}O_4$	17*R*,18*R*-dihydroxy-EPE	Resolvin E3	17*R*,18*R*-dihydroxy-5*Z*,8*Z*,11*Z*,13*E*,15*E*-eicosapentaenoic acid
5-HEPE		$C_{20}H_{30}O_3$	5-hydroxy-EPE	5-hydroxy-eicosapentaenoic acid	5-hydroxy-6*E*,8*Z*,11*Z*,14*Z*,17*Z*-eicosapentaenoic acid

Abbreviation	Structure	Formula	Chemical Name	Trivial Name	Complete Stereochemistry
12-HEPE		$C_{20}H_{30}O_3$	12-hydroxy-EPE	12-hydroxy-eicosapentaenoic acid	12-hydroxy-5*Z*,8*Z*,10*E*,14*Z*,17*Z*-eicosapentaenoic acid
15-HEPE		$C_{20}H_{30}O_3$	15-hydroxy-EPE	15-hydroxy-eicosapentaenoic acid	15-hydroxy-5*Z*,8*Z*,11*Z*,13*E*,17*Z*-eicosapentaenoic acid
18-HEPE		$C_{20}H_{30}O_3$	18-hydroxy-EPE	18-hydroxy-eicosapentaenoic acid	18-hydroxy-5*Z*,8*Z*,11*Z*,14*Z*,16*E*-eicosapentaenoic acid

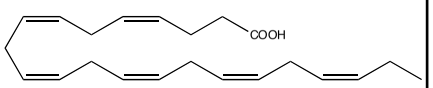
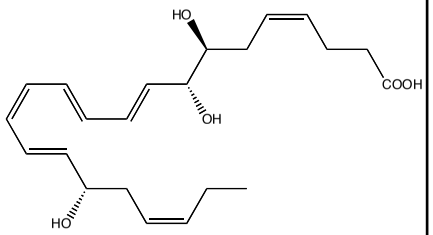
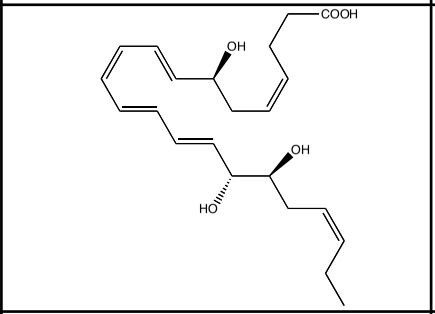
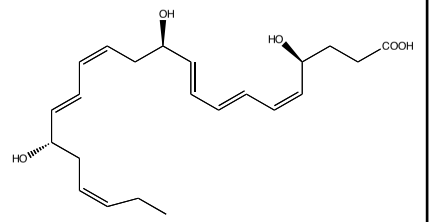
Prof. Charles N. SERHAN Laboratory
Brigham & Women's Hospital
Center for Experimental Therapeutics
and Reperfusion Injury
Harvard Medical School

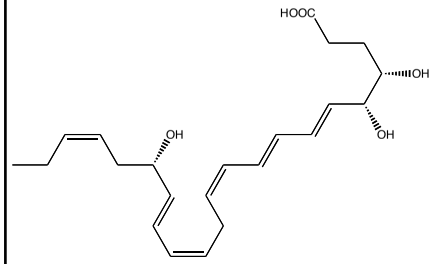
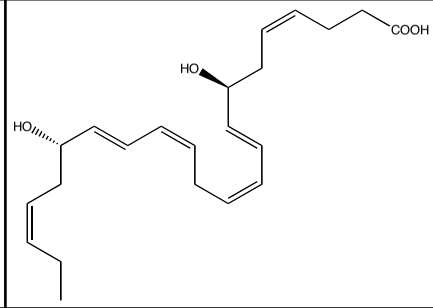
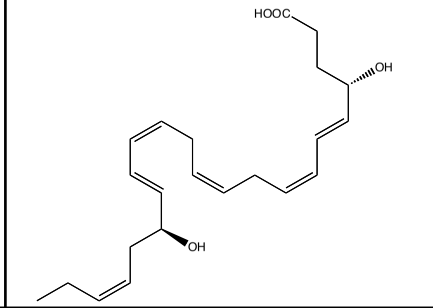
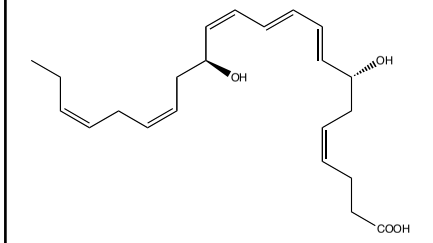


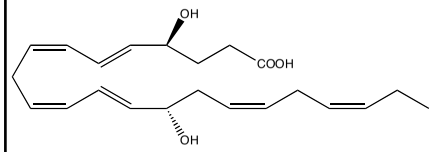
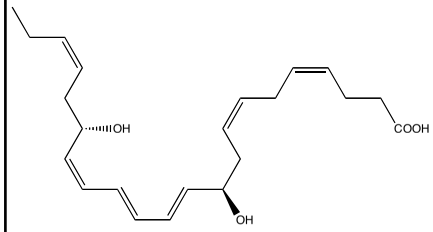
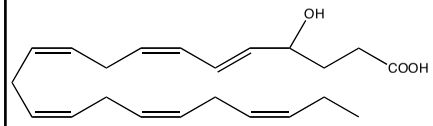
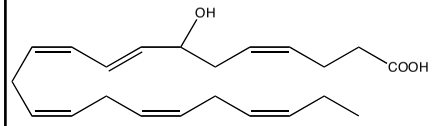
CETRI
Center for Experimental Therapeutics
and Reperfusion Injury

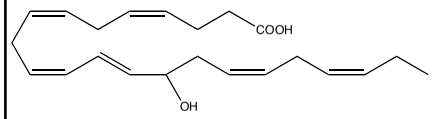
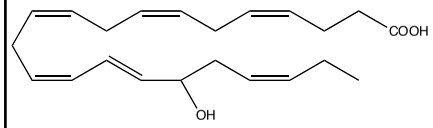
CNSerhanlab

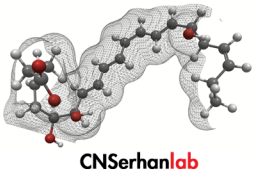
Docosahexaenoic Acid Metabolome

Abbreviation	Structure	Formula	Chemical Name	Trivial Name	Complete Stereochemistry
DHA		$C_{22}H_{32}O_2$	Docosahexaenoic acid	Docosahexaenoic acid	4*Z*,7*Z*,10*Z*,13*Z*,16*Z*,19*Z*-docosahexaenoic acid
RvD1		$C_{22}H_{32}O_5$	7*S*,8*R*,17*S*-trihydroxy-DHA	Resolvin D1	7*S*,8*R*,17*S*-trihydroxy-4*Z*,9*E*,11*E*,13*Z*,15*E*,19*Z*-docosahexaenoic acid
RvD2		$C_{22}H_{32}O_5$	7*S*,16*R*,17*S*-trihydroxy-DHA	Resolvin D2	7*S*,16*R*,17*S*-trihydroxy-4*Z*,8*E*,10*Z*,12*E*,14*E*,19*Z*-docosahexaenoic acid
RvD3		$C_{22}H_{32}O_5$	4*S*,11*R*,17*S*-trihydroxy-DHA	Resolvin D3	4*S*,11*R*,17*S*-trihydroxy-5*Z*,7*E*,9*E*,13*Z*,15*E*,19*Z*-docosahexaenoic acid

Abbreviation	Structure	Formula	Chemical Name	Trivial Name	Complete Stereochemistry
RvD4		$C_{22}H_{32}O_5$	4*S*,5R,17*S*-trihydroxy-DHA	Resolvin D4	4*S*,5*R*,17*S*-trihydroxy-6*E*,8*E*,10*Z*,13*Z*,15*E*,19*Z*-docosahexaenoic acid
RvD5		$C_{22}H_{32}O_4$	7*S*,17*S*-dihydroxy-DHA	Resolvin D5	7*S*,17*S*-dihydroxy-4*Z*,8*E*,10*Z*,13*Z*,15*E*,19*Z*-docosahexaenoic acid
RvD6		$C_{22}H_{32}O_4$	4*S*,17*S*-dihydroxy-DHA	Resolvin D6	4*S*,17*S*-dihydroxy-5*E*,7*Z*,10*Z*,13*Z*,15*E*,19*Z*-docosahexaenoic acid
MaR1		$C_{22}H_{32}O_4$	7*R*,14*S*-dihydroxy-DHA	Maresin 1	7*R*,14*S*-dihydroxy-4*Z*,8*E*,10*E*,12*Z*,16*Z*,19*Z*-docosahexaenoic acid

Abbreviation	Structure	Formula	Chemical Name	Trivial Name	Complete Stereochemistry
4S,14S-diHDHA		$C_{22}H_{32}O_4$	4*S*,14*S*-dihydroxy-DHA	4,14-diHDHA	4*S*,14*S*-dihydroxy-5*E*,7*Z*,10*Z*,12*E*,16*Z*,19*Z*-docosahexaenoic acid
PD1		$C_{22}H_{32}O_4$	10*R*,17*S*-dihydroxy-DHA	[Neuro] Protectin D1	10*R*,17*S*-dihydroxy-4*Z*,7*Z*,11*E*,13*E*,15*Z*,19*Z*-docosahexaenoic acid
4-HDHA		$C_{22}H_{32}O_3$	4-hydroxy-DHA	4-hydroxy-DHA	4-hydroxy-5*E*,7*Z*,10*Z*,13*Z*,16*Z*,19*Z*-docosahexaenoic acid
7-HDHA		$C_{22}H_{32}O_3$	7-hydroxy-DHA	7-hydroxy-DHA	7-hydroxy-4*Z*,8*E*,10*Z*,13*Z*,16*Z*,19*Z*-docosahexaenoic acid

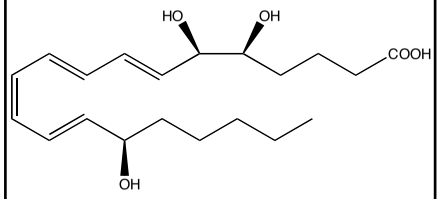
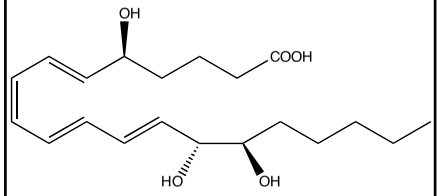
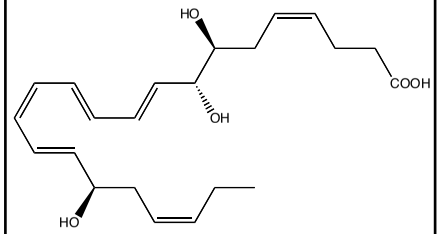
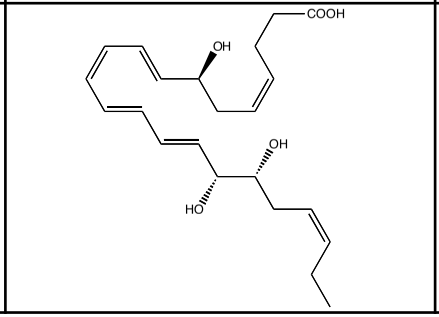
Abbreviation	Structure	Formula	Chemical Name	Trivial Name	Complete Stereochemistry
14-HDHA		$C_{22}H_{32}O_3$	14-hydroxy-DHA	14-hydroxy-DHA	14-hydroxy-4*Z*,7*Z*,10*Z*,12*E*,16*Z*,19*Z*-docosahexaenoic acid
17-HDHA		$C_{22}H_{32}O_3$	17-hydroxy-DHA	17-hydroxy-DHA	17-hydroxy-4*Z*,7*Z*,10*Z*,13*Z*,15*E*,19*Z*-docosahexaenoic acid

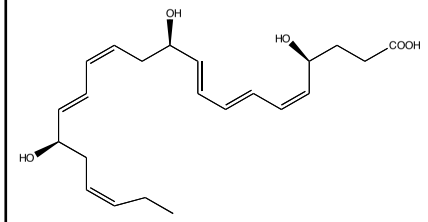
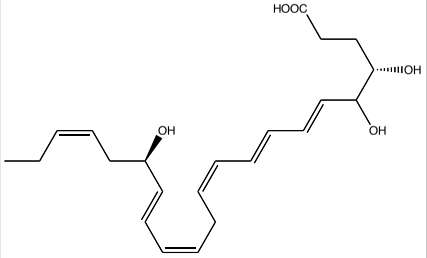
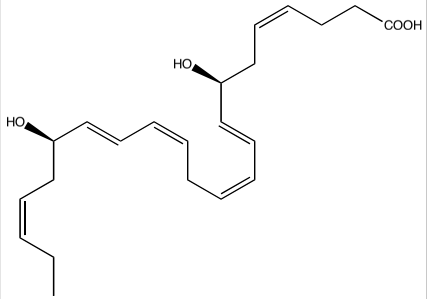
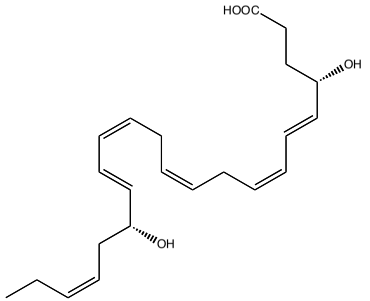


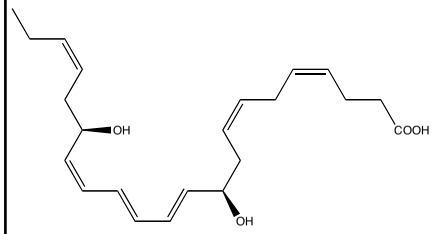
CETRI
Center for Experimental Therapeutics
and Reperfusion Injury

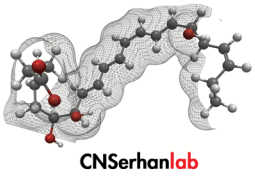
CNSerhanlab

Aspirin-Triggered Specialized Pro-resolving Mediators

Abbreviation	Structure	Formula	Chemical Name	Trivial Name	Complete Stereochemistry
AT-LXA$_4$		$C_{20}H_{32}O_5$	5*S*,6*R*,15*R*-trihydroxy-LXA$_4$	15-epi-Lipoxin A$_4$	5*S*,6*R*,15*R*-trihydroxy-7*E*,9*E*,11*Z*,13*E*-eicosatetraenoic acid
AT-LXB$_4$		$C_{20}H_{32}O_5$	5*S*,14*R*,15*R*-trihydroxy-LXB$_4$	15-epi-Lipoxin B$_4$	5*S*,14*R*,15*R*-trihydroxy-6*E*,8*Z*,10*E*,12*E*-eicosatetraenoic acid
AT-RvD1		$C_{22}H_{32}O_5$	7*S*,8*R*,17*R*-trihydroxy-DHA	Aspirin-triggered Resolvin D1	7*S*,8*R*,17*R*-trihydroxy-4*Z*,9*E*,11*E*,13*Z*,15*E*,19*Z*-docosahexaenoic acid
AT-RvD2		$C_{22}H_{32}O_5$	7*S*,16*R*,17*R*-trihydroxy-DHA	Aspirin-triggered Resolvin D2	7*S*,16*R*,17*R*-trihydroxy-4*Z*,8*E*,10*Z*,12*E*,14*E*,19*Z*-docosahexaenoic acid

Abbreviation	Structure	Formula	Chemical Name	Trivial Name	Complete Stereochemistry
AT-RvD3		$C_{22}H_{32}O_5$	4*S*,11*R*,17*R*-trihydroxy-DHA	Aspirin-triggered Resolvin D3	4*S*,11*R*,17*R*-trihydroxy-5*Z*,7*E*,9*E*,13*Z*,15*E*,19*Z*-docosahexaenoic acid
AT-RvD4		$C_{22}H_{32}O_5$	4*S*,5,17*R*-trihydroxy-DHA	Aspirin-triggered Resolvin D4	4*S*,5*R*,17*R*-trihydroxy-6*E*,8*E*,10*Z*,13*Z*,15*E*,19*Z*-docosahexaenoic acid
AT-RvD5		$C_{22}H_{32}O_4$	7*S*,17*R*-dihydroxy-DHA	Aspirin-triggered Resolvin D5	7*S*,17*R*-dihydroxy-4*Z*,8*E*,10*Z*,13*Z*,15*E*,19*Z*-docosahexaenoic acid
AT-RvD6		$C_{22}H_{32}O_4$	4*S*,17*R*-dihydroxy-DHA	Aspirin-triggered Resolvin D6	4*S*,17*R*-dihydroxy-5*E*,7*Z*,10*Z*,13*Z*,15*E*,19*Z*-docosahexaenoic acid

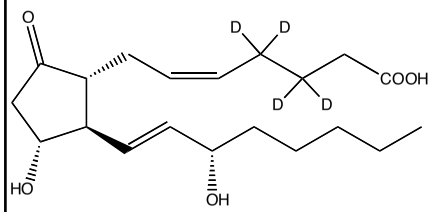
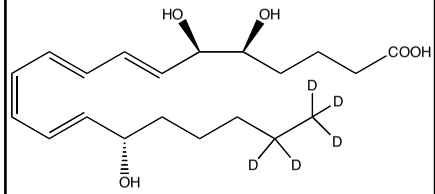
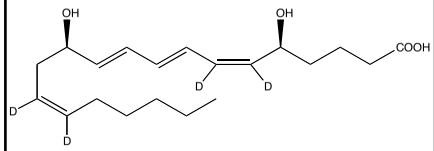
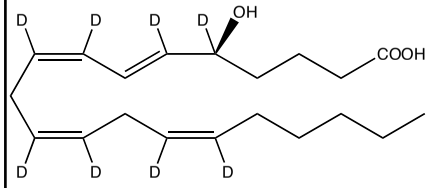
Abbreviation	Structure	Formula	Chemical Name	Trivial Name	Complete Stereochemistry
AT-PD1		$C_{22}H_{32}O_4$	10*R*,17*R*-dihydroxy-DHA	Aspirin-triggered [Neuro] Protectin D1	10*R*,17*R*-dihydroxy-4*Z*,7*Z*,11*E*,13*E*,15*Z*,19*Z*-docosahexaenoic acid

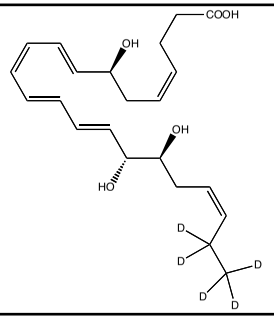


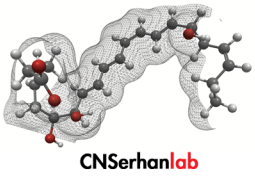
CETRI
Center for Experimental Therapeutics
and Reperfusion Injury

CNSerhanlab

Deuterium Labeled Internal Standards

Abbreviation	Structure	Formula	Chemical Name	Trivial Name	Complete Stereochemistry
d_4-PGE$_2$		$C_{20}H_{28}D_4O_5$	9-dehydro-prostaglandin $F_{2\alpha}$-d_4	d_4-Prostaglandin E_2	9-oxo-11α,15*S*-dihydroxy-5*Z*,13*E*-3,3,4,4-d_4-prostadienoic acid
d_5-LXA$_4$		$C_{20}H_{27}D_5O_5$	5*S*,6*R*,15*S*-trihydroxy-LXA_4-d_5	d_5-Lipoxin A_4	5*S*,6*R*,15*S*-trihydroxy-7*E*,9*E*,11*Z*,13*E*-19,19,20,20,20-d_5-eicosatetraenoic acid
d_4-LTB$_4$		$C_{20}H_{28}D_4O_4$	5*S*,12*R*-dihydroxy-LTB_4-d_4	d_4-Leukotriene B_4	5*S*,12*R*-dihydroxy-6*Z*,8*E*,10*E*,14*Z*-6,7,14,15-d_4-eicosatetraenoic acid
d_8-5S-HETE		$C_{20}H_{24}D_8O_3$	5*S*-hydroxy-ETE-d_8	d_8-5S-hydroxy-eicosatetraenoic acid	5*S*-hydroxy-6*E*,8*Z*,11*Z*,14*Z*-5,6,8,9,11,12,14,15-d_8-eicosatetraenoic acid

Abbreviation	Structure	Formula	Chemical Name	Trivial Name	Complete Stereochemistry
d_5-RvD2		$C_{22}H_{27}D_5O_5$	7*S*,16*R*,17*S*-trihydroxy-DHA -d_5	d_5-Resolvin D2	7*S*,16*R*,17*S*-trihydroxy-4*Z*,8*E*,10*Z*,12*E*,14*E*,19*Z*-19,19,20,20,20,20-d_5-docosahexaenoic acid



CNSerhanlab

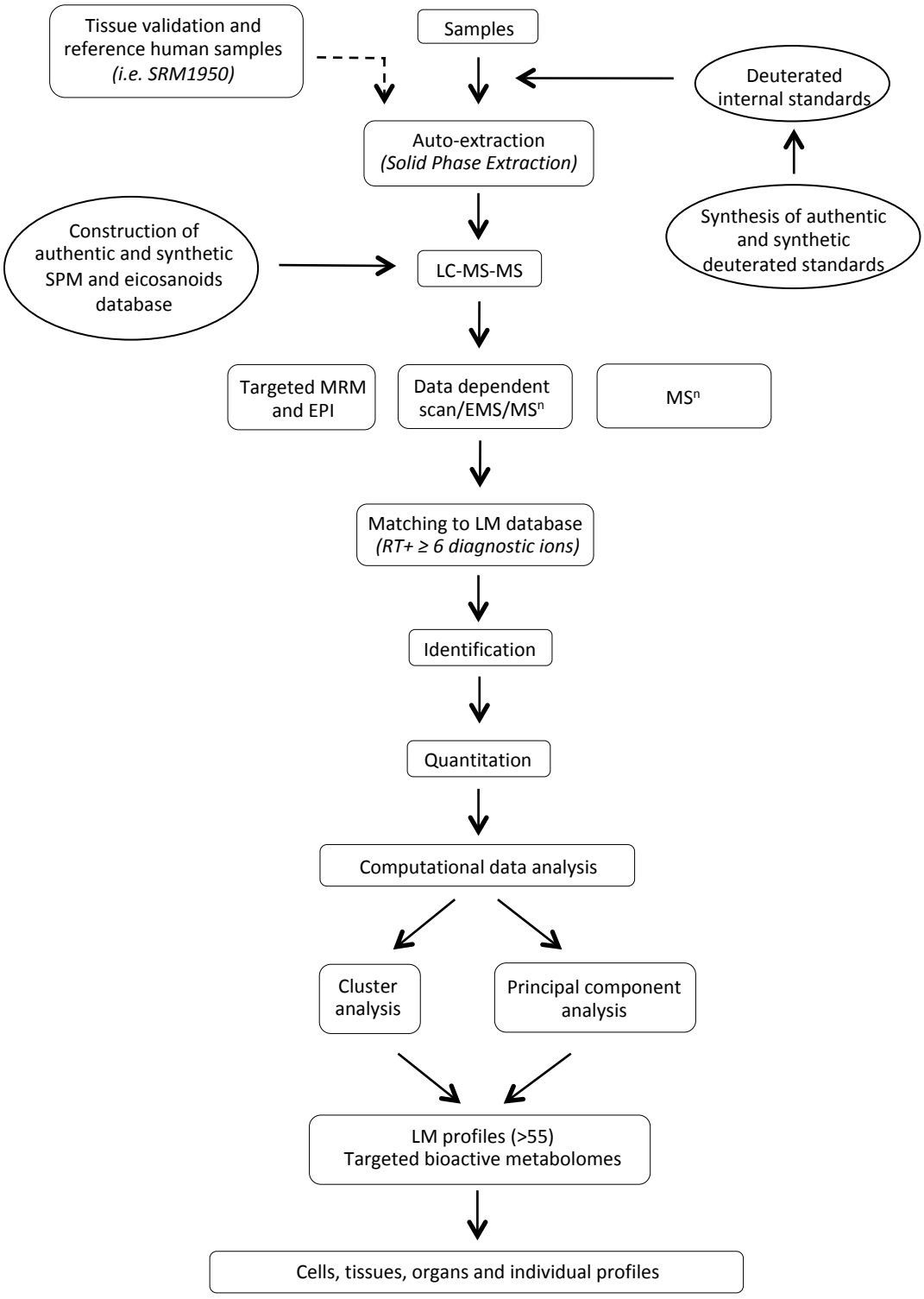
CETRI
Center for Experimental Therapeutics
and Reperfusion Injury

Methods

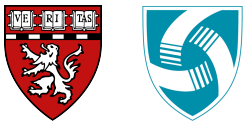
Prof. Charles N. SERHAN Laboratory
Brigham & Women's Hospital
Center for Experimental Therapeutics
and Reperfusion Injury
Harvard Medical School

Lipid Mediator Metabololipidomics
LC-MS-MS workup

Operationalization of lipid mediator metabololipidomics



Identification and signature profiles for pro-resolving and inflammatory lipid mediators in human tissue
Colas RA, Shinohara M, Dalli J, Chiang N, Serhan CN. Am J Physiol Cell Physiol. **2014**



Shimadzu LC20AD HPLC Settings		
Solvent A	H_2O (0.01% acetic acid)	
Solvent B	Methanol (0.01% acetic acid)	
Flow rate	0.4mL/min	
Column	Eclipse plus C18, 1.8 µm, 4.6 x 100 mm, AGILENT	
Column Heater	ThermaSphere TS-130, 50°C, PHENOMENEX	
Gradient	Time (min)	Solvent (%B)
	0.0	55
	2.0	55
	10.0	85
	18.0	98
	20.0	98
	20.1	55
	24.5	End

Table 1: High Performance Liquid Chromatography settings.

AB Sciex 5500 Q TRAP MS setting for MRM and EPI		
	MRM	EPI
Curtain Gas	25	25
Collision Gas	Medium	Medium
Ion Spray Voltage	-4000	-4000
Temperature	500 °C	500 °C
Ion Source Gas 1	40	40
Ion Source Gas 2	40	40

Table 2: Mass Spectrometer settings for Multiple Reaction Monitoring and Enhance Product Ion for AB Sciex 5500 Q TRAP.

AB Sciex 6500 Q TRAP MS setting for MRM and EPI		
	MRM	EPI
Curtain Gas	30	30
Collision Gas	Medium	Medium
Ion Spray Voltage	-4500	-4500
Temperature	580 °C	580 °C
Ion Source Gas 1	80	80
Ion Source Gas 2	70	70

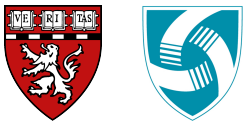
Table 3: Mass Spectrometer settings for Multiple Reaction Monitoring and Enhance Product Ion for AB Sciex 6500 Q TRAP.

AB Sciex 5500 Q TRAP Individual lipid mediator, pathway marker, and further metabolite setting for MRM (V)				
Compound	DP	EP	CE	CXP
AA	-90	-10	-41	-22
PGD_2	-80	-10	-30	-15
PGE_2	-80	-10	-30	-15
$PGF_{2\alpha}$	-80	-10	-30	-15
TxB_2	-80	-10	-30	-15
LTB_4	-110	-10	-22	-17
20-OH-LTB_4	-100	-10	-26	-17
20-COOH-LTB_4	-90	-10	-26	-17
LXA_4	-95	-10	-22	-11
LXB_4	-90	-10	-30	-21
5,15-diHETE	-110	-10	-22	-17
5-HETE	-90	-10	-21	-22
12-HETE	-70	-10	-21	-15
15-HETE	-65	-10	-19	-18
EPA	-90	-10	-41	-22
LXA_5	-95	-10	-22	-11
LXB_5	-90	-10	-30	-21
5,15-diHEPE	-110	-10	-22	-17
RvE1	-80	-10	-24	-16
RvE2	-40	-10	-22	-17
RvE3	-40	-10	-22	-17
5-HEPE	-60	-10	-22	-11
12-HEPE	-70	-10	-21	-15
15-HEPE	-65	-10	-19	-8
18-HEPE	-65	-10	-18	-23
DHA	-90	-10	-41	-22
RvD1	-90	-10	-22	-13
RvD2	-90	-10	-24	-13
RvD3	-80	-10	-28	-19
RvD5	-40	-10	-22	-17
RvD6	-40	-10	-22	-17
MaR1	-40	-10	-22	-17
4,14-diHDHA	-40	-10	-22	-17
PD1	-40	-10	-22	-17
4-HDHA	-40	-10	-18	-13
7-HDHA	-40	-10	-18	-13
14-HDHA	-70	-10	-21	-15
17-HDHA	-80	-10	-20	-18
d_4-PGE_2	-80	-10	-30	-11
d_4-LXA_4	-135	-10	-24	-17
d_4-LTB_4	-135	-10	-24	-17
d_8-5S-HETE	-100	-10	-22	-11
d_5-RvD2	-90	-10	-24	-13

Table 4: Mass Spectrometer settings for Multiple Reaction Monitoring forAB Sciex 5500 Q TRAP. DP=Declustering Potential, EP=Entrance potential, CE=Collision Energy, CXP=Collision Cell Exit Potential

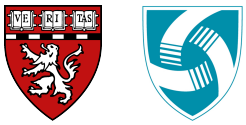
AB Sciex 6500 Q TRAP Individual lipid mediator, pathway marker, and further metabolite setting for MRM (V)				
Compound	DP	EP	CE	CXP
AA	-10.0	-10.0	-10.0	-12.0
PGD_2	-30.0	-10.0	-22.0	-8.0
PGE_2	-30.0	-10.0	-22.0	-8.0
PGF_{2a}	-60.0	-10.0	-33.3	-12.0
TxB_2	-70.0	-10.0	-25.0	-22.0
LTB_4	-45.0	-10.0	-21.0	-10.8
20-OH-LTB_4	-60.0	-10.0	-22.0	-8.0
20-COOH-LTB_4	-60.0	-10.0	-22.0	-8.0
LXA_4	-30.0	-10.0	-18.2	-10.0
LXB_4	-45.0	-10.0	-21.5	-12.0
5,15-diHETE	-35.0	-10.0	-18.0	-12.0
5-HETE	-30.0	-10.0	-18.0	-9.5
12-HETE	-30.0	-10.0	-18.0	-9.5
15-HETE	-30.0	-10.0	-18.0	-9.5
EPA	-10.0	-10.0	-10.0	-12.0
LXA_5	-30.0	-10.0	-18.6	-10.0
LXB_5	-30.0	-10.0	-21.2	-10.0
5,15-diHEPE	-30.0	-12.0	-17.0	-12.0
RvE1	-50.0	-10.0	-21.1	-10.0
RvE2	-30.0	-12.0	-18.6	-10.0
RvE3	-35.0	-10.0	-20.5	-14.0
5-HEPE	-30.0	-10.0	-18.0	-9.5
12-HEPE	-30.0	-10.0	-18.0	-9.5
15-HEPE	-30.0	-10.0	-18.0	-9.5
18-HEPE	-30.0	-10.0	-18.0	-9.5
DHA	-10.0	-10.0	-10.0	-12.0
RvD1	-40.0	-10.0	-19.0	-9.0
RvD2	-40.0	-10.0	-27.8	-10.0
RvD3	-55.0	-10.0	-24.6	-9.5
RvD5	-42.0	-10.0	-18.0	-11.0
RvD6	-50.0	-10.0	-20.0	-11.0
MaR1	-38.0	-10.0	-19.6	-11.0
4,14-diHDHA	-50.0	-10.0	-20.0	-11.0
PD1	-45.0	-10.0	-20.8	-9.5
4-HDHA	-30.0	-10.0	-18.0	-9.5
7-HDHA	-30.0	-10.0	-18.0	-9.5
14-HDHA	-30.0	-10.0	-18.0	-9.5
17-HDHA	-30.0	-10.0	-18.0	-9.5
d_4-PGE_2	-30.0	-10.0	-26.0	-14.0
d_4-LXA_4	-40.0	-10.0	-17.5	-7.5
d_4-LTB_4	-55.0	-10.0	-21.7	-10.0
d_8-5S-HETE	-40.0	-10.0	-24.0	-12.0
d_5-RvD2	-40.0	-10.0	-20.3	-13.0

Table 5: Mass Spectrometer settings for Multiple Reaction Monitoring for AB Sciex 5500 Q TRAP. DP=Declustering Potential, EP=Entrance potential, CE=Collision Energy, CXP=Collision Cell Exit Potential



Compound name	Linear equation	r^2	Internal standard
RvD1	y=0.00002081x	0.999	d_5-RvD2
RvD2	y=0.00009118x	0.997	d_5-RvD2
RvD3	y=0.000008163x	0.990	d_5-RvD2
RvD5	y=0.00001137x	0.993	d_4-LTB_4
PD1	y=0.00001425x	0.995	d_4-LTB_4
MaR1	y=0.00007534x	0.999	d_4-LTB_4
17-HDHA	y=0.00001132x	0.996	d_8-5HETE
14-HDHA	y=0.00001916x	0.999	d_8-5HETE
7-HDHA	y=0.00001096x	0.996	d_8-5HETE
4-HDHA	y=0.00001040x	0.996	d_8-5HETE
LXA_5	y=0.00002043x	0.999	d_5-LXA_4
RvE1	y=0.00004358x	0.999	d_5-LXA_4
RvE2	y=0.0001647x	0.996	d_4-LTB_4
RvE3	y=0.0001047x	0.998	d_4-LTB_4
18-HEPE	y=0.00002131x	0.998	d_8-5HETE
15-HEPE	y=0.00002002x	0.999	d_8-5HETE
12-HEPE	y=0.00001899x	0.997	d_8-5HETE
5-HEPE	y=0.00001301x	0.998	d_8-5HETE
LXA_4	y=0.00001607x	0.996	d_5-LXA_4
LXB_4	y=0.00007325x	0.995	d_5-LXA_4
5S,15S-diHETE	y=0.00008011x	0.997	d_4-LTB_4
LTB_4	y=0.00001074x	0.991	d_4-LTB_4
20-OH-LTB_4	y=0.00001524x	0.999	d_4-LTB_4
20-COOH-LTB_4	y=0.00007931x	0.999	d_4-LTB_4
PGD_2	y=0.00001019x	0.993	d_4-PGE_2
PGE_2	y=0.00001698x	0.998	d_4-PGE_2
$PGF_{2\alpha}$	y=0.00001643x	0.997	d_4-PGE_2
TXB_2	y=0.00001054x	0.997	d_4-PGE_2
15-HETE	y=0.00001377x	0.999	d_8-5HETE
12-HETE	y=0.00001142x	0.994	d_8-5HETE
5-HETE	y=0.00001010x	0.990	d_8-5HETE

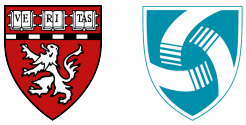
Table 6: Linear equation, coefficient of determination (r^2) and attributed internal standard



Internal standards	Recovery (%) in plasma	Interference (% signal remaining)	LOD 5500 (pg) in phase	LOD 5500 (pg) in plasma	LOD 6500 (pg) in phase	LOD 6500 (pg) in plasma
d_5-RvD2	73 ± 16	28 ± 9	0.17	0.43	0.04	0.09
d_5-LXA_4	75 ± 2	7 ± 2	0.10	0.25	0.02	0.05
d_4-LTB_4	80 ± 3	18 ± 2	0.10	0.51	0.05	0.22
d_4-PGE_2	63 ± 13	21 ± 6	0.19	0.47	0.09	0.23
d_8-5S-HETE	68 ± 3	28 ± 5	0.10	0.26	0.07	0.15
d_5-authentic lipid mediators						
d_5-10S,17S-diHDHA	88 ± 4	8 ± 2	0.06	0.45	0.03	0.15
d_5-5S,15S-diHEPE	94 ± 6	9 ± 5	0.13	0.95	0.03	0.15
d_5-15-HEPE	63 ± 12	26 ± 4	0.09	0.23	0.10	0.19

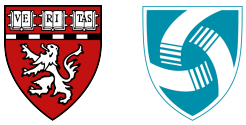
Table 7: Determination of recovery, interference and LOD. Plasma recoveries, interference and limit of detection (LOD) of the internal standards and authentic d_5-lipid mediators used as representative of specific chromatographic regions and physical properties of compounds. LOD was assessed in phase and in the matrix. Mean ± SEM, n = 6 for recoveries and interference, d = 3 for LOD.

Lipid Mediator Metabololipidomics
LC-MS-MS workup



Compound name	Nominal concentration (pg)	Intraday (repeatability)			Interday (reproducibility)		
		Measured (pg)	%RSD	Accuracy	Measured (pg)	%RSD	Accuracy
RvD1	5	5.0 ± 0.1	1%	99%	4.6 ± 0.1	2%	92%
	20	17.2 ± 0.7	7%	86%	23.2 ± 0.7	5%	84%
	100	109.8 ± 2.2	4%	90%	118.5 ± 1.8	3%	82%
	200	227.8 ± 2.5	2%	86%	233.5 ± 1.8	2%	83%
RvD2	5	5.2 ± 0.1	4%	96%	4.6 ± 0.1	4%	93%
	20	16.9 ± 0.7	7%	85%	22.6 ± 0.5	4%	87%
	100	113.0 ± 2.3	4%	87%	116.5 ± 1.6	3%	84%
	200	227.8 ± 3.1	3%	86%	235.5 ± 2.5	2%	82%
RvD3	5	5.7 ± 0.1	3%	86%	5.4 ± 0.1	4%	91%
	20	19.8 ± 0.5	4%	99%	26.0 ± 0.3	2%	70%
	100	118.3 ± 1.4	2%	82%	125.3 ± 0.9	2%	75%
	200	226.8 ± 2.2	2%	87%	226.8 ± 1.7	1%	87%
RvD5	5	4.8 ± 0.1	5%	97%	4.6 ± 0.04	2%	93%
	20	16.7 ± 0.6	6%	83%	24.0 ± 0.3	2%	80%
	100	114.3 ± 0.8	1%	86%	117.0 ± 0.0	0%	83%
	200	217.0 ± 2.6	2%	92%	217.0 ± 2.4	2%	92%
PD1	5	4.6 ± 0.1	5%	93%	4.4 ± 0.05	2%	88%
	20	16.7 ± 0.3	3%	84%	23.7 ± 0.5	4%	82%
	100	114.0 ± 0.7	1%	86%	118.5 ± 1.0	2%	82%
	200	218.5 ± 1.3	1%	91%	220.0 ± 1.3	1%	90%
MaR1	5	4.6 ± 0.2	9%	91%	4.2 ± 0.02	1%	85%
	20	16.1 ± 0.1	1%	81%	22.3 ± 0.5	4%	89%
	100	107.0 ± 3.5	7%	93%	110.8 ± 1.4	3%	89%
	200	221.3 ± 2.3	2%	89%	229.0 ± 1.8	2%	86%
17-HDHA	5	3.5 ± 0.1	4%	69%	4.4 ± 0.05	2%	87%
	20	17.8 ± 0.3	3%	89%	23.7 ± 0.8	6%	82%
	100	115.0 ± 1.1	2%	85%	120.0 ± 0.6	1%	80%
	200	222.0 ± 1.2	1%	89%	227.5 ± 1.6	1%	86%
14-HDHA	5	3.2 ± 0.1	5%	64%	4.3 ± 0.02	1%	86%
	20	17.3 ± 0.4	4%	86%	22.2 ± 0.4	3%	89%
	100	114.8 ± 2.4	4%	85%	109.5 ± 2.3	4%	91%
	200	212.3 ± 2.3	2%	94%	233.5 ± 0.9	1%	83%
7-HDHA	5	3.5 ± 0.1	4%	70%	4.5 ± 0.1	3%	90%
	20	19.7 ± 0.1	1%	99%	25.8 ± 0.4	3%	71%
	100	123.8 ± 2.3	4%	76%	128.0 ± 2.5	4%	72%
	200	236.5 ± 1.0	1%	82%	240.5 ± 2.4	2%	80%
4-HDHA	5	3.6 ± 0.1	6%	73%	4.6 ± 0.02	1%	91%
	20	19.8 ± 0.3	2%	99%	26.0 ± 0.3	2%	70%
	100	125.8 ± 2.0	3%	74%	128.5 ± 1.6	2%	72%
	200	236.3 ± 2.1	2%	82%	240.3 ± 1.4	1%	80%

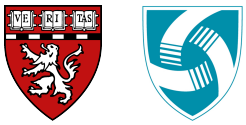
Table 8 (part 1/4): Precision and accuracy of LM metabololipidomics. %RSD, relative standard deviation; %RSD = (SEM/mean) x 100; mean ± SEM; n = 4



Compound name	Nominal concentration (pg)	Intraday (repeatability) Measured (pg)	%RSD	Accuracy	Interday (reproducibility) Measured (pg)	%RSD	Accuracy
LXA_5	5	5.6 ± 0.1	2%	87%	5.0 ± 0.1	4%	99%
	20	18.0 ± 0.3	3%	90%	21.6 ± 0.7	6%	92%
	100	111.0 ± 2.6	5%	89%	110.3 ± 1.9	3%	90%
	200	212.3 ± 2.8	3%	94%	240.3 ± 3.3	3%	80%
RvE1	5	5.8 ± 0.1	4%	85%	5.1 ± 0.1	3%	99%
	20	17.4 ± 0.1	1%	87%	24.3 ± 0.3	2%	78%
	100	113.3 ± 2.3	4%	87%	121.8 ± 1.7	3%	78%
	200	242.0 ± 3.7	3%	79%	241.8 ± 1.7	1%	79%
RvE2	5	5.5 ± 0.4	14%	91%	4.7 ± 0.1	3%	93%
	20	16.4 ± 0.7	8%	82%	20.7 ± 0.7	6%	97%
	100	108.8 ± 2.8	5%	91%	101.2 ± 2.9	6%	99%
	200	199.3 ± 3.3	3%	100%	240.0 ± 1.1	1%	80%
RvE3	5	6.1 ± 0.3	8%	78%	5.0 ± 0.4	16%	99%
	20	17.8 ± 1.7	17%	89%	23.7 ± 1.6	12%	81%
	100	105.0 ± 3.0	6%	95%	116.5 ± 1.0	2%	84%
	200	218.5 ± 3.4	3%	91%	227.8 ± 3.1	3%	86%
18-HEPE	5	4.1 ± 0.1	3%	82%	4.3 ± 0.03	2%	87%
	20	17.6 ± 0.2	2%	88%	23.3 ± 0.5	4%	83%
	100	117.5 ± 2.0	3%	83%	121.3 ± 1.5	2%	79%
	200	239.0 ± 2.3	2%	81%	241.8 ± 1.5	1%	79%
15-HEPE	5	4.3 ± 0.1	3%	87%	4.4 ± 0.04	2%	88%
	20	17.6 ± 0.2	2%	88%	22.8 ± 0.5	4%	86%
	100	115.5 ± 2.5	4%	85%	122.3 ± 1.1	2%	78%
	200	231.3 ± 1.7	1%	84%	239.3 ± 1.7	1%	80%
12-HEPE	5	4.6 ± 0.04	2%	93%	4.4 ± 0.1	3%	88%
	20	18.1 ± 0.2	1%	91%	22.6 ± 0.4	3%	87%
	100	122.5 ± 1.3	2%	78%	117.3 ± 1.7	3%	83%
	200	231.3 ± 2.3	2%	84%	227.8 ± 1.3	1%	86%
5-HEPE	5	4.6 ± 0.05	2%	91%	4.6 ± 0.04	2%	92%
	20	18.1 ± 0.2	2%	91%	23.4 ± 0.5	4%	83%
	100	115.3 ± 1.3	2%	85%	119.5 ± 0.5	1%	81%
	200	223.5 ± 2.0	2%	88%	233.3 ± 1.0	1%	83%

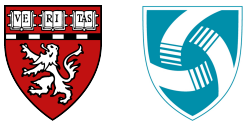
Table 8 (part 2/4): Precision and accuracy of LM metabololipidomics. %RSD, relative standard deviation; %RSD = (SEM/mean) x 100; mean ± SEM; n = 4

Lipid Mediator Metabololipidomics LC-MS-MS workup



Compound name	Nominal concentration (pg)	Intraday (repeatability) Measured (pg)	%RSD	Accuracy	Interday (reproducibility) Measured (pg)	%RSD	Accuracy
LXA_4	5	5.5 ± 0.1	2%	90%	4.9 ± 0.1	4%	98%
	20	17.7 ± 0.8	8%	88%	23.5 ± 0.1	1%	82%
	100	114.0 ± 2.1	4%	86%	119.0 ± 1.1	2%	81%
	200	226.3 ± 2.1	2%	87%	233.5 ± 1.5	1%	83%
LXB_4	5	6.2 ± 0.1	4%	76%	5.4 ± 0.1	3%	92%
	20	18.4 ± 0.6	6%	92%	22.9 ± 0.4	3%	85%
	100	116.8 ± 1.7	3%	83%	114.5 ± 1.2	2%	86%
	200	220.0 ± 4.5	4%	90%	231.8 ± 1.6	1%	84%
5S,15S-diHETE	5	5.1 ± 0.1	4%	98%	5.4 ± 0.1	2%	93%
	20	20.3 ± 0.5	4%	98%	26.4 ± 0.2	1%	68%
	100	123.3 ± 1.4	2%	77%	129.0 ± 2.0	3%	71%
	200	246.5 ± 3.7	3%	77%	251.0 ± 6.1	5%	75%
LTB_4	5	5.0 ± 0.03	1%	99%	5.8 ± 0.1	2%	84%
	20	22.3 ± 0.3	2%	89%	27.5 ± 0.4	3%	63%
	100	125.0 ± 0.6	1%	75%	130.0 ± 1.8	3%	70%
	200	231.0 ± 3.2	3%	85%	226.8 ± 2.9	3%	87%
20-OH-LTB_4	5	5.4 ± 0.1	4%	93%	4.9 ± 0.03	1%	98%
	20	17.1 ± 0.5	6%	86%	22.6 ± 0.4	3%	87%
	100	111.3 ± 2.7	5%	89%	116.0 ± 0.8	1%	84%
	200	223.3 ± 1.1	1%	88%	229.0 ± 3.0	3%	86%
20-COOH-LTB_4	5	5.1 ± 0.4	16%	99%	5.2 ± 0.1	2%	97%
	20	18.0 ± 0.9	9%	90%	21.4 ± 1.6	13%	93%
	100	117.5 ± 1.3	2%	83%	114.0 ± 2.2	4%	86%
	200	227.3 ± 2.3	2%	86%	253.8 ± 3.4	3%	73%
PGD_2	5	5.7 ± 0.1	2%	87%	5.2 ± 0.1	4%	96%
	20	17.1 ± 0.2	2%	86%	22.8 ± 0.3	3%	86%
	100	105.5 ± 2.3	4%	95%	115.5 ± 1.0	2%	85%
	200	213.3 ± 3.1	3%	93%	218.3 ± 3.7	3%	91%
PGE_2	5	5.5 ± 0.1	4%	90%	4.9 ± 0.2	6%	97%
	20	18.0 ± 0.5	4%	90%	22.9 ± 0.9	7%	86%
	100	116.8 ± 4.0	7%	83%	113.8 ± 2.3	4%	86%
	200	206.5 ± 1.2	1%	97%	209.5 ± 4.6	4%	95%
$PGF_{2\alpha}$	5	5.5 ± 0.1	2%	90%	5.2 ± 0.1	3%	97%
	20	19.2 ± 0.5	4%	96%	25.6 ± 0.4	3%	72%
	100	120.3 ± 1.7	3%	80%	123.8 ± 1.1	2%	76%
	200	233.3 ± 3.1	3%	83%	220.8 ± 0.9	1%	90%
TXB_2	5	4.7 ± 0.2	7%	94%	5.0 ± 0.01	0.3%	100%
	20	16.8 ± 0.8	8%	84%	22.2 ± 1.3	10%	89%
	100	111.2 ± 4.1	7%	89%	118.0 ± 4.5	8%	82%
	200	219.0 ± 3.6	3%	91%	189.5 ± 6.9	7%	95%

Table 8 (part 3/4): Precision and accuracy of LM metabololipidomics. %RSD, relative standard deviation; %RSD = (SEM/mean) x 100; mean ± SEM; n = 4



Compound name	Nominal concentration (pg)	Intraday (repeatability) Measured (pg)	%RSD	Accuracy	Interday (reproducibility) Measured (pg)	%RSD	Accuracy
15-HETE	5	4.1 ± 0.1	2%	82%	4.2 ± 0.05	2%	84%
	20	16.6 ± 0.2	2%	83%	21.8 ± 0.4	3%	91%
	100	109.3 ± 2.6	5%	91%	110.8 ± 1.3	2%	89%
	200	212.8 ± 2.3	2%	94%	219.0 ± 0.8	1%	91%
12-HETE	5	4.5 ± 0.04	2%	89%	4.8 ± 0.04	2%	95%
	20	17.7 ± 0.3	2%	89%	23.1 ± 0.6	4%	85%
	100	113.8 ± 1.8	3%	86%	117.0 ± 1.1	2%	83%
	200	216.5 ± 1.8	2%	92%	220.0 ± 1.7	2%	90%
5-HETE	5	4.9 ± 0.05	2%	97%	5.0 ± 0.1	4%	100%
	20	20.3 ± 0.3	3%	98%	25.7 ± 0.3	2%	72%
	100	125.0 ± 3.0	5%	75%	127.8 ± 1.4	2%	72%
	200	232.5 ± 1.4	1%	84%	239.5 ± 4.7	4%	80%

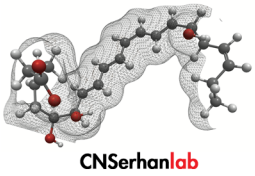
Table 8 (part 4/4): Precision and accuracy of LM metabololipidomics. %RSD, relative standard deviation; %RSD = (SEM/mean) x 100; mean ± SEM; n = 4

Prof. Charles N. SERHAN Laboratory
Brigham & Women's Hospital
Center for Experimental Therapeutics
and Reperfusion Injury
Harvard Medical School

Lipid Mediator Metabololipidomics LC-MS-MS workup

	Day-0	Day-1	Day-2	Day-3	Day-4	Day-5	2-weeks	4-weeks
DHA bioactive metabolome		Δ change (%)						
RvD1	49.5±1.3							
with		-1.0	-0.2	+3.2	-3.2	-1.1		
without		+0.8	-2.0	+2.4	-13.2	-15.5	-33.4	-56.8
RvD2	13.4±0.8							
with		-1.3	+1.1	+6.4	+2.3	+1.1		
without		-9.2	+2.5	+2.2	-1.2	-19.2	-39.5	-59.0
RvD3	68.5±7.5							
with		-3.1	+8.0	+6.6	-4.4	+6.0		
without		-6.8	-8.5	-3.3	-11.3	-12.6	-18.9	-25.4
RvD5	24.9±2.2							
with		+6.5	-10.5	-18.0	-8.4	-16.8		
without		-16.4	-14.8	-15.1	-20.2	-17.0	-38.7	-44.0
RvD6	21.6±2.0							
with		+1.7	+2.2	-1.1	+0.4	-11.8		
without		+4.6	-9.8	-12.5	-17.2	-14.3	-19.5	-85.3
PD1	6.1±0.1							
with		-3.0	+5.5	-8.3	-11.9	-10.4		
without		+6.7	+1.7	-18.3	-26.8	-23.7	-33.0	-36.7
MaR1	9.0±0.8							
with		+2.3	+0.9	-8.0	-6.5	-17.8		
without		-6.5	-2.5	-2.6	-15.7	-24.7	-67.9	-73.1
EPA bioactive metabolome								
RvE1	13.1±0.9							
with		-3.3	-9.0	-13.9	-9.4	-13.1		
without		+7.6	-4.2	-22.0	-23.7	-24.6	-50.7	-57.1
RvE3	686.3±51.0							
with		-6.4	-7.6	-5.1	-7.2	-4.4		
without		-1.3	-5.2	-4.5	-3.5	-18.7	-40.9	-42.2
AA bioactive metabolome								
LXA_4	11.2±1.0							
with		1.8	+0.5	-8.6	-8.3	-5.9		
without		4.4	-3.8	-5.2	-5.7	-3.8	-14.4	-39.9
LXB_4	117.6±2.6							
with		-1.7	-3.6	-1.9	+5.2	-5.5		
without		-1.3	-5.8	-3.6	-5.8	-11.3	-17.7	-57.1
PGD_2	55.1±1.9							
with		+9.8	-6.8	+3.3	+3.7	-15.5		
without		+7.6	-2.8	+5.8	+2.1	-16.9	-10.9	-26.7
PGE_2	199.2±10.2							
with		+3.7	-1.2	+0.1	-11.8	-15.6		
without		-6.2	-12.2	-14.3	-20.8	-15.3	-11.9	-29.8
PGF_{2a}	143.3±2.5							
with		-0.4	-0.2	+1.2	+8.9	-13.6		
without		+8.0	+1.1	+1.6	+4.8	-9.0	-15.6	-34.1
TxB_2	6067.0±128.4							
with		-5.2	-7.8	+7.4	-5.5	-12.5		
without		+9.2	-3.2	+7.3	-16.6	-19.2	-17.8	-34.2

Table 9: Human serum (250µl) was stored under -30°C with or without 1ml methanol. On the designated day (Day-0 to 4-weeks), LC-MS-MS based LM metabololipidomics were carried out. Results are expressed as pg/ml serum; mean ± SEM of d = 3 determinations and Δ change (%) from Day-0.



CETRI
Center for Experimental Therapeutics
and Reperfusion Injury

CNSerhanlab

Multiple Reaction Monitoring Transitions

Lipid Mediator Metabololipidomics LC-MS-MS workup

Compound	Q1	Q3	Compound	Q1	Q3
AA	303.3	259.1	15-HEPE	317.2	219.1
PGD_2	351.3	233.2	18-HEPE	317.2	259.1
PGE_2	351.3	175.1			
$PGF_{2\alpha}$	353.3	193.1	DHA	327.3	283.1
TXB_2	369.3	169.1	RvD1	375.2	215.2
LTB_4	335.3	195.2	RvD2	375.2	141.2
LXA_4	351.2	235.1	RvD3	375.2	147.1
LXB_4	351.2	221.1	RvD5	359.2	199.1
5,15-diHETE	335.2	115.2	RvD6	359.2	159.1
5-HETE	319.2	115.1	MaR1	359.2	250.1
12-HETE	319.2	179.1	4,14-diHDHA	359.2	221.1
15-HETE	319.2	219.1	PD1	359.2	153.1
			4-HDHA	343.2	101.1
EPA	301.3	257.1	7-HDHA	343.2	141.1
LXA_5	349.2	215.1	14-HDHA	343.2	205.1
LXB_5	349.2	221.2	17-HDHA	343.2	245.1
5,15-diHEPE	333.2	235.2			
RvE1	349.2	195.1	d_4-PGE_2	355.3	193.2
RvE2	333.3	253.2	d_5-LXA_4	356.3	115.2
RvE3	333.3	201.1	d_4-LTB_4	339.3	197.2
5-HEPE	317.2	115.1	d_8-5S-HETE	327.3	116.1
12-HEPE	317.2	179.1	d_5-RvD2	380.2	141.2

Table 1: MRM transition for LC-MS-MS based metabololipidomics of AA, EPA and DHA derived lipid mediators.

Compound	Q1	Q3	Compound	Q1	Q3
AT-LXA_4	351.2	235.1	AT-RvD3	375.2	147.1
AT-LXB_4	351.2	221.1	AT-RvD5	359.2	199.1
			AT-RvD6	359.2	159.1
AT-RvD1	375.2	215.2			
AT-RvD2	375.2	141.2	AT-PD1	359.2	153.1

Table 2: MRM transition for LC-MS-MS based metabololipidomics of AA and DHA derived aspirin-triggered lipid mediators.

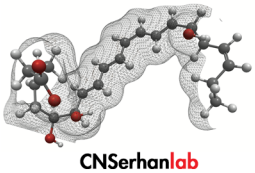
Compound	Q1	Q3	Compound	Q1	Q3
20-OH-LTB_4	351.2	195.1	Dihydro-RvD1	377.2	279.1
20-COOH-LTB_4	365.2	195.1	7-oxo-RvD2	373.2	260.1
15-oxo-LXA_4	349.2	233.1	16-oxo-RvD2	373.2	260.1
13,14-dihydro-LXA_4	353.2	253.1	22-OH-RvD2	391.2	249.1
5-oxo-LXB_4	349.2	219.1	Dihydro-RvD2	377.2	279.1
			14-oxo-MaR1	357.2	221.1
18-oxo-RvE1	347.2	213.1	7-oxo-MaR1	357.2	219.1
19-OH-RvE1	365.2	205.1	22-OH-MaR1	375.2	177.1
20-OH-RvE1	365.2	291.1	22-COOH-MaR1	389.2	177.1
20-COOH-RvE1	379.2	237.1	Dihydro-MaR1	361.2	223.1
Dihydro-RvE1	351.2	225.1	22-OH-PD1	375.2	153.1
			22-COOH-PD1	389.2	153.1
8-oxo-RvD1	373.2	231.1	Dihydro-PD1	361.2	263.1
17-oxo-RvD1	373.2	231.1			

Table 3: MRM transition for LC-MS-MS based metabololipidomics of further metabolites of AA, EPA and DHA derived lipid mediators.

Additional references:

Specific lipid mediator signatures of human phagocytes: microparticles stimulate macrophage efferocytosis and pro-resolving mediators. *Dalli J, Serhan CN.* **Blood**. 2012 120(15)

Metabolomics-lipidomics of eicosanoids and docosanoids generated by phagocytes. *Yang R, Chiang N, Oh SF, Serhan CN.* **Curr Protoc Immunol**. 2011 Chapter 14:Unit 14.26.



CETRI
Center for Experimental Therapeutics
and Reperfusion Injury

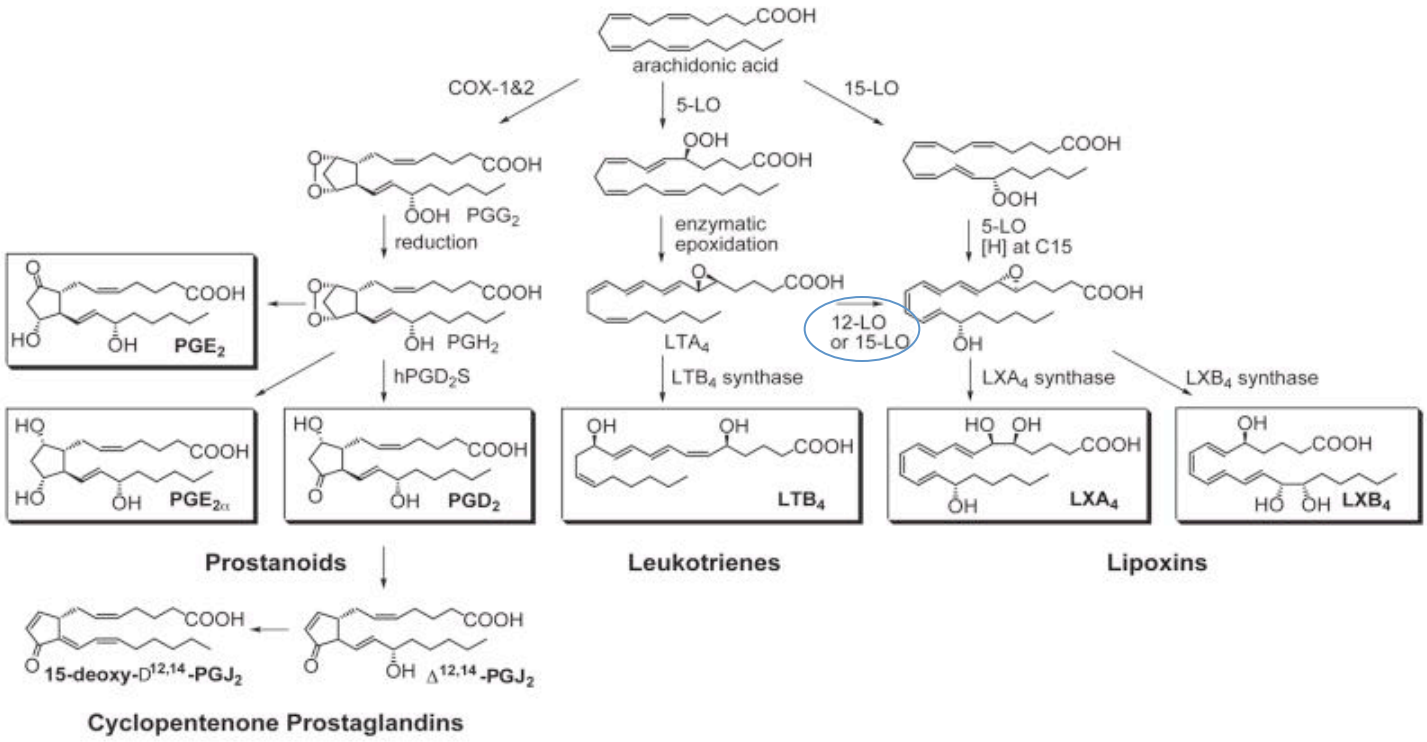
CNSerhanlab

Lipid Mediator Biosynthetic Pathways

Prof. Charles N. SERHAN Laboratory
Brigham & Women's Hospital
Center for Experimental Therapeutics
and Reperfusion Injury
Harvard Medical School

Lipid Mediator Metabololipidomics LC-MS-MS workup

Biosynthetic scheme for the Prostaglandins, Leukotrienes and Lipoxins



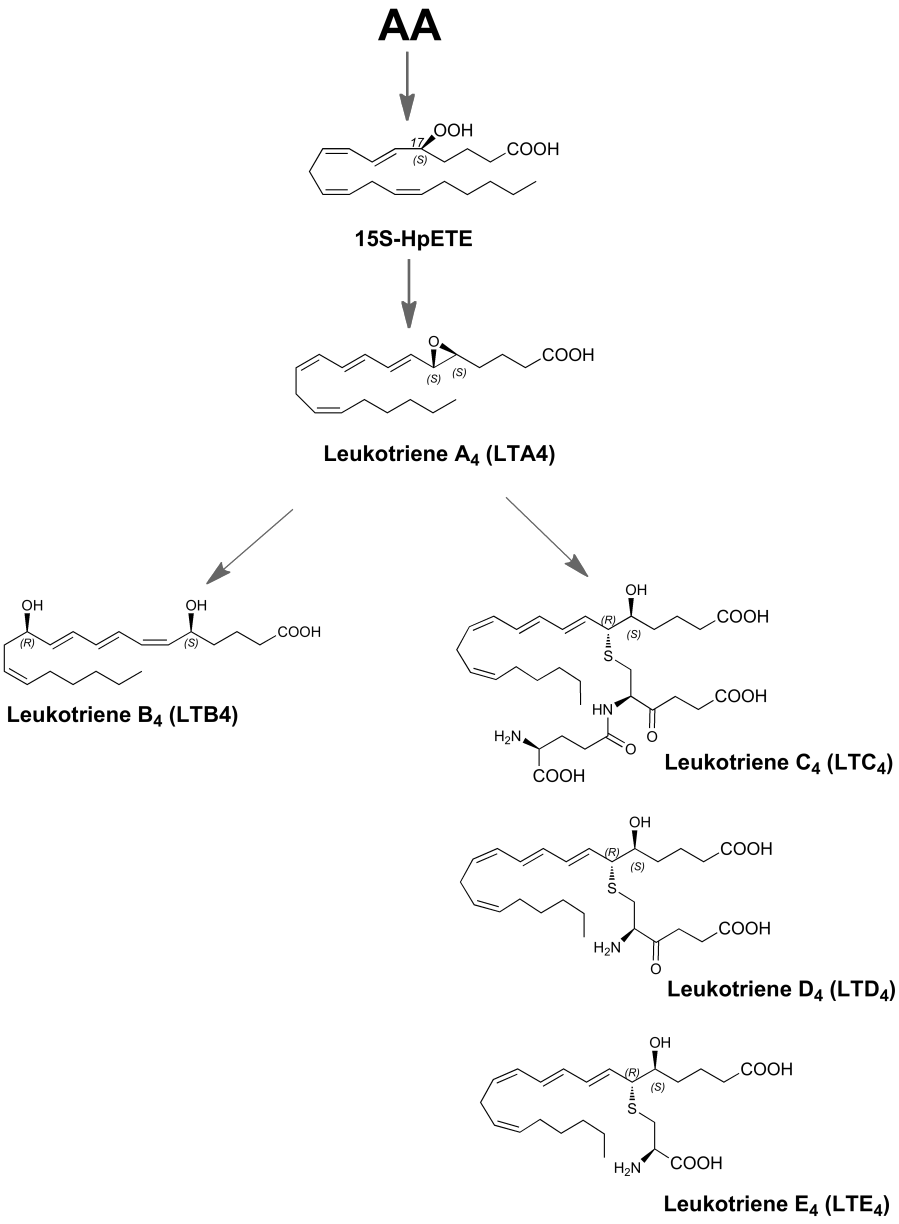
Key eicosanoids that play pivotal roles in initiating inflammation and its resolution.

◯ Circle denotes human lipoxygenases, in mice 12/15 LOX is a single enzyme NOT found in human tissue.

Anti-Inflammatory and Pro-Resolving Lipid Mediators. *Charles N. Serhan, Stephanie Yacoubian and Rong Yang* **Annu Rev Pathol.** 2008 3: 279–312. PMCID: PMC2739396

Lipid Mediator Metabololipidomics
LC-MS-MS workup

Biosynthetic scheme for the Leukotrienes

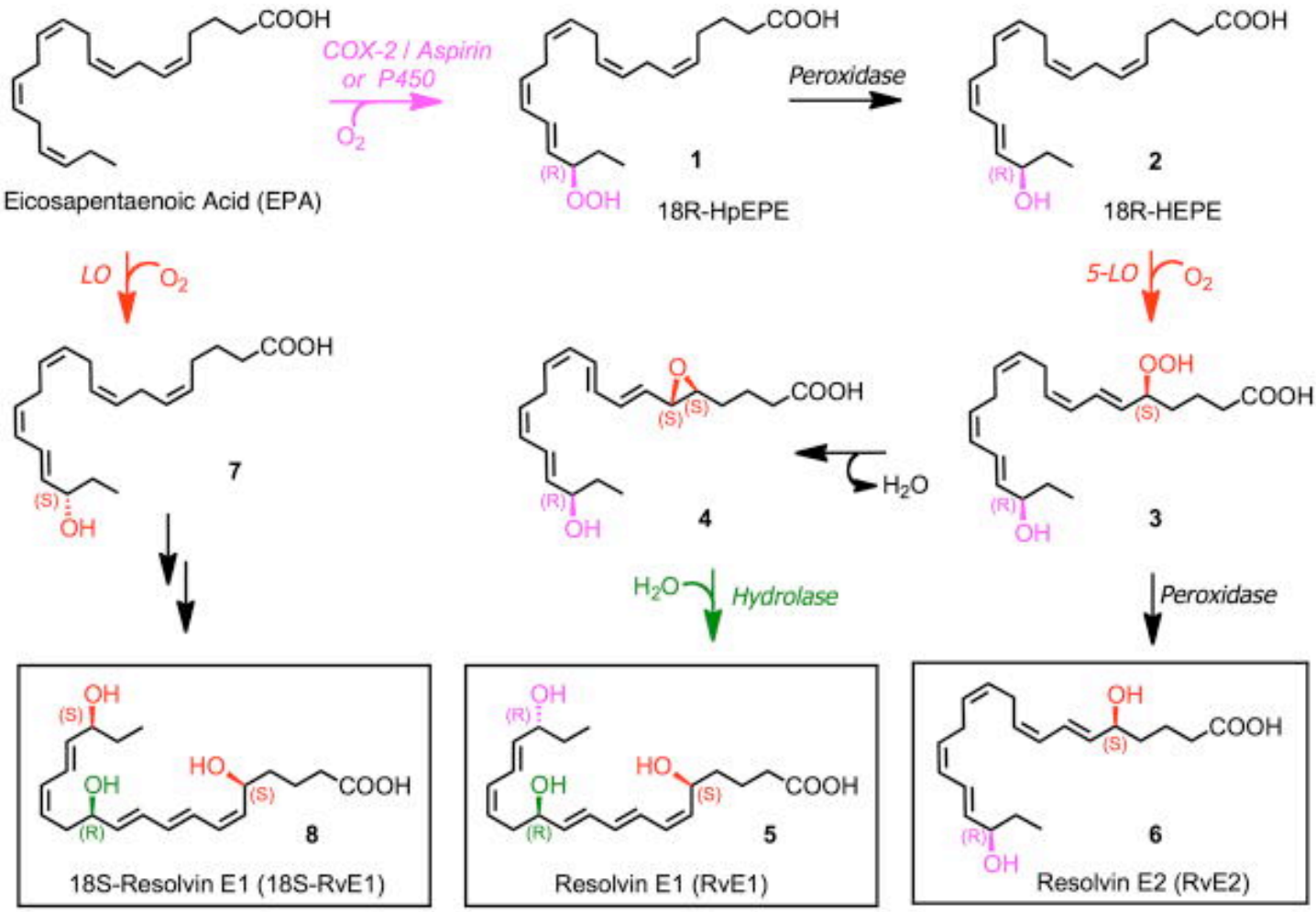


Leukotrienes and lipoxins: structures, biosynthesis, and biological effects. *Samuelsson B, Dahlén SE, Lindgren JA, Rouzer CA, Serhan CN* **Science.** 1987 237(4819):1171-6 PMID:2820055

Prof. Charles N. SERHAN Laboratory
Brigham & Women's Hospital
Center for Experimental Therapeutics
and Reperfusion Injury
Harvard Medical School

Lipid Mediator Metabololipidomics LC-MS-MS workup

Biosynthetic scheme for the E-series Resolvins



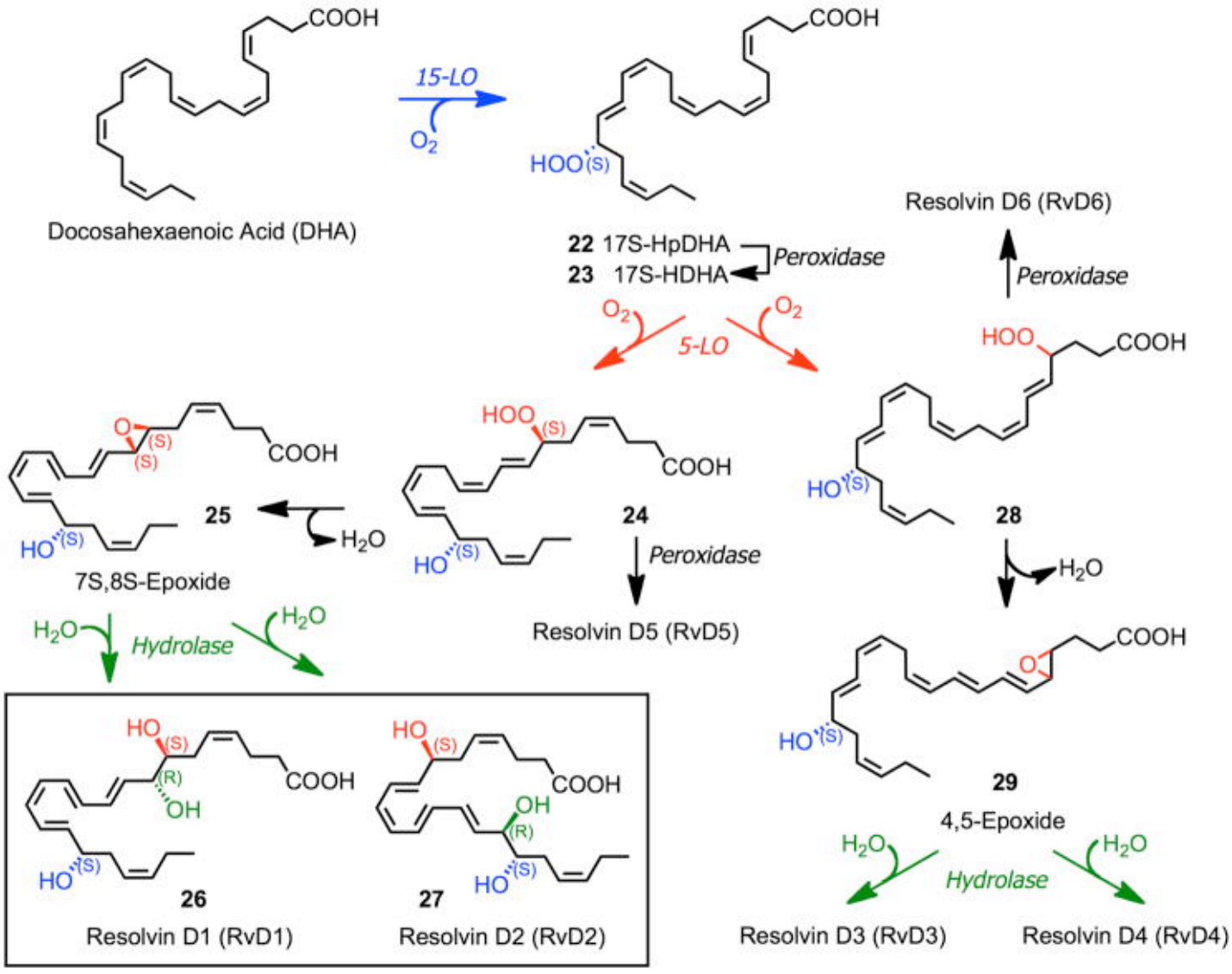
E-series resolvin biosynthesis and metabolic inactivation.

Resolvins and Protectins in Inflammation-Resolution *Charles N. Serhan, Nicos A. Petasis* Chem Rev. **2011** 12 111(10): 5922–5943. PMCID: PMC3192290

Prof. Charles N. SERHAN Laboratory
Brigham & Women's Hospital
Center for Experimental Therapeutics
and Reperfusion Injury
Harvard Medical School

Lipid Mediator Metabololipidomics
LC-MS-MS workup

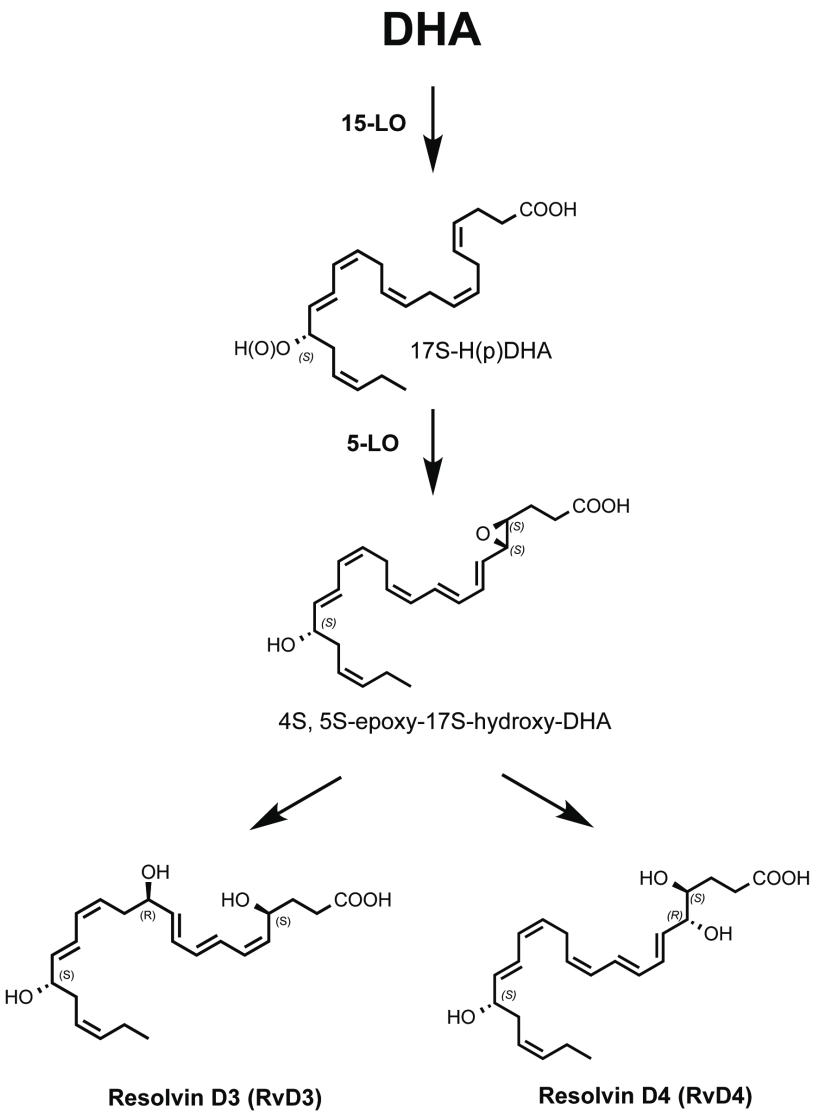
Biosynthetic scheme for the D-series Resolvins



Pathways and enzymes in the biosynthesis of D-series resolvins.

Resolvins and Protectins in Inflammation-Resolution *Charles N. Serhan, Nicos A. Petasis* Chem Rev. **2011** 12 111(10): 5922–5943. PMCID: PMC3192290

Biosynthetic scheme for the D-series Resolvins



Resolvin D3 and aspirin-triggered resolvin D3 are potent immunoresolvents *Dalli J, Winkler JW, Colas RA, Arnardottir H, Cheng CY, Chiang N, Petasis NA, Serhan CN* **Chem Biol. 2013** 20(2):188-201. PMCID: 23438748

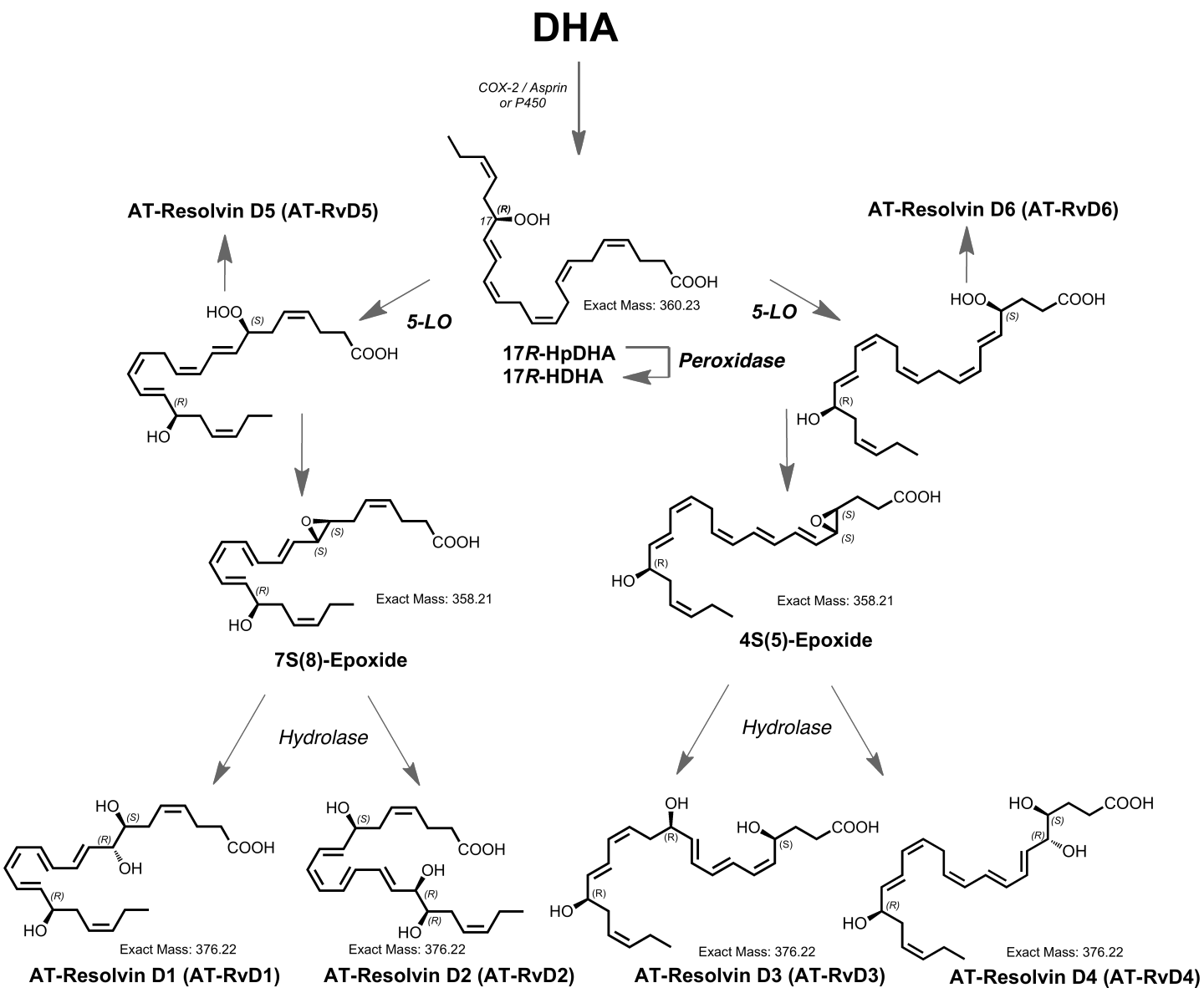
Resolvin D4 stereoassignment and its novel actions in host protection and bacterial clearance *Winkler JW, Orr SK, Dalli J, Cheng CY, Sanger JM, Chiang N, Petasis NA, Serhan CN* **Sci Rep. 2016** 6:18972. PMCID: 26743932

Resolvin D3 multi-level proresolving actions are host protective during infection *Paul C. Norris, Hildur Arnardottir, Julia M. Sanger, David Fichtner, Gregory S. Keyes, Charles N. Serhan* **PLEFA. 2016** In Press, Accepted Manuscript

Prof. Charles N. SERHAN Laboratory
Brigham & Women's Hospital
Center for Experimental Therapeutics
and Reperfusion Injury
Harvard Medical School

Lipid Mediator Metabololipidomics
LC-MS-MS workup

Biosynthetic scheme for the aspirin triggered D-series Resolvins



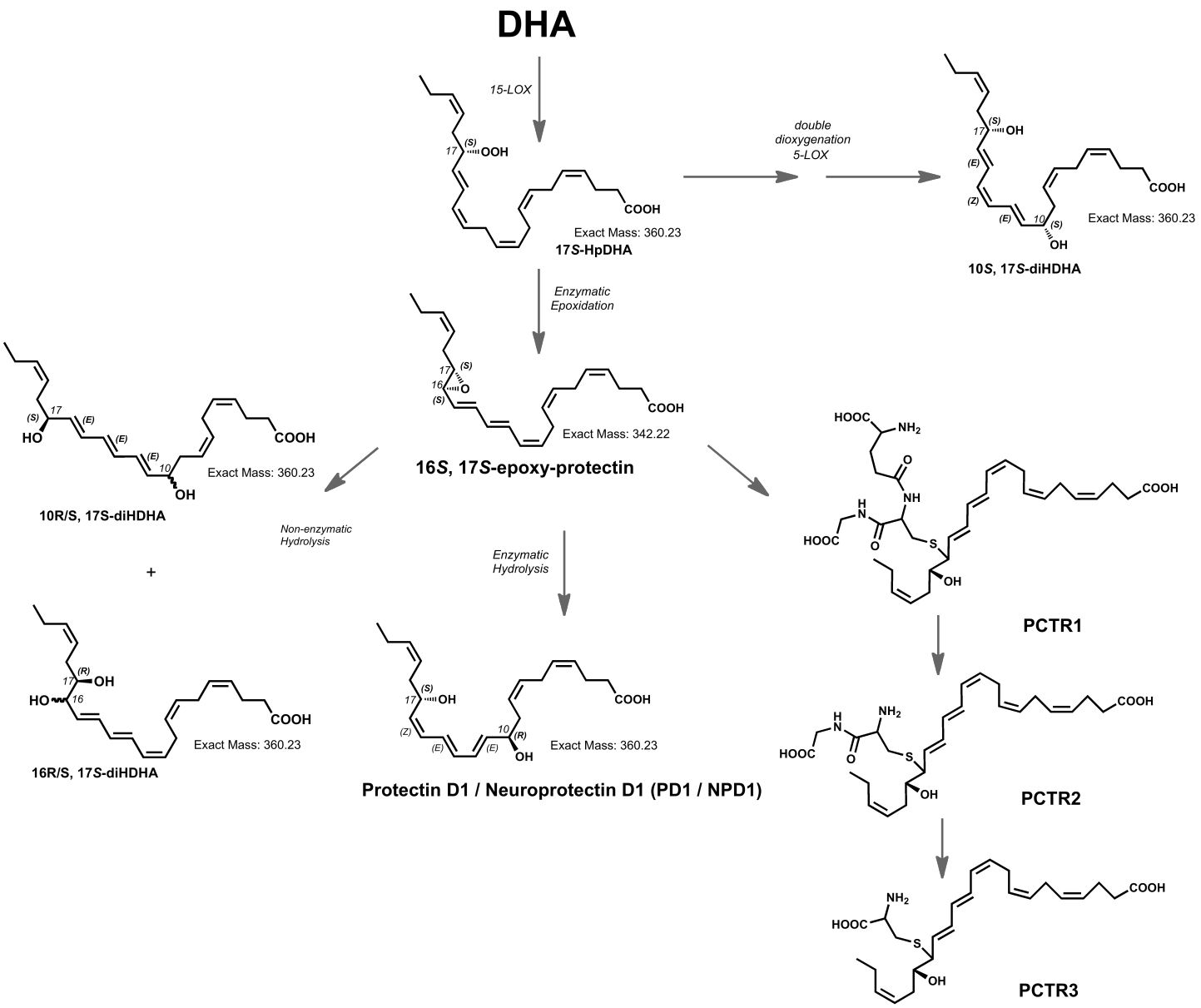
Pathways and enzymes involved in the biosynthesis of aspirin-triggered D-series resolvins.

Resolvins and Protectins in Inflammation-Resolution *Charles N. Serhan, Nicos A. Petasis* Chem Rev. **2011** 12 111(10): 5922–5943. PMCID: PMC3192290

Prof. Charles N. SERHAN Laboratory
Brigham & Women's Hospital
Center for Experimental Therapeutics
and Reperfusion Injury
Harvard Medical School

Lipid Mediator Metabololipidomics
LC-MS-MS workup

Biosynthetic scheme for the Protectins



Resolvins and Protectins in Inflammation-Resolution *Charles N. Serhan, Nicos A. Petasis* Chem Rev. **2011** 12 111(10): 5922–5943. PMCID: PMC3192290

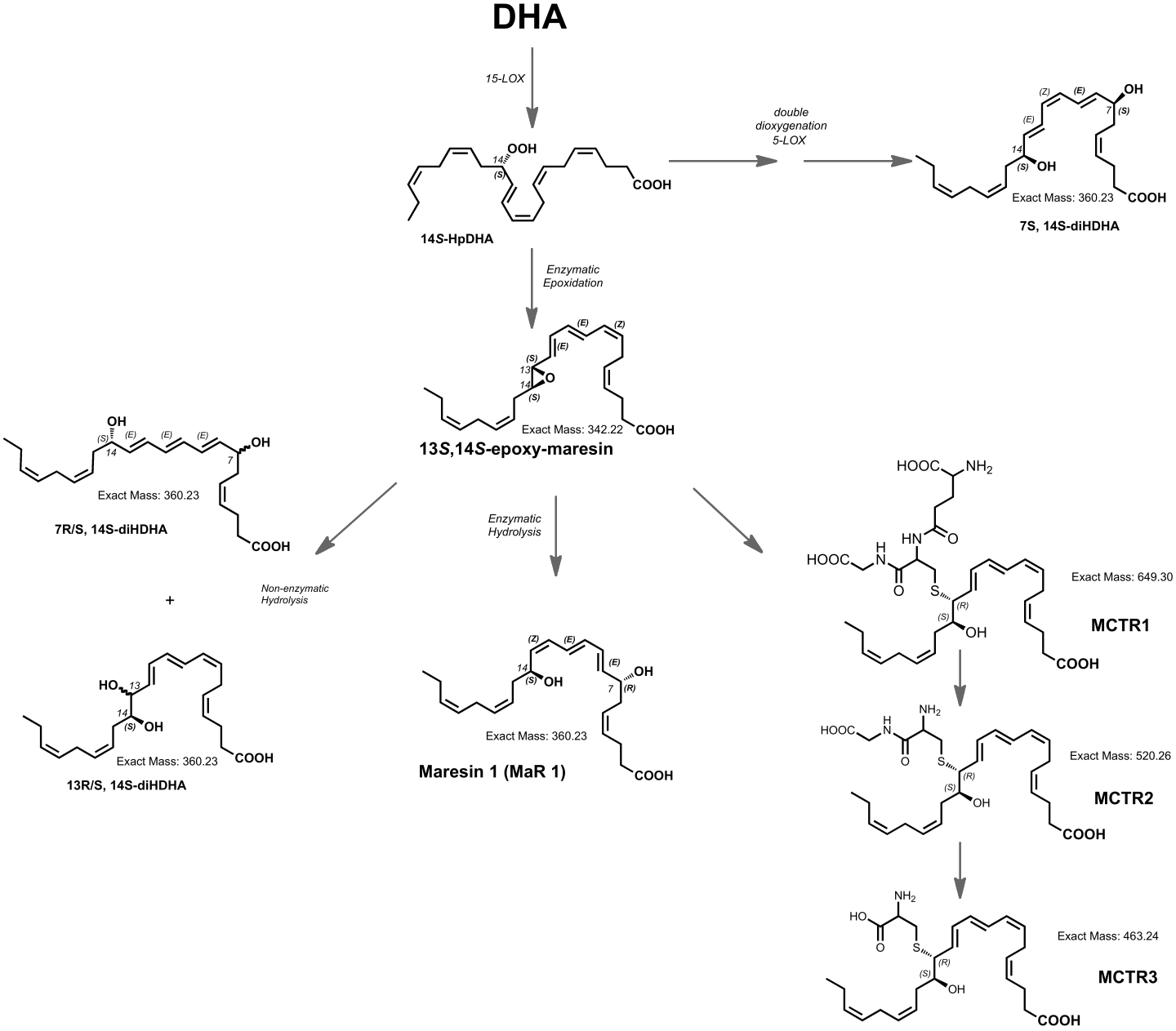
Novel proresolving and tissue-regenerative resolvin and protectin sulfido-conjugated pathways *Dalli J, Ramon S, Norris PC, Colas RA, Serhan CN* **FASEB J. 2015** 29(5):2120-36 PMCID: 25713027

Identification and Actions of A Novel Third Maresin Conjugate in Tissue Regeneration: MCTR3. *Dalli J, Sanger JM, Rodriguez AR, Chiang N, Spur BW, Serhan CN.* **PLOS ONE 2016**; in press.

Prof. Charles N. SERHAN Laboratory
Brigham & Women's Hospital
Center for Experimental Therapeutics
and Reperfusion Injury
Harvard Medical School

Lipid Mediator Metabololipidomics
LC-MS-MS workup

Biosynthetic scheme for the Maresins



Maresins: novel macrophage mediators with potent antiinflammatory and proresolving actions. *Serhan CN, Yang R, Martinod K, Kasuga K, Pillai PS, Porter TF, Oh SF, Spite M*. **J Exp Med**. 2009 206(1):15-23. PMCID: PMC2626672

Novel proresolving and tissue-regenerative resolvin and protectin sulfido-conjugated pathways *Dalli J, Ramon S, Norris PC, Colas RA, Serhan CN* **FASEB J. 2015** 29(5):2120-36 PMCID: 25713027

The Protectin PCTR1 enhances resolution of infectious inflammation and is produced by human M2 macrophages. *Ramon S, Dalli J, Sanger JM, Winkler JW, Aursnes M, Tungen JE, Hansen TV, Serhan CN.* **Am J Pathol. 2016**; in press.