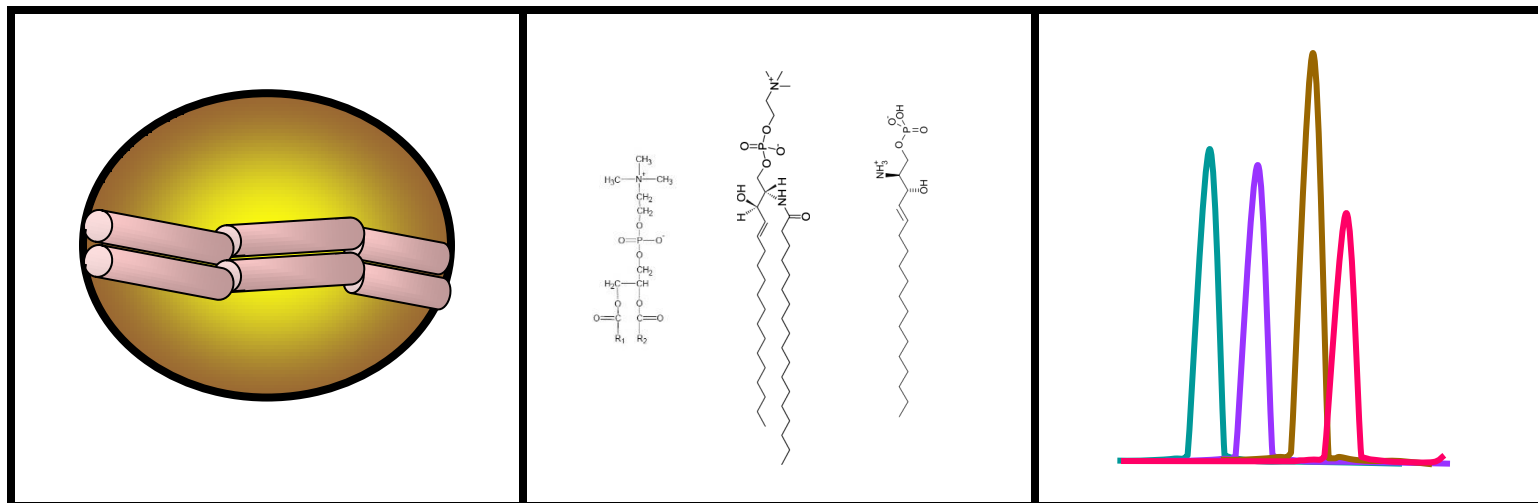# Using Skyline for Targeted Lipidomics
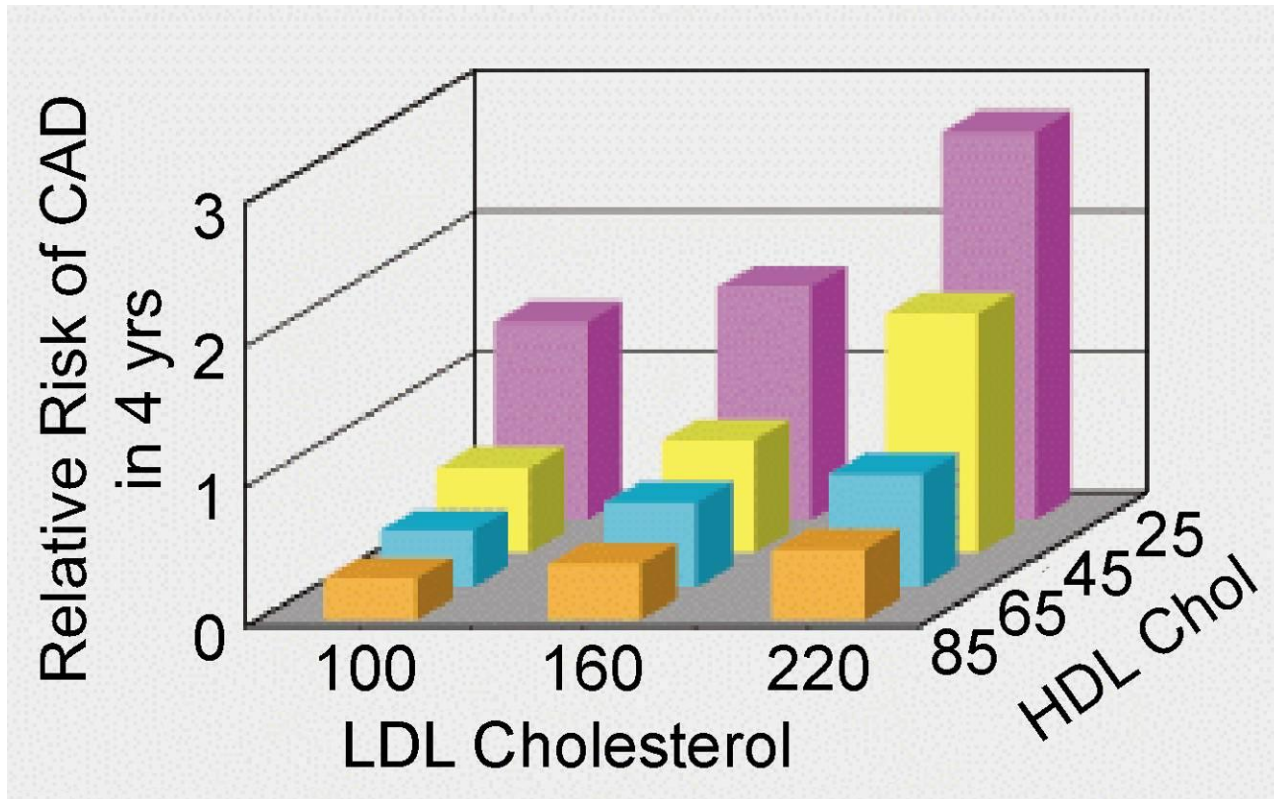

User's Group Meeting
June 9, 2013

Hari Nair, PhD
Andy Hoofnagle, MD PhD
University of Washington

HDL is the Good Cholesterol

The Framingham Heart Study

Castelli, *Can J Cardiol*, 1988

HDL Cholesterol is Not the Whole Story

Torcetrapib (ILLUMINATE)
   60% increase in HDL cholesterol
   40% increase in poor outcomes

Dalcetrapib (dal-OUTCOMES)
   35% increase in HDL cholesterol
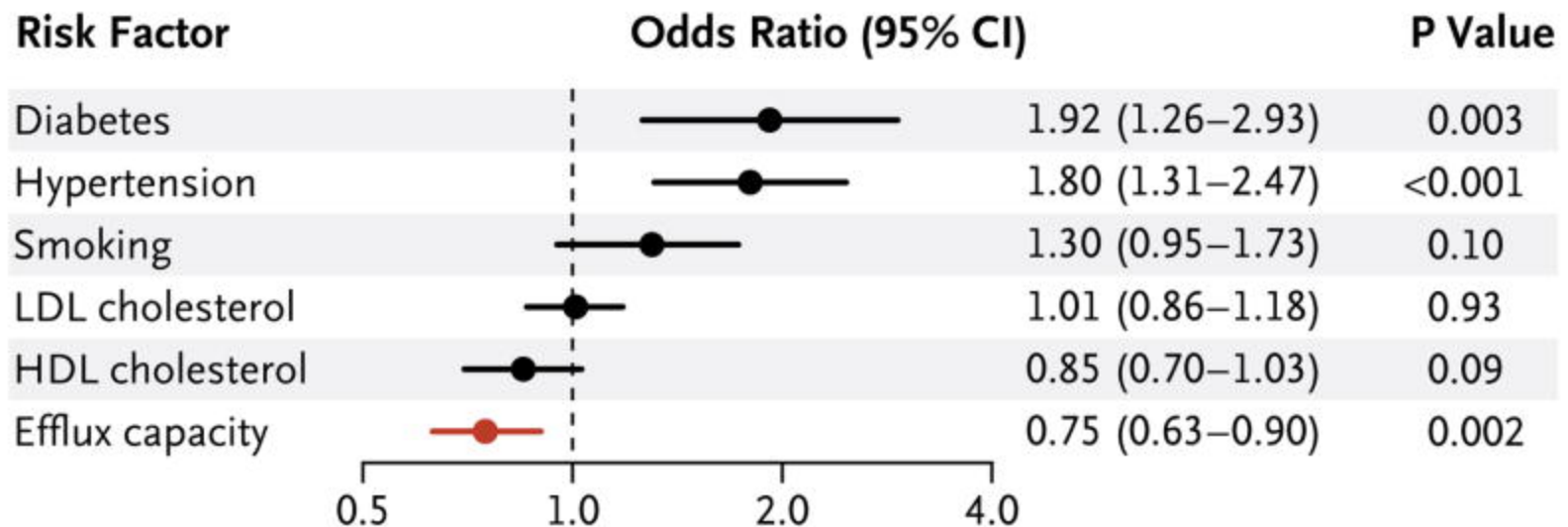   No improvement in outcomes

Niacin (AIM-HIGH)
   30% increase in HDL cholesterol
   Twice as many strokes

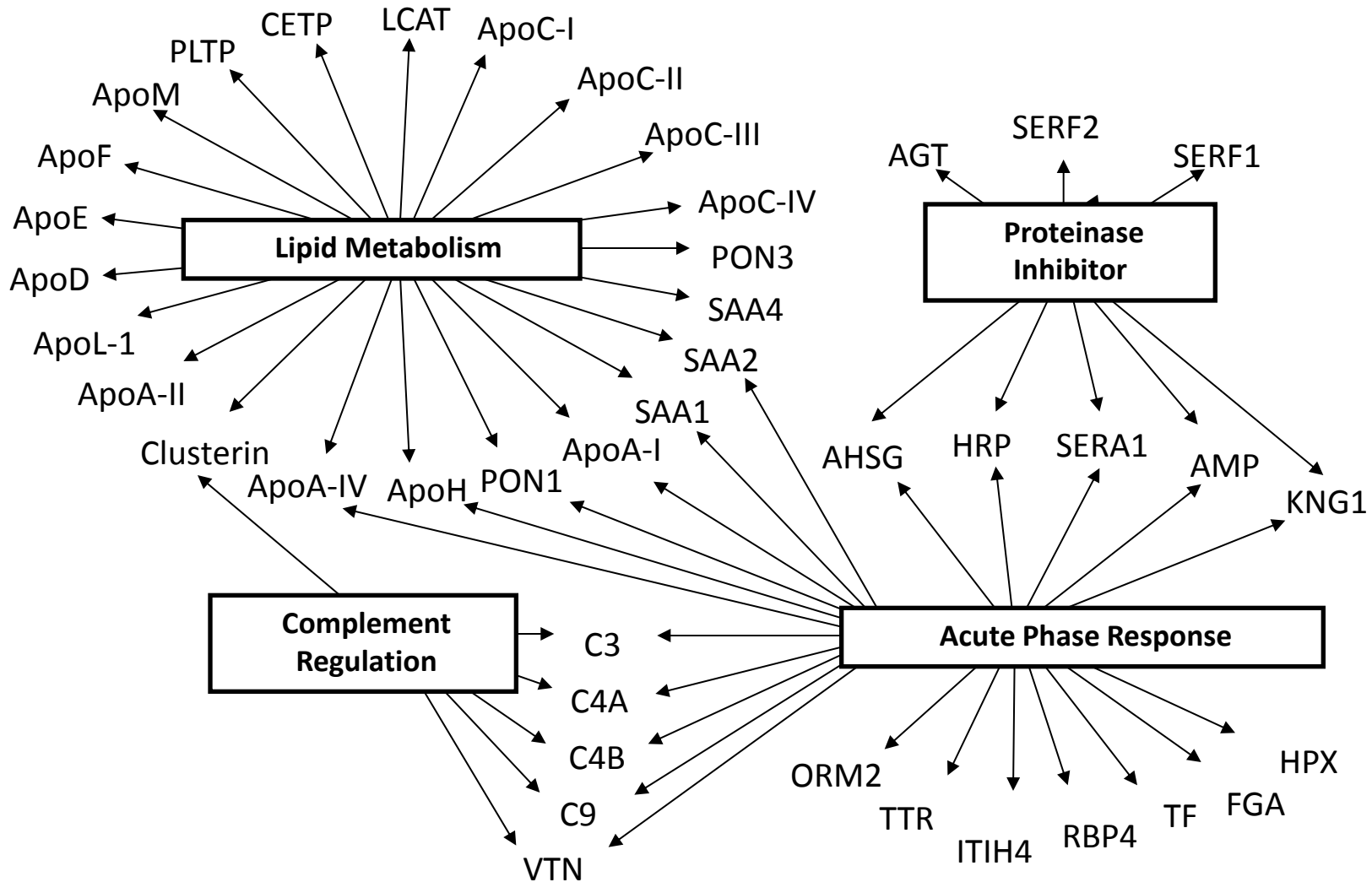*Three HDL-C raising studies terminated early*

HDL Does More than Just Carry Lipids
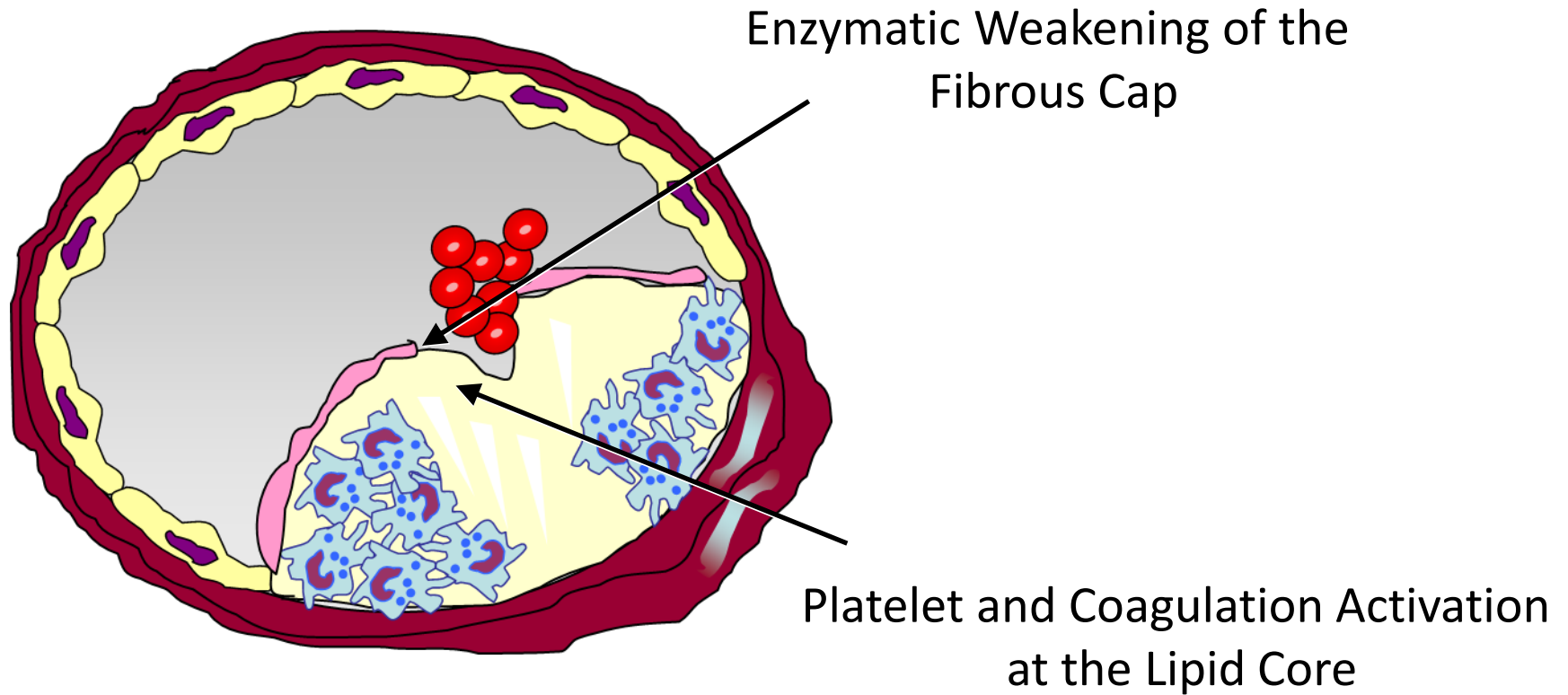## *Functional Assays of HDL*

Particles remove lipids from lipid-laden macrophages
HDL activity in vitro is predictive of coronary artery disease

Risk Factor	Odds Ratio (95% CI)	P Value
Diabetes	1.92 (1.26–2.93)	0.003
Hypertension	1.80 (1.31–2.47)	<0.001
Smoking	1.30 (0.95–1.73)	0.10
LDL cholesterol	1.01 (0.86–1.18)	0.93
HDL cholesterol	0.85 (0.70–1.03)	0.09
Efflux capacity	0.75 (0.63–0.90)	0.002

Khera, NEJM, 2011

The HDL Proteome is Vast

Vaisar, JCI, 2007

Thrombotic Occlusion of Coronary Artery

Enzymatic Weakening of the Fibrous Cap
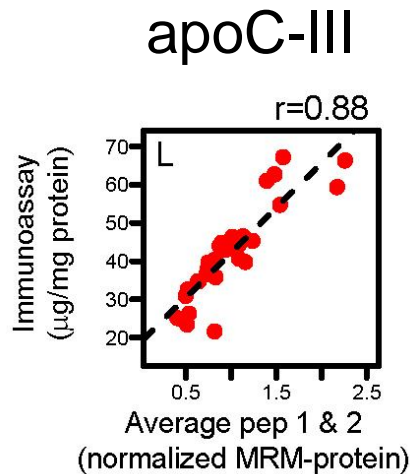
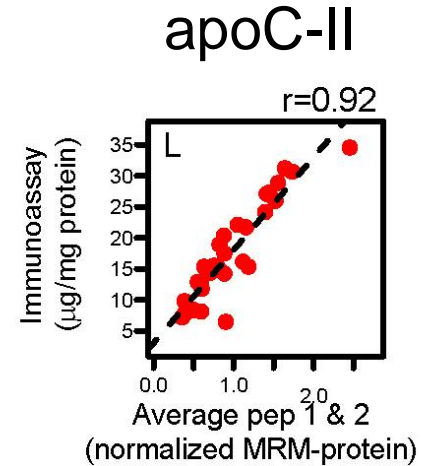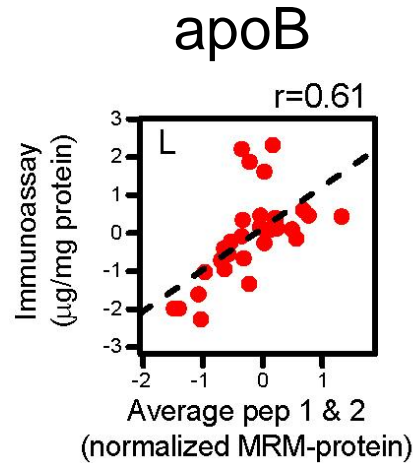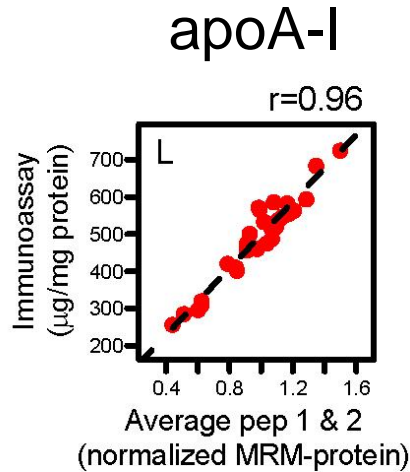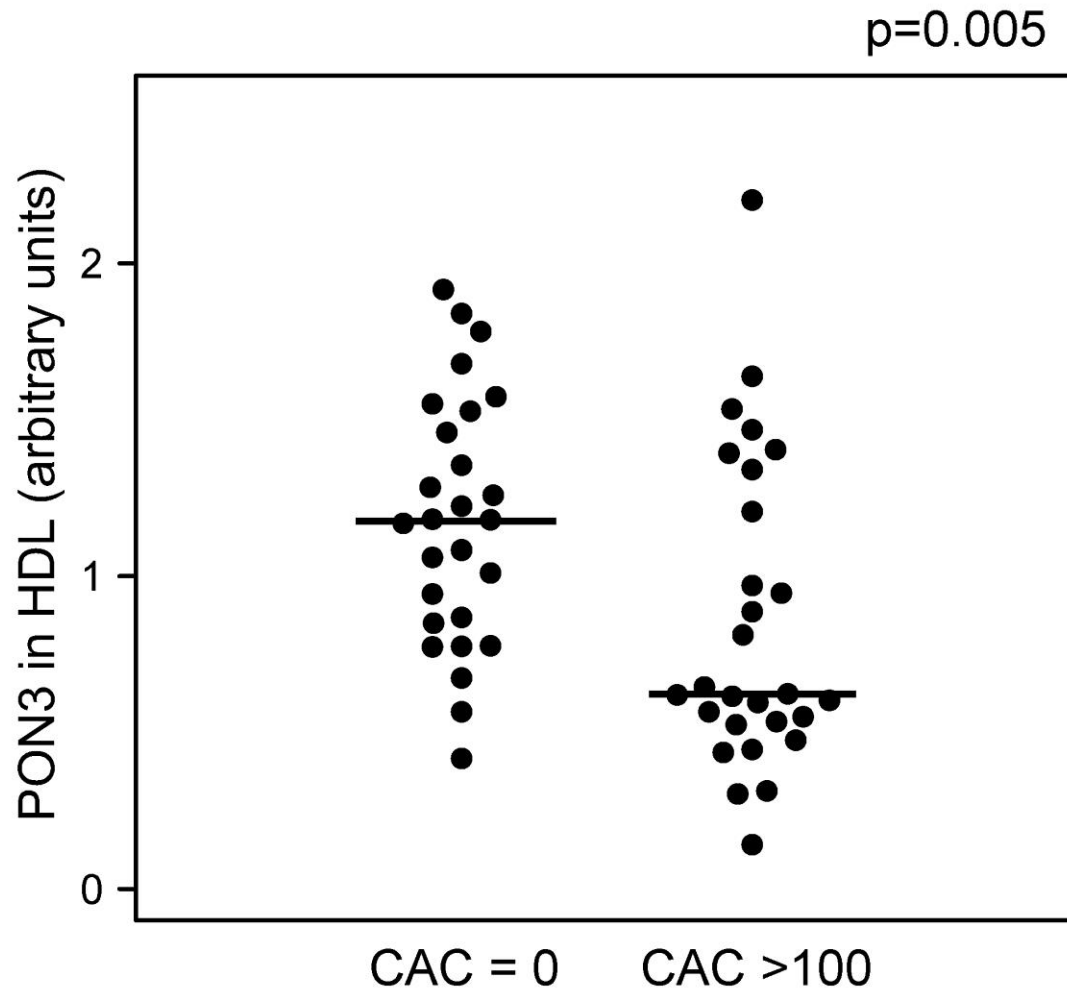Platelet and Coagulation Activation at the Lipid Core

Acute Coronary Syndrome and Sudden Death
Proteolysis
Complement Activation

Targeted Proteomics for HDL
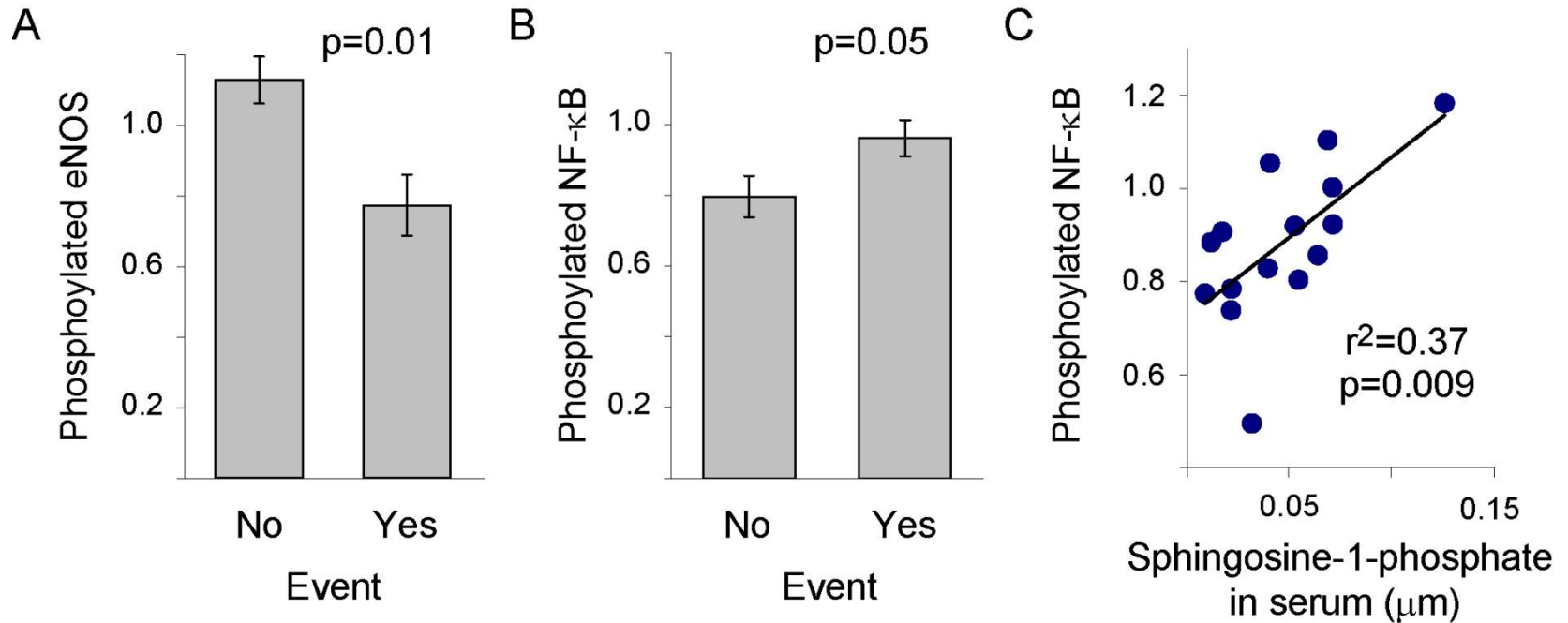## *Internal Standard Protein*
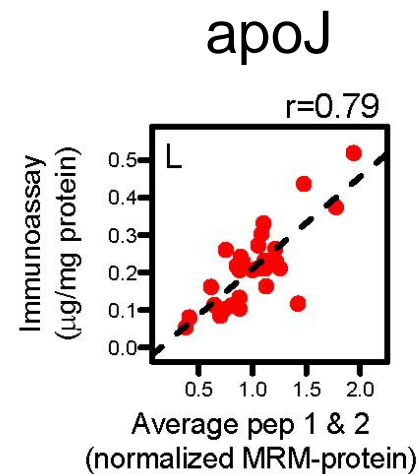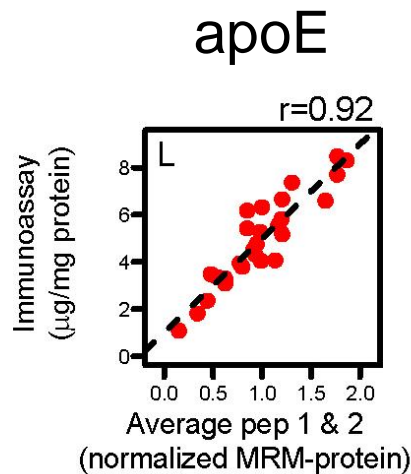
Paraoxonase 3 is Depleted in Patients with Type 1 Diabetes and Artery Calcification
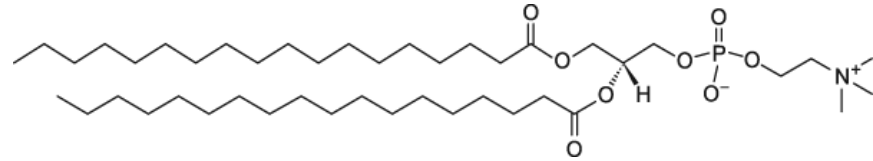
Endothelial Cell Function
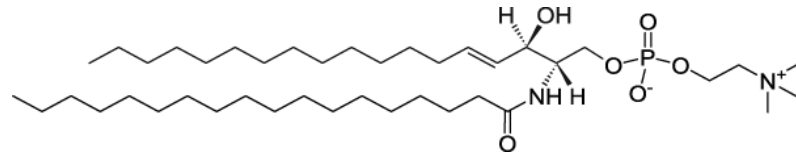## *Implicating HDL Lipids*
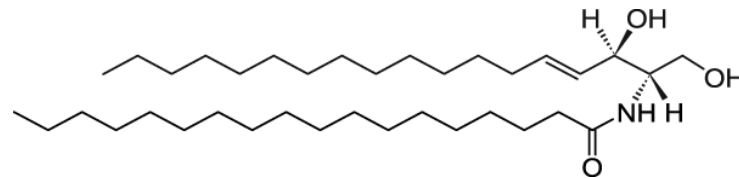
Thinking Beyond Cholesterol
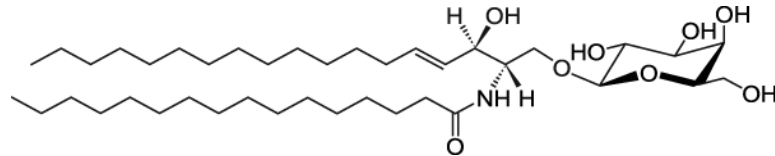## *Phospholipids and sphingolipids*

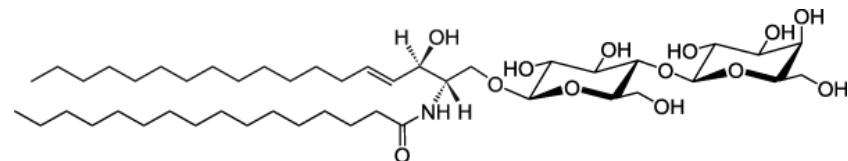Phosphotidylcholine

Sphingomyelin

Ceramide

Glucosylceramide

Lactosylceramide

Common Fragment Ions

Phosphotidylcholine
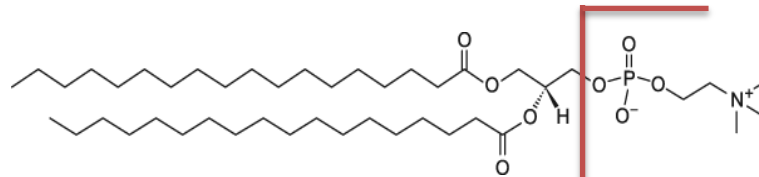
Sphingomyelin

Common Head Group Fragment (m/z)

184

Ceramide

Glucosylceramide
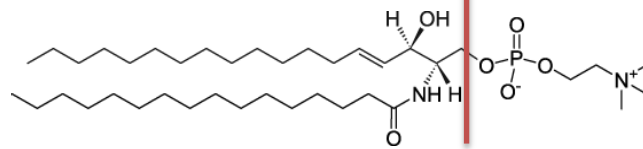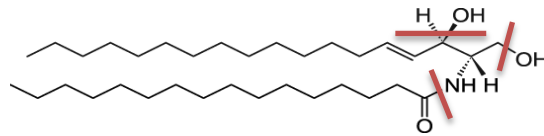
Lactosylceramide

Common Backbone Fragment (m/z)

264
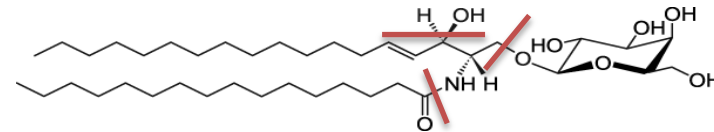
Typical Chromatogram
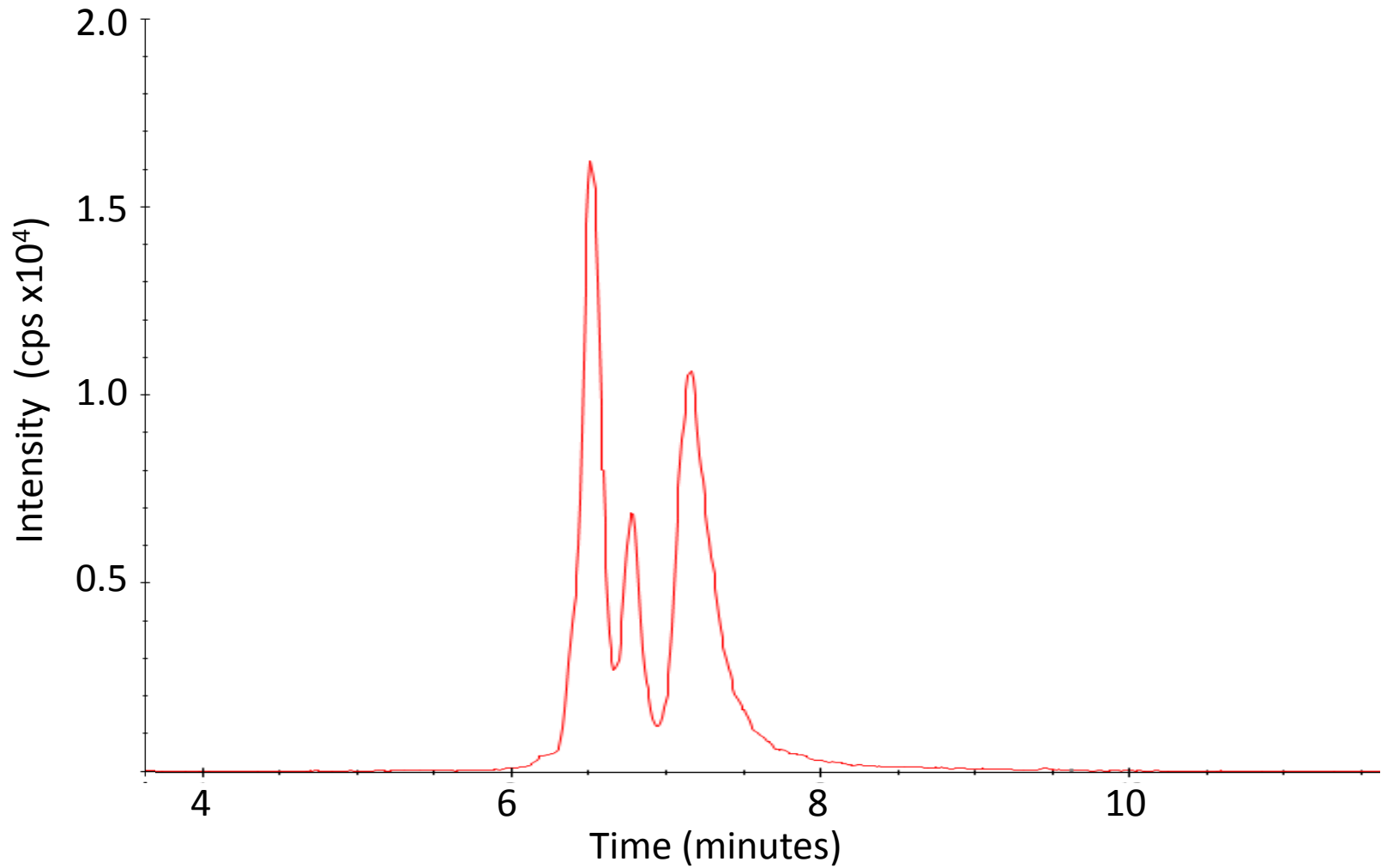
Biological Complexity

Step 1: Design Data Processing Method

Assign transitions

Step 2: Design Data Processing Method

Set RT and Integration window
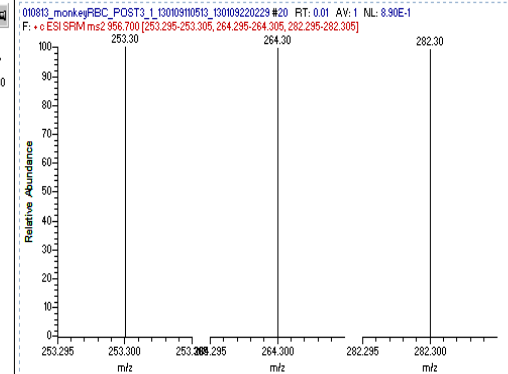
Step 3: Design Data Processing Method

Assign smoothing parameters

Step 4: Design Data Processing Method

Manually pick peak

Step 5: Process the Data

Step 5: Export the Data

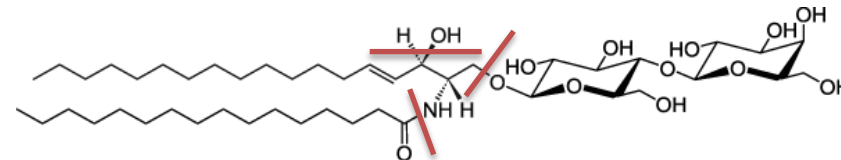| Component Name		Curve Index		Weighting Index	Origin Index	Equation						
788						NA						

Filename	Sample Type	Sample Name	Sample ID	Exp Amnt	Calc Amnt	Units	%Diff	Level	%RSD-RESP	Peak Status	Response Ratio	Response Type	Equation	Area	Height	ISTD Area	ISTI
010813_monkeyRBC_PRE1_1	Unknown Sample	010813_monkeyRBC_PRE1_1	1	NA	NA		NA	NA			NF	Area	NA	136487.59	28560.30	??	
010813_monkeyRBC_PRE1_2	Unknown Sample	010813_monkeyRBC_PRE1_2	1	NA	NA		NA	NA			NF	Area		145206.09	30314.25	??	
010813_monkeyRBC_PRE1_3	Unknown Sample	010813_monkeyRBC_PRE1_3	1	NA	NA		NA	NA			NF	Area		149180.09	30249.19	??	
010813_monkeyRBC_POST1_1	Unknown Sample	010813_monkeyRBC_POST1_1	1	NA	NA		NA	NA			NF	Area		182658.50	38818.23	??	
010813_monkeyRBC_POST1_2	Unknown Sample	010813_monkeyRBC_POST1_2	1	NA	NA		NA	NA			NF	Area		190733.55	39764.02	??	
010813_monkeyRBC_POST1_3	Unknown Sample	010813_monkeyRBC_POST1_3	1	NA	NA		NA	NA			NF	Area		173889.14	34070.94	??	
010813_monkeyRBC_PRE2_1	Unknown Sample	010813_monkeyRBC_PRE2_1	1	NA	NA		NA	NA			NF	Area		189164.62	40319.93	??	
010813_monkeyRBC_PRE2_2	Unknown Sample	010813_monkeyRBC_PRE2_2	1	NA	NA		NA	NA			NF	Area		167379.77	34061.77	??	
010813_monkeyRBC_PRE2_3	Unknown Sample	010813_monkeyRBC_PRE2_3	1	NA	NA		NA	NA			NF	Area		193727.86	39262.33	??	
010813_monkeyRBC_POST2_1	Unknown Sample	010813_monkeyRBC_POST2_1	1	NA	NA		NA	NA			NF	Area		168495.02	31550.61	??	
010813_monkeyRBC_POST2_2	Unknown Sample	010813_monkeyRBC_POST2_2	1	NA	NA		NA	NA			NF	Area		188380.69	36724.43	??	
010813_monkeyRBC_POST2_3	Unknown Sample	010813_monkeyRBC_POST2_3	1	NA	NA		NA	NA			NF	Area		170135.61	33313.56	??	
010813_monkeyRBC_PRE3_1	Unknown Sample	010813_monkeyRBC_PRE3_1	1	NA	NA		NA	NA			NF	Area		135639.70	29214.99	??	
010813_monkeyRBC_PRE3_2	Unknown Sample	010813_monkeyRBC_PRE3_2	1	NA	NA		NA	NA			NF	Area		134814.65	28652.55	??	
010813_monkeyRBC_PRE3_3	Unknown Sample	010813_monkeyRBC_PRE3_3	1	NA	NA		NA	NA			NF	Area		138835.41	27577.20	??	
010813_monkeyRBC_POST3_1	Unknown Sample	010813_monkeyRBC_POST3_1	1	NA	NA		NA	NA			NF	Area		187401.10	35500.49	??	
010813_monkeyRBC_POST3_2	Unknown Sample	010813_monkeyRBC_POST3_2	1	NA	NA		NA	NA			NF	Area		175078.66	34355.31	??	
010813_monkeyRBC_POST3_3	Unknown Sample	010813_monkeyRBC_POST3_3	1	NA	NA		NA	NA			NF	Area		181405.21	36857.98	??	
010813_monkeyRBC_PRE4_1	Unknown Sample	010813_monkeyRBC_PRE4_1	1	NA	NA		NA	NA			NF	Area		160985.25	32890.93	??	
010813_monkeyRBC_PRE4_2	Unknown Sample	010813_monkeyRBC_PRE4_2	1	NA	NA		NA	NA			NF	Area		182843.09	40270.93	??	
010813_monkeyRBC_PRE4_3	Unknown Sample	010813_monkeyRBC_PRE4_3	1	NA	NA		NA	NA			NF	Area		171569.68	34277.97	??	
010813_monkeyRBC_POST4_1	Unknown Sample	010813_monkeyRBC_POST4_1	1	NA	NA		NA	NA			NF	Area		192123.97	38070.61	??	
010813_monkeyRBC_POST4_2	Unknown Sample	010813_monkeyRBC_POST4_2	1	NA	NA		NA	NA			NF	Area		169116.74	34466.66	??	
010813_monkeyRBC_POST4_3	Unknown Sample	010813_monkeyRBC_POST4_3	1	NA	NA		NA	NA			NF	Area		167021.19	33967.62	??	
010813_monkeyRBC_PRE5_1	Unknown Sample	010813_monkeyRBC_PRE5_1	1	NA	NA		NA	NA			NF	Area		148311.81	28999.64	??	
010813_monkeyRBC_PRE5_2	Unknown Sample	010813_monkeyRBC_PRE5_2	1	NA	NA		NA	NA			NF	Area		149206.67	28003.17	??	
010813_monkeyRBC_PRE5_3	Unknown Sample	010813_monkeyRBC_PRE5_3	1	NA	NA		NA	NA			NF	Area		156327.86	32923.56	??	
010813_monkeyRBC_POST5_1	Unknown Sample	010813_monkeyRBC_POST5_1	1	NA	NA		NA	NA			NF	Area		188079.90	38208.70	??	
010813_monkeyRBC_POST5_2	Unknown Sample	010813_monkeyRBC_POST5_2	1	NA	NA		NA	NA			NF	Area		177395.34	38309.32	??	
010813_monkeyRBC_POST5_3	Unknown Sample	010813_monkeyRBC_POST5_3	1	NA	NA		NA	NA			NF	Area		218723.46	43443.40	??	

Created By:

User Name	Full Name	Date
QUANTUM		5/18/2013 5:49:47 PM

An Excel spreadsheet with a single transition per tab

Tabs: 794_2 / 794_3 / 844_1 / 844_2 / 844_3 / 900_2 / 900_3 / 956_4 / **788** / 701 / 729 / 492_2 / 702 / 730 / 732 / 758_1 / 872 / 758 / 492_1 / 520_2 / 730_1 / 757 / 928_3 / 956_5 / 786 / 928_1 / 768 / 691 / 789 / 759 / 785
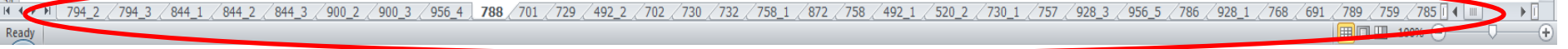
Step 6: Manual Integration & Re-process

	Component Name		Curve Index		Weighting Index	Origin Index	Equation									
2	Component Name		Curve Index		Weighting Index	Origin Index	Equation									
3	788						NA									
4																

	Filename	Sample Type	Sample Name	Sample ID	Exp Amnt	Calc Amnt	Units	%Diff	Level	%RSD-RESP	Peak Status	Response Ratio	Response Type	Equation	Area	Height	ISTD Area	ISTD
6	010813_monkeyRBC_PRE1_1	Unknown Sample	010813_monkeyRBC_PRE1_1	1	NA	NA		NA	NA			NF	Area	NA	136487.59	28560.30	??	
7	010813_monkeyRBC_PRE1_2	Unknown Sample	010813_monkeyRBC_PRE1_2	1	NA	NA		NA	NA			NF	Area		145206.09	30314.25	??	
8	010813_monkeyRBC_PRE1_3	Unknown Sample	010813_monkeyRBC_PRE1_3	1	NA	NA		NA	NA			NF	Area		149180.09	30249.19	??	
9	010813_monkeyRBC_POST1_1	Unknown Sample	010813_monkeyRBC_POST1_1	1	NA	NA		NA	NA			NF	Area		182658.50	38818.23	??	
10	010813_monkeyRBC_POST1_2	Unknown Sample	010813_monkeyRBC_POST1_2	1	NA	NA		NA	NA			NF	Area		190733.55	39764.02	??	
11	010813_monkeyRBC_POST1_3	Unknown Sample	010813_monkeyRBC_POST1_3	1	NA	NA		NA	NA			NF	Area		173889.14	34070.94	??	
12	010813_monkeyRBC_PRE2_1	Unknown Sample	010813_monkeyRBC_PRE2_1	1	NA	NA		NA	NA			NF	Area		189164.62	40319.93	??	
13	010813_monkeyRBC_PRE2_2	Unknown Sample	010813_monkeyRBC_PRE2_2	1	NA	NA		NA	NA			NF	Area		167379.77	34061.77	??	
14	010813_monkeyRBC_PRE2_3	Unknown Sample	010813_monkeyRBC_PRE2_3	1	NA	NA		NA	NA			NF	Area		193727.86	39262.33	??	
15	010813_monkeyRBC_POST2_1	Unknown Sample	010813_monkeyRBC_POST2_1	1	NA	NA		NA	NA			NF	Area		168495.02	31550.61	??	
16	010813_monkeyRBC_POST2_2	Unknown Sample	010813_monkeyRBC_POST2_2	1	NA	NA		NA	NA			NF	Area		188380.69	36724.43	??	
17	010813_monkeyRBC_POST2_3	Unknown Sample	010813_monkeyRBC_POST2_3	1	NA	NA		NA	NA			NF	Area		170135.61	33313.56	??	
18	010813_monkeyRBC_PRE3_1	Unknown Sample	010813_monkeyRBC_PRE3_1	1	NA	NA		NA	NA			NF	Area		135639.70	29214.99	??	
19	010813_monkeyRBC_PRE3_2	Unknown Sample	010813_monkeyRBC_PRE3_2	1	NA	NA		NA	NA			NF	Area		134814.65	28652.55	??	
20	010813_monkeyRBC_PRE3_3	Unknown Sample	010813_monkeyRBC_PRE3_3	1	NA	NA		NA	NA			NF	Area		138835.41	27577.20	??	
21	010813_monkeyRBC_POST3_1	Unknown Sample	010813_monkeyRBC_POST3_1	1	NA	NA		NA	NA			NF	Area		187401.10	35500.49	??	
22	010813_monkeyRBC_POST3_2	Unknown Sample	010813_monkeyRBC_POST3_2	1	NA	NA		NA	NA			NF	Area		175078.66	34355.31	??	
23	010813_monkeyRBC_POST3_3	Unknown Sample	010813_monkeyRBC_POST3_3	1	NA	NA		NA	NA			NF	Area		181405.21	36857.98	??	
24	010813_monkeyRBC_PRE4_1	Unknown Sample	010813_monkeyRBC_PRE4_1	1	NA	NA		NA	NA			NF	Area		160985.25	32890.93	??	
25	010813_monkeyRBC_PRE4_2	Unknown Sample	010813_monkeyRBC_PRE4_2	1	NA	NA		NA	NA			NF	Area		182843.09	40270.93	??	
26	010813_monkeyRBC_PRE4_3	Unknown Sample	010813_monkeyRBC_PRE4_3	1	NA	NA		NA	NA			NF	Area		171569.68	34277.97	??	
27	010813_monkeyRBC_POST4_1	Unknown Sample	010813_monkeyRBC_POST4_1	1	NA	NA		NA	NA			NF	Area		192123.97	38070.61	??	
28	010813_monkeyRBC_POST4_2	Unknown Sample	010813_monkeyRBC_POST4_2	1	NA	NA		NA	NA			NF	Area		169116.74	34466.66	??	
29	010813_monkeyRBC_POST4_3	Unknown Sample	010813_monkeyRBC_POST4_3	1	NA	NA		NA	NA			NF	Area		167021.19	33967.62	??	
30	010813_monkeyRBC_PRE5_1	Unknown Sample	010813_monkeyRBC_PRE5_1	1	NA	NA		NA	NA			NF	Area		148311.81	28999.64	??	
31	010813_monkeyRBC_PRE5_2	Unknown Sample	010813_monkeyRBC_PRE5_2	1	NA	NA		NA	NA			NF	Area		149206.67	28003.17	??	
32	010813_monkeyRBC_PRE5_3	Unknown Sample	010813_monkeyRBC_PRE5_3	1	NA	NA		NA	NA			NF	Area		156327.86	32923.56	??	
33	010813_monkeyRBC_POST5_1	Unknown Sample	010813_monkeyRBC_POST5_1	1	NA	NA		NA	NA			NF	Area		188079.90	38208.70	??	
34	010813_monkeyRBC_POST5_2	Unknown Sample	010813_monkeyRBC_POST5_2	1	NA	NA		NA	NA			NF	Area		177395.34	38309.32	??	
35	010813_monkeyRBC_POST5_3	Unknown Sample	010813_monkeyRBC_POST5_3	1	NA	NA		NA	NA			NF	Area		218723.46	43443.40	??	

Created By:
User Name
QUANTUM

Identify samples with wrong retention time, etc.

Fix processing parameters, reprocess

There are 100 tabs here!

Tabs: 794_2 / 794_3 / 844_1 / 844_2 / 844_3 / 900_2 / 900_3 / 956_4 / **788** / 701 / 729 / 492_2 / 702 / 730 / 732 / 758_1 / 872 / 758 / 492_1 / 520_2 / 730_1 / 757 / 928_3 / 956_5 / 786 / 928_1 / 768 / 691 / 789 / 759 / 785

Ready

Step 7: Compile Data: A Single Spreadsheet

	3	4	5	6	7	8	9	10	11	12	13	14
1	Day	Patient	Injection	788	758	626	492	464	759	647	520	65
2				PC(18:0/18:1(9Z))	PC(16:0/18:2(10E, 12Z))	GluCer(d18:1/12:0)	Cer(d18:1/14:0)	Cer(d18:1/12:0)	SM(d18:1/20:0)	SM(d18:1/12:0)	Cer(d18:0/16:0)	PC(10:0/16:0)
3	1	P14	1	0.29	134.16	0.60	0.01	0.59	1.22	1.28	0.12	0.0:
4	1	P14	2	0.33	135.95	0.72	0.01	0.61	1.29	1.20	0.14	0.0:
5	1	P14	3	0.27	124.54	0.62	0.01	0.54	1.10	1.15	0.11	0.0!
6	1	P16	1	0.26	41.82	0.61	0.00	0.53	0.46	1.11	0.10	0.0:
7	1	P16	2	0.32	46.51	0.68	0.01	0.63	0.53	1.24	0.16	0.0
8	1	P16	3	0.28	46.05	0.64	0.01	0.65	0.40	1.15	0.14	0.0:
9	1	P21	1	0.34	63.23	0.72	0.00	0.60	0.55	1.27	0.14	0.0:
10	1	P21	2	0.29	62.54	0.72	0.01	0.63	0.63	1.20	0.11	0.0:
11	1	P21	3	0.27	58.79	0.68	0.00	0.57	0.52	1.21	0.14	0.0:
12	1	P26	1	0.28	54.43	0.60	0.01	0.52	0.52	1.02	0.13	0.0:
13	1	P26	2	0.33	58.25	0.67	0.02	0.53	0.55	1.15	0.13	0.0:
14	1	P26	3	0.31	58.01	0.70	0.01	0.61	0.44	1.11	0.13	0.0:
15	1	P33	1	0.28	67.67	0.64	0.01	0.58	0.61	1.16	0.12	0.0:
16	1	P33	2	0.27	64.86	0.61	0.01	0.47	0.53	1.16	0.18	0.0:
17	1	P33	3	0.28	63.04	0.67	0.00	0.57	0.50	1.09	0.12	0.0:
18	1	P34	1	0.32	57.93	0.68	0.00	0.59	0.53	1.09	0.14	0.0:

6HDL summary | Sheet1

Ready 200%

Workflow Complexity

Three different LC-MS runs

Create a processing method for each transition

Using a previously acquired data file:

       Assign RT, peak width, smoothing parameters.

Process data using this processing method

Adjust processing parameters as needed

Export data:

       One worksheet in Excel per transition

       Random order of worksheets in Excel

       Compile data into a single file

Perform QC:

       Manually ensure proper integration (retention time, peak shape)

       Export data

       Perform QC

Lather, Rinse, Repeat...

Possible Solution: Skyline

Easy to manipulate large data sets

Chromatographic alignment

Automated peak integration

Simple report configuration

Simple data export

Possible Solution: Skyline
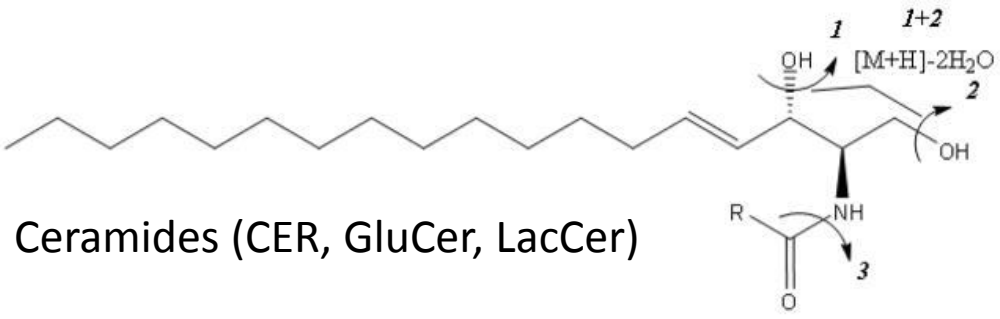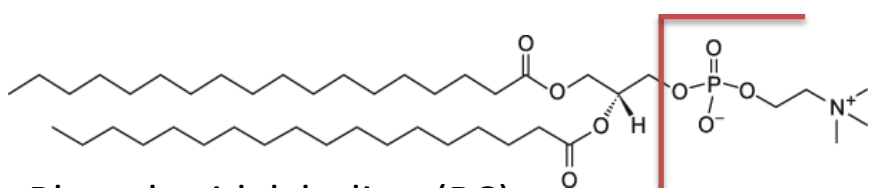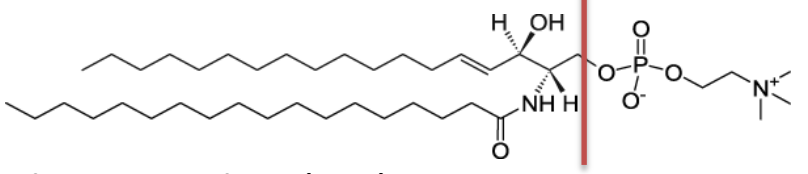
Tricking Skyline

Set one amino acid to be the fragment for the class of lipids
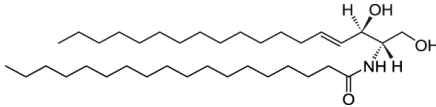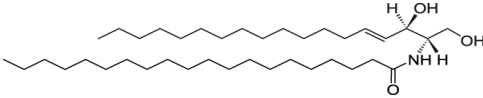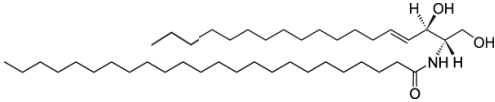
Set another amino acid to add up to the right precursor mass

Pretend the two amino acids are a peptide

Inventing Amino Acids: The Constant Part

Modified Glycine

M+H$^+$ Chemical Formula

Ceramides (CER, GluCer, LacCer)

264      $C_{15}H_{24}$

Phosphatidylcholine (PC)

184      $C_{10}H_{4}$

Sphingomyelins (SM)

Inventing Amino Acids: The Variable Part

	M+H$^+$	Modified Alanine Chemical Formula
C18 Ceramide (d18:1/18:0)	548	$C_{13}H_{13}$
C20 Ceramide (d18:1/20:0)	576	$C_{20}H_{13}$
C22 Ceramide (d18:1/22:0)	604	$C_{17}H_{21}$

Step 1: Invent Modified Amino Acids
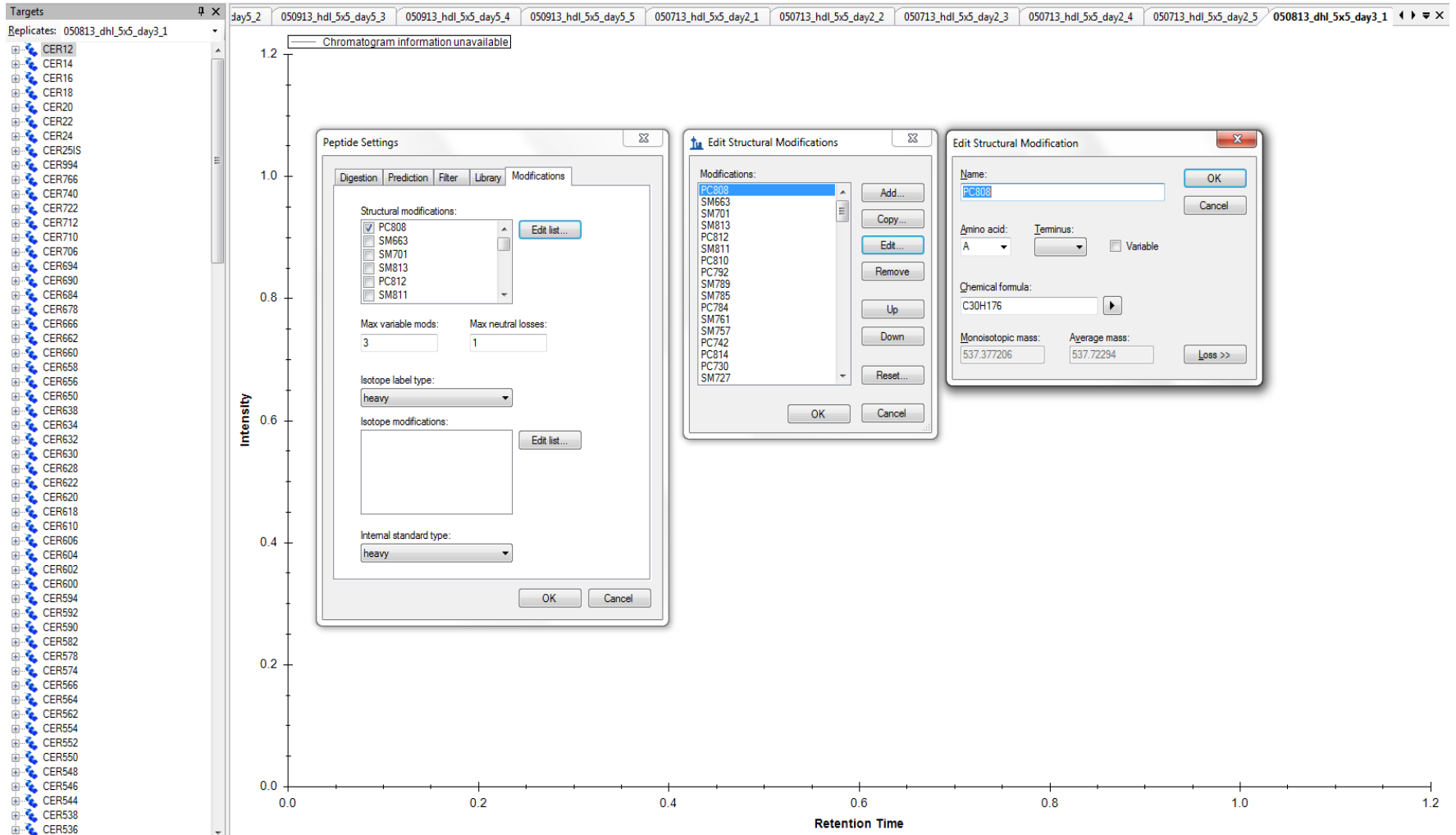
Step 2: Invent New "Proteins" and "Peptides"

Step 3: Modify the Amino Acids on the "Peptides"

Step 4: Import Data

Step 5: Manually Pick Peaks if Needed
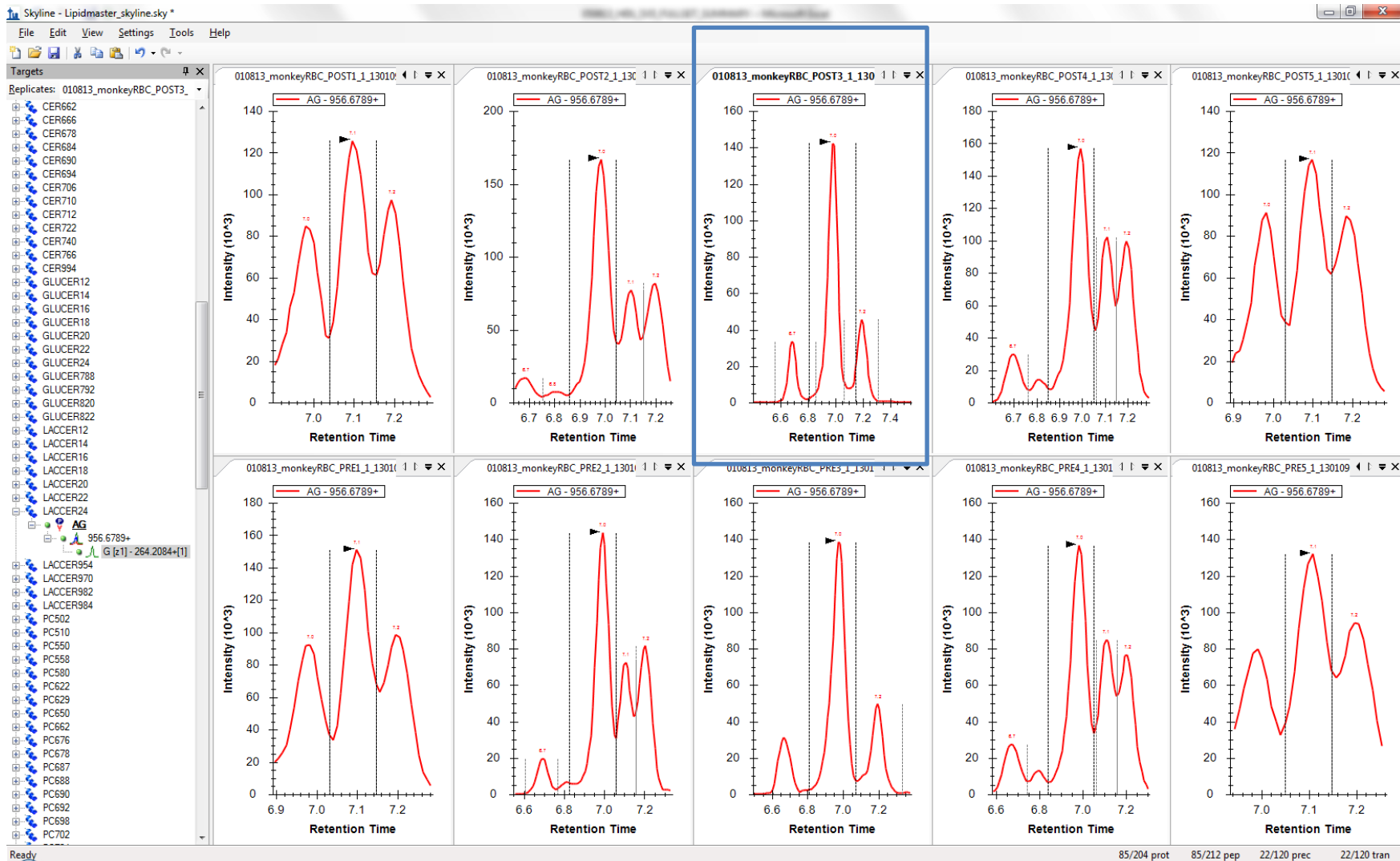
Step 6: Export the Data
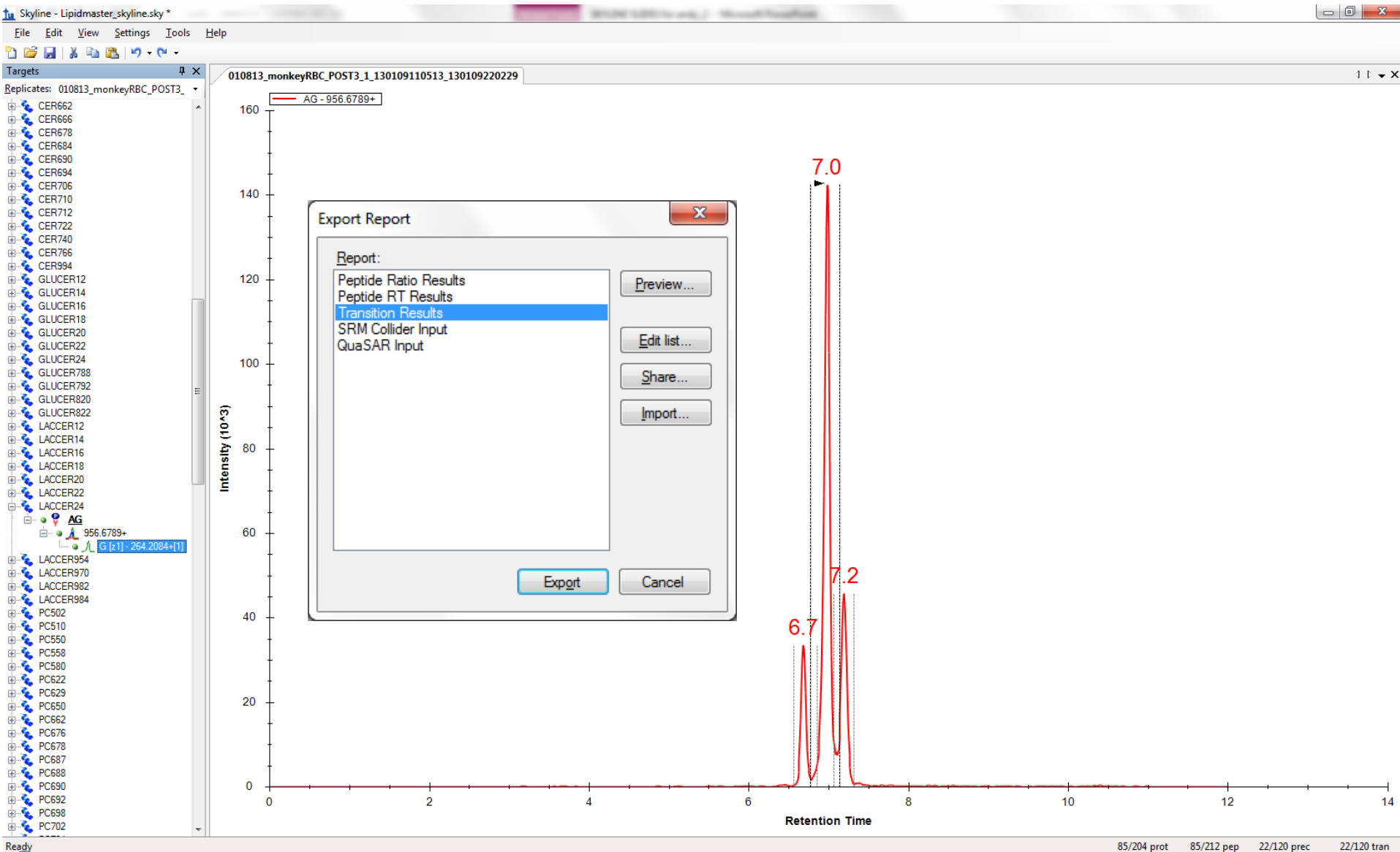
A Deliverable: One Tab on One Spreadsheet

An Experiment: The Data First

Lipid in HDL extract	Within Day CV	Between Day CV	Lipid in HDL extract	Within Day CV	Between Day CV
Cer(d18:1/12:0)	9%	9%	PC(18:0/20:3)	12%	15%
Cer(d18:1/24:0)	12%	11%	PC(18:0/22:5)	11%	16%
Glucosylceramide (d18:1/12:0)	8%	9%	PC(18:0/22:6)	10%	15%
PC(10:0/16:0)	12%	30%	PC(18:1/22:6)	8%	14%
PC(10:0/18:1)	10%	19%	PC(20:5/16:0)	13%	15%
PC(12:0/17:2)	11%	35%	SM(d18:0/16:0)	9%	14%
PC(12:0/18:1)	9%	14%	SM(d18:0/18:0)	10%	17%
PC(12:0/18:2)	10%	14%	SM(d18:0/20:0)	11%	15%
PC(13:0/20:3)	11%	14%	SM(d18:0/22:0)	9%	15%
PC(14:0/18:1)	11%	13%	SM(d18:1/12:0)	8%	14%
PC(14:0/18:2)	9%	14%	SM(d18:1/12:0)-18	11%	22%
PC(14:0/18:3)	13%	11%	SM(d18:1/16:0)	10%	13%
PC(15:0/20:5)	11%	14%	SM(d18:1/16:1)	11%	15%
PC(15:0/22:6)	11%	15%	SM(d18:1/18:1)	11%	13%
PC(16:0/18:1)	11%	15%	SM(d18:1/20:0)	11%	14%
PC(16:0/18:2)	10%	13%	SM(d18:1/22:0)	11%	14%
PC(16:0/20:3)	10%	14%	SM(d18:1/22:1)	10%	14%
PC(16:0/20:4)	10%	13%	SM(d18:1/24:0)	10%	16%
PC(16:0/22:4)	11%	15%	SM(d18:1/24:1)	8%	15%
PC(16:0/22:5)	10%	14%	SM(d18:2/18:1)	13%	14%
PC(18:0/11:1)	14%	17%	SM(d18:2/22:1)	11%	13%
PC(18:0/18:2)	11%	15%	SM(d18:2/24:1)	10%	17%
PC(18:0/20:2)	10%	17%			

How long would you be willing to spend to get this amount of data?

Vendor Software: 15 hours, Skyline: 4 hours

Actively collaborating with the author of the software: Priceless