Lipidomics and food metabolites

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&

Molecular Discovery, London

Photos from Perugia, Umbria, Italy

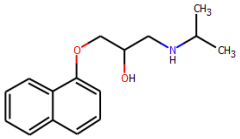
The limits of QSAR (QSPR)

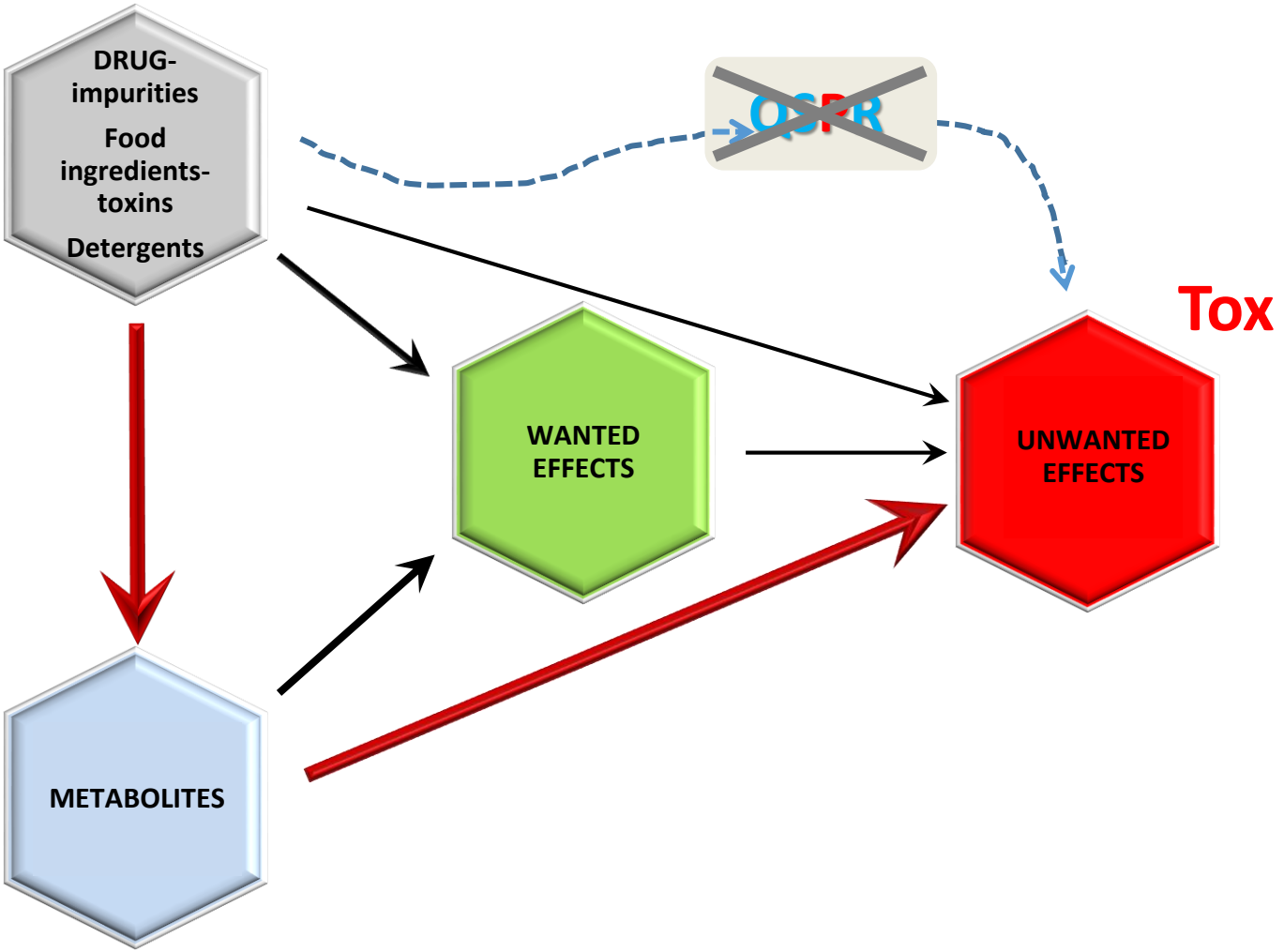
1. Local models (plastics and drugs cannot be located in the same model)
2. Activity (Property) must be generated by the same mechanism
3. Similarity methods depend on descriptors
4. Chemical descriptors must be relevant to P
5. Biological descriptors must be relevant to P
6. Pruning descriptors very dangerous !!
7. Combination of methods better than consensus methods
8. AI << IA (interpretation …interpretation …interpretation)

Struture-Property Relationships

Chemical description
0110001110010101

xenobiotics

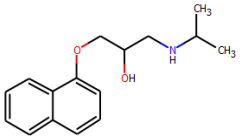


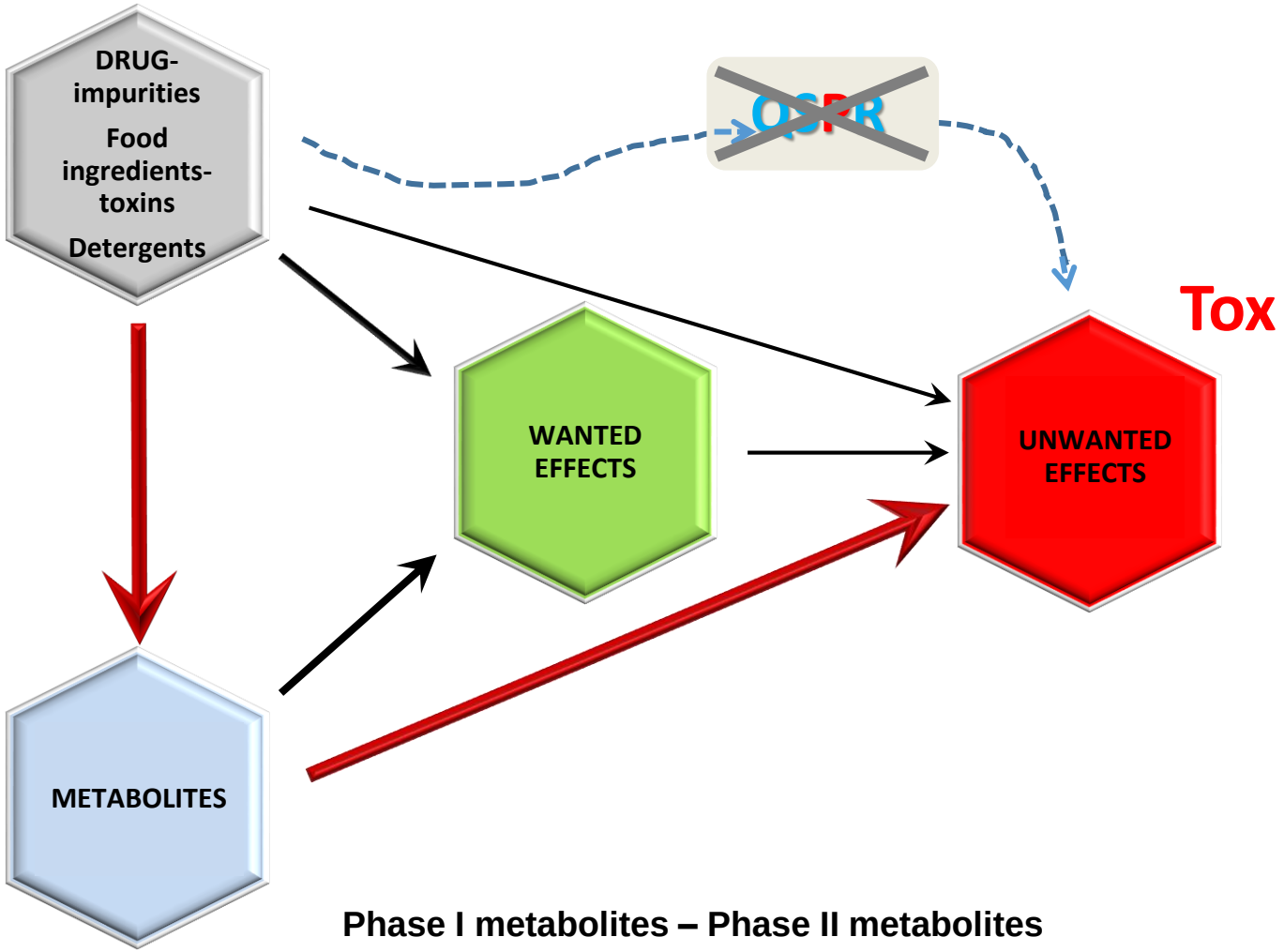


Struture-Property Relationships

Chemical description
0110001110001010 1

xenobiotics

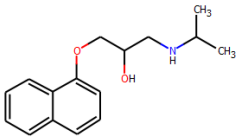




Phase I metabolites – Phase II metabolites

Struture-Toxicity Relationships

xenobiotics



DRUG-
impurities

Food
ingredients-
toxins

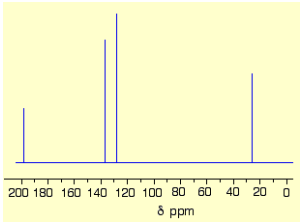
Detergents

QSPR

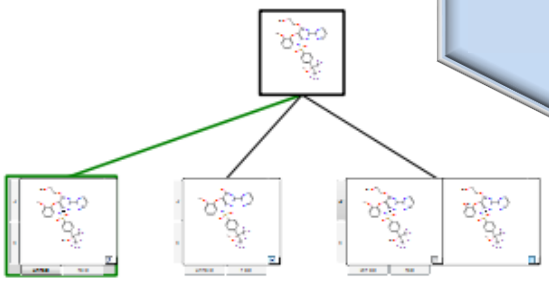
WANTED
EFFECTS

UNWANTED
EFFECTS

Tox



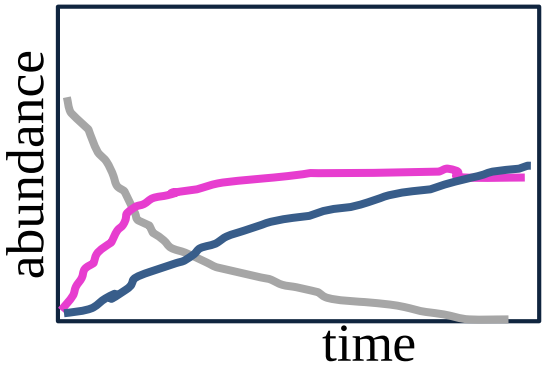
METABOLITES

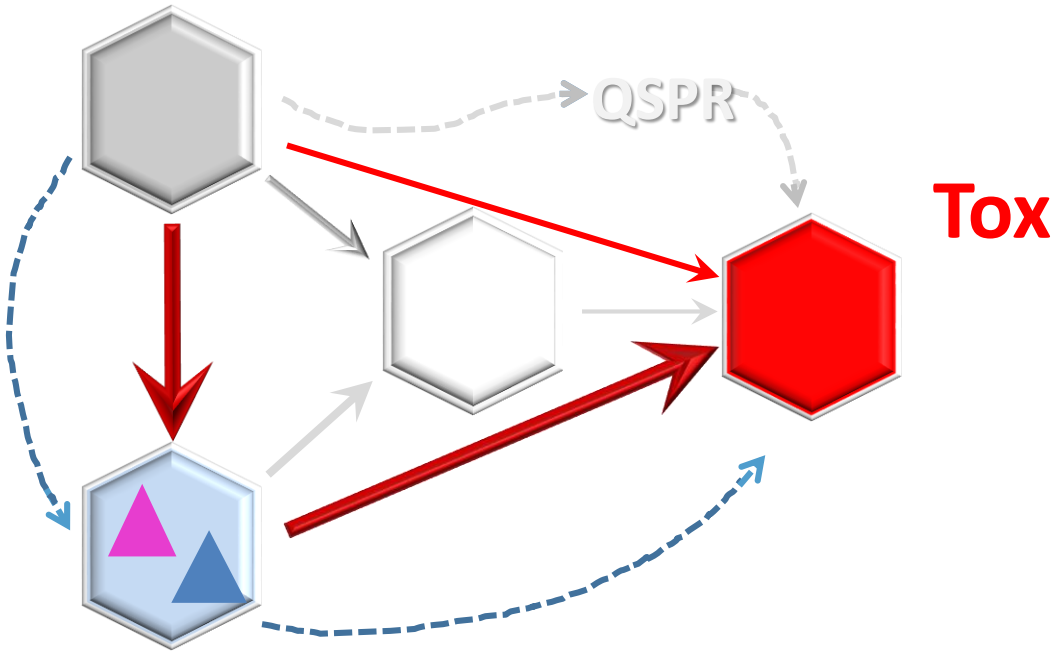


NO metabolomics !

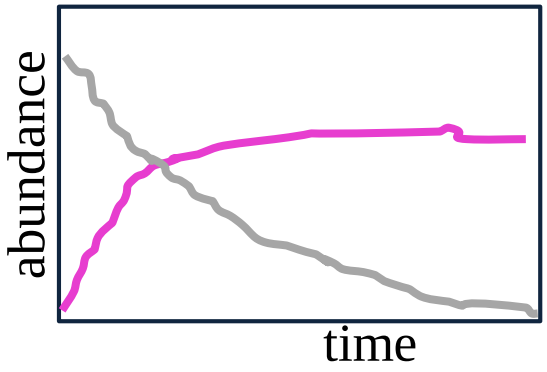
Chemical description
0110001110001010 1

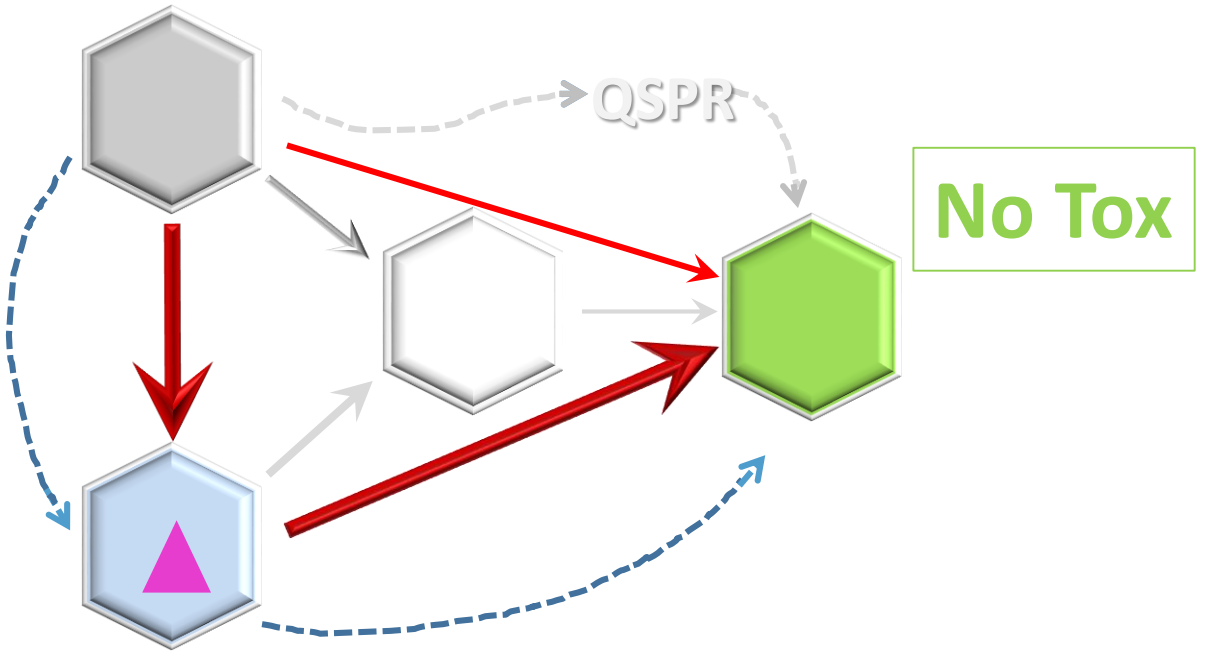
Identification of metabolites chemical structure, abundance, kinetics





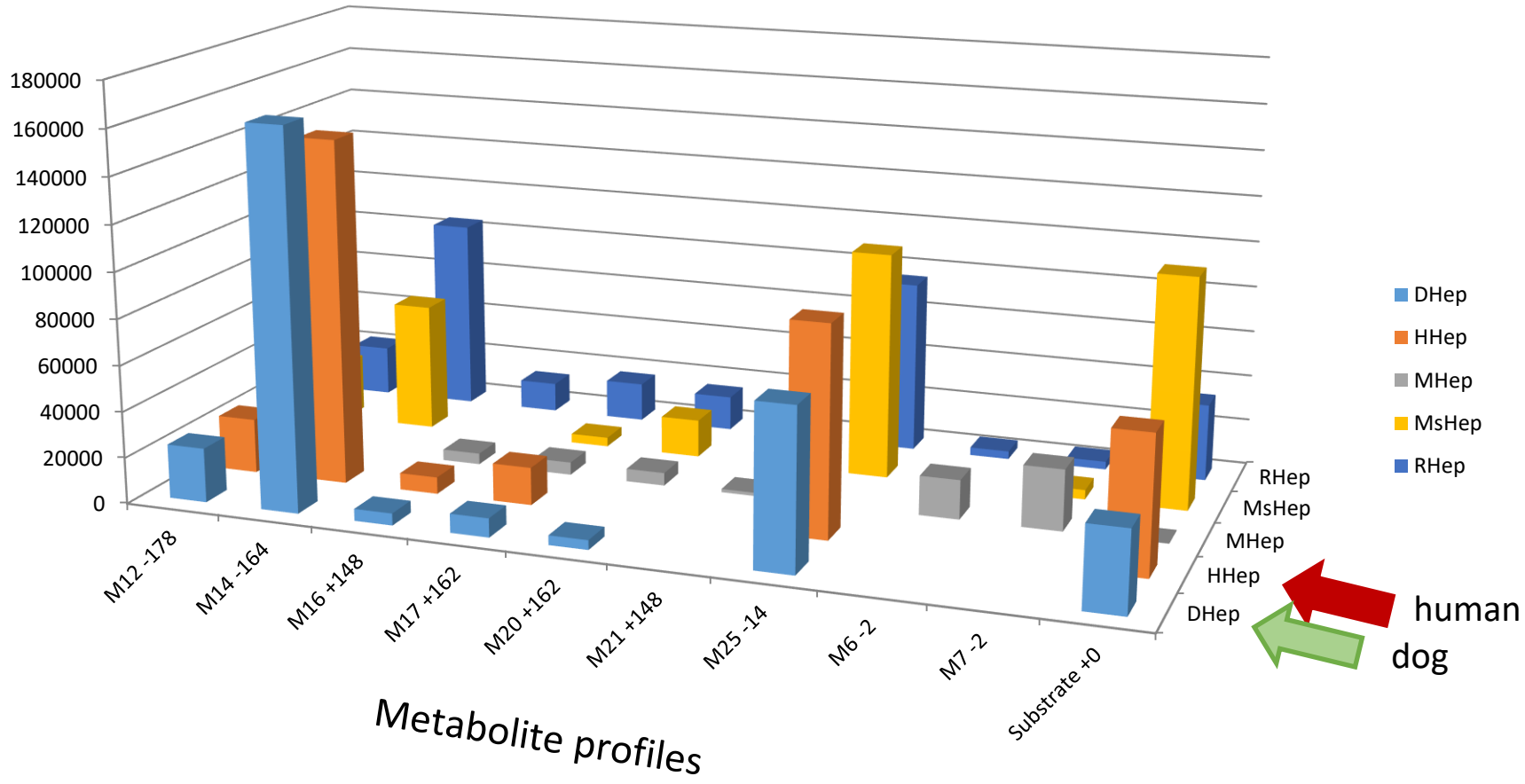
Identification of metabolites chemical structure, abundance, kinetics

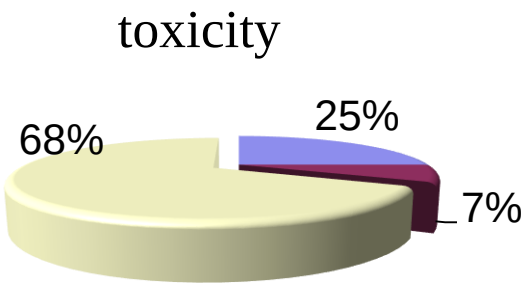


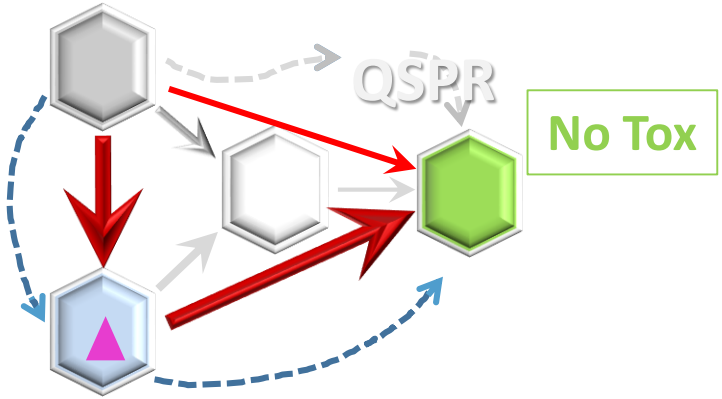


BioMatrix analysis

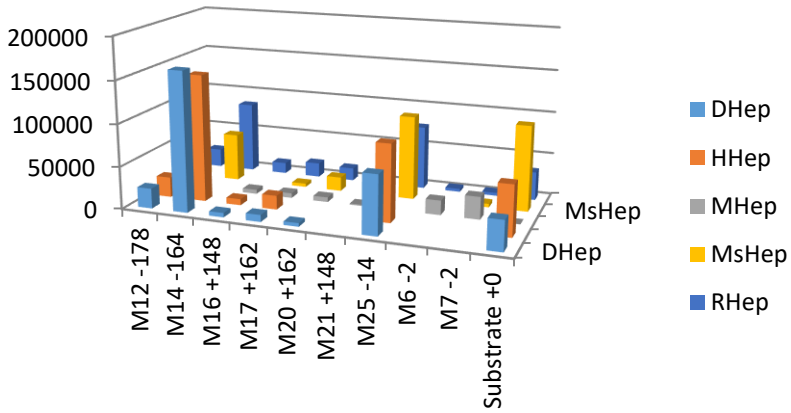
Hepatocytes across species Analysis

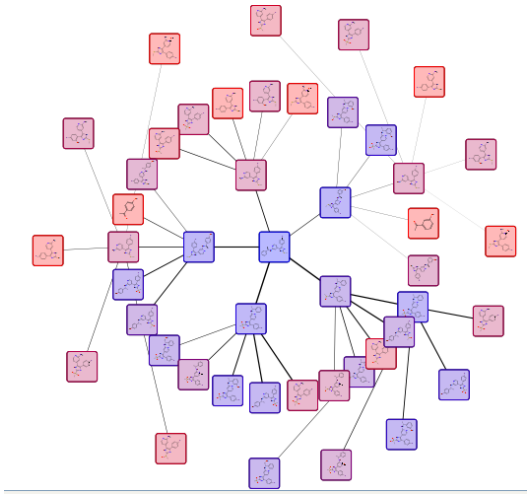




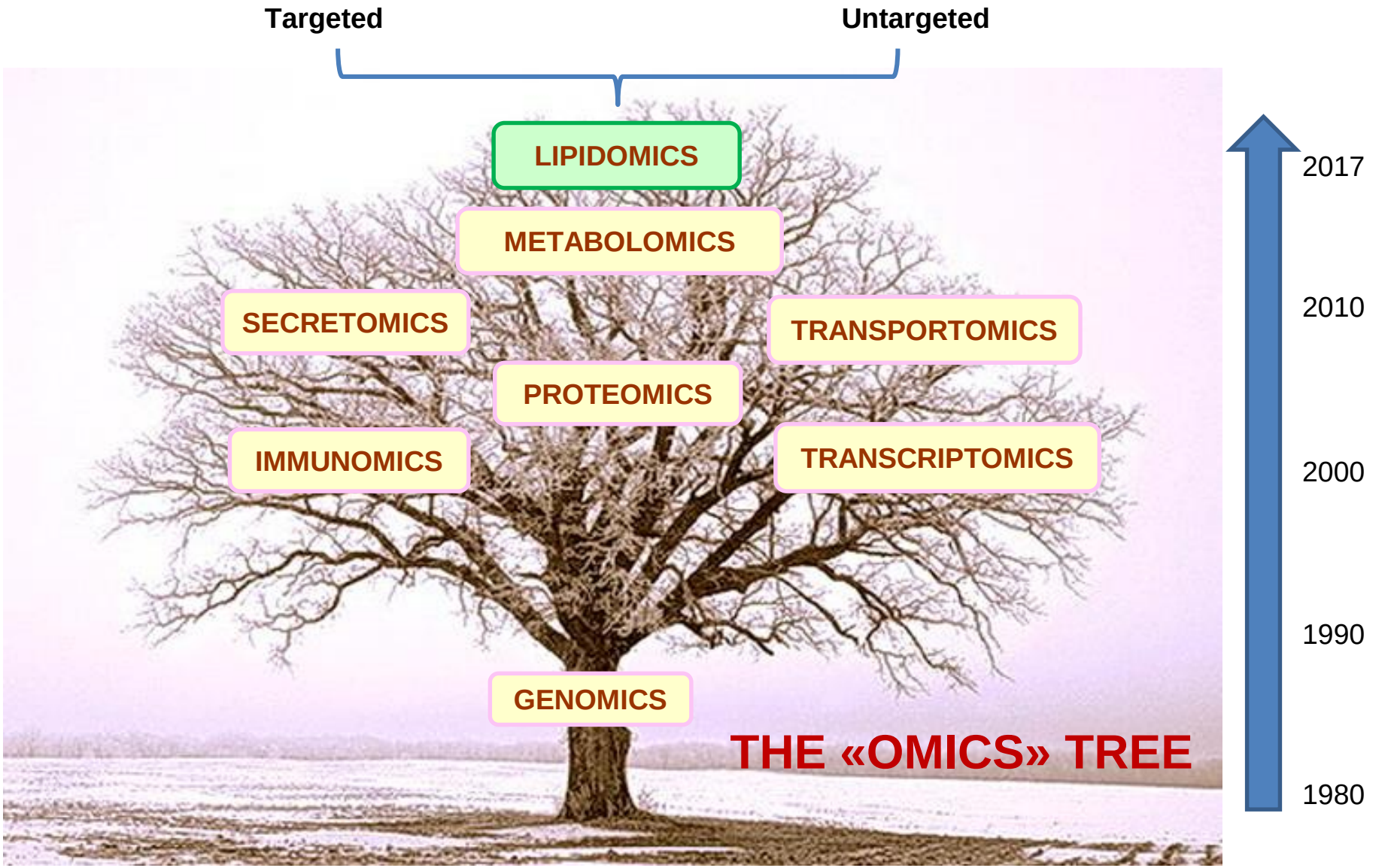


Take home messages



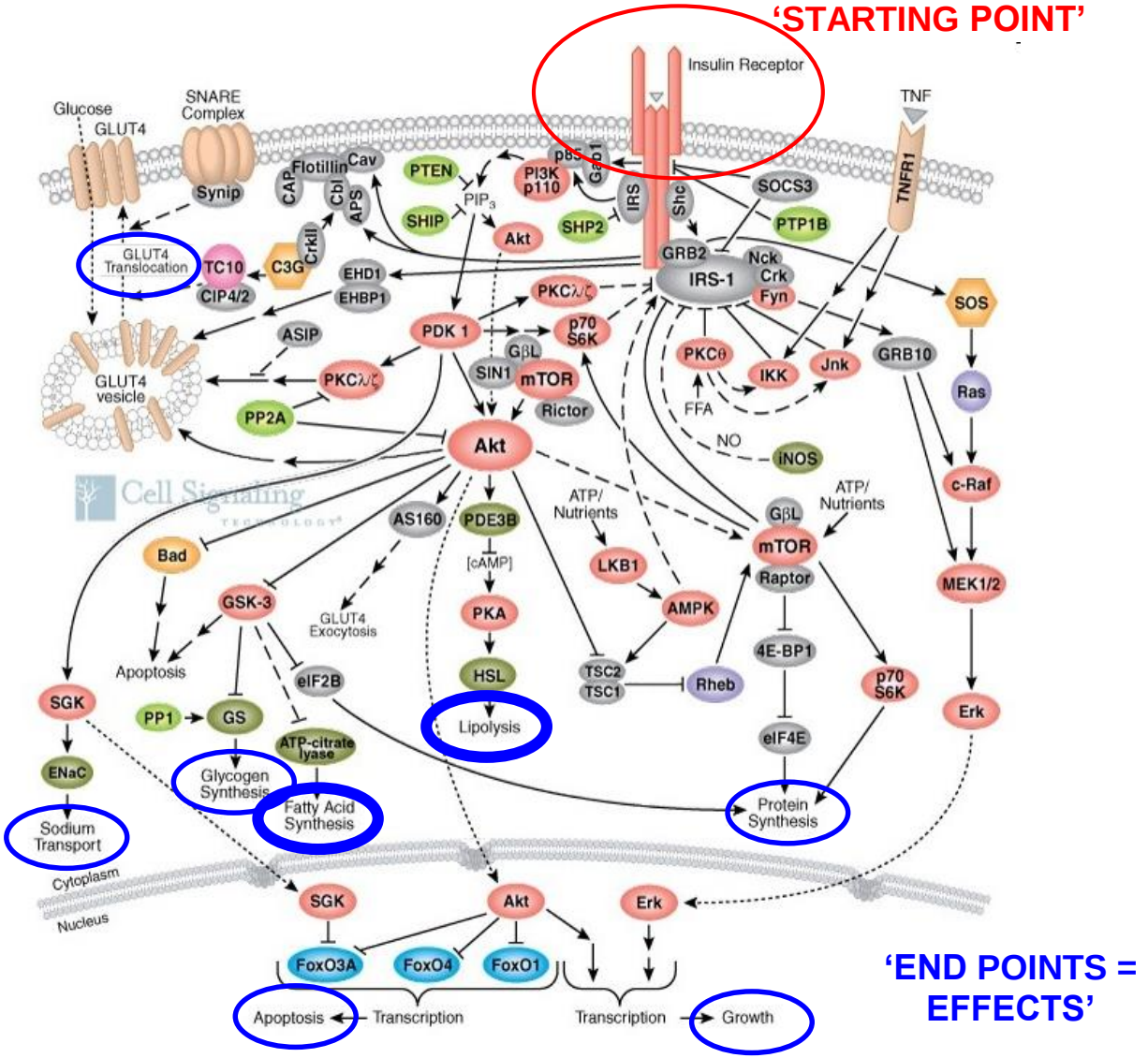


Metabolic patways

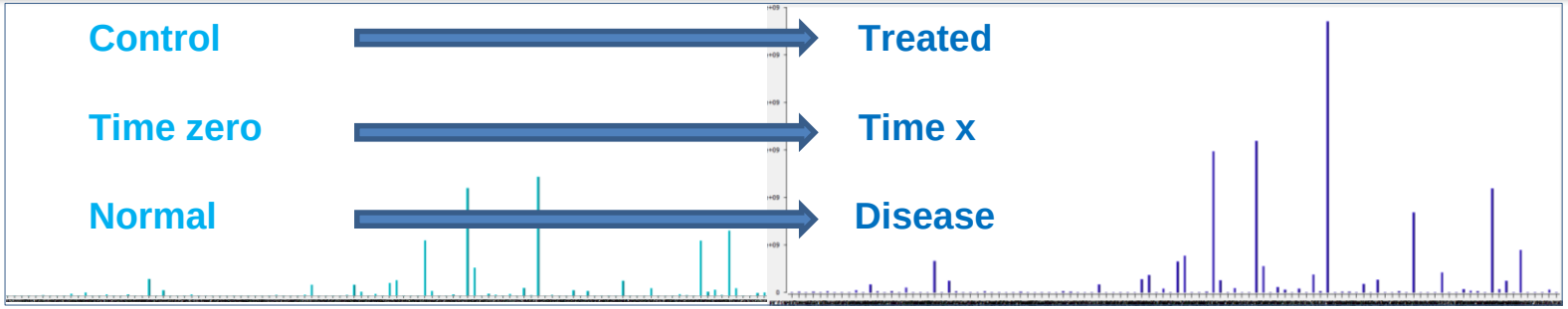


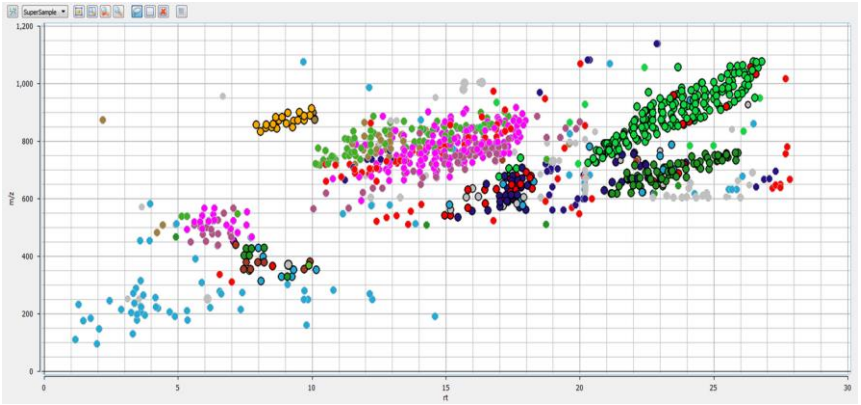
According to SciFinder only 18 publications are reported in Untargeted Lipidomics till today (2017)

Why untargeted Lipidomics ?

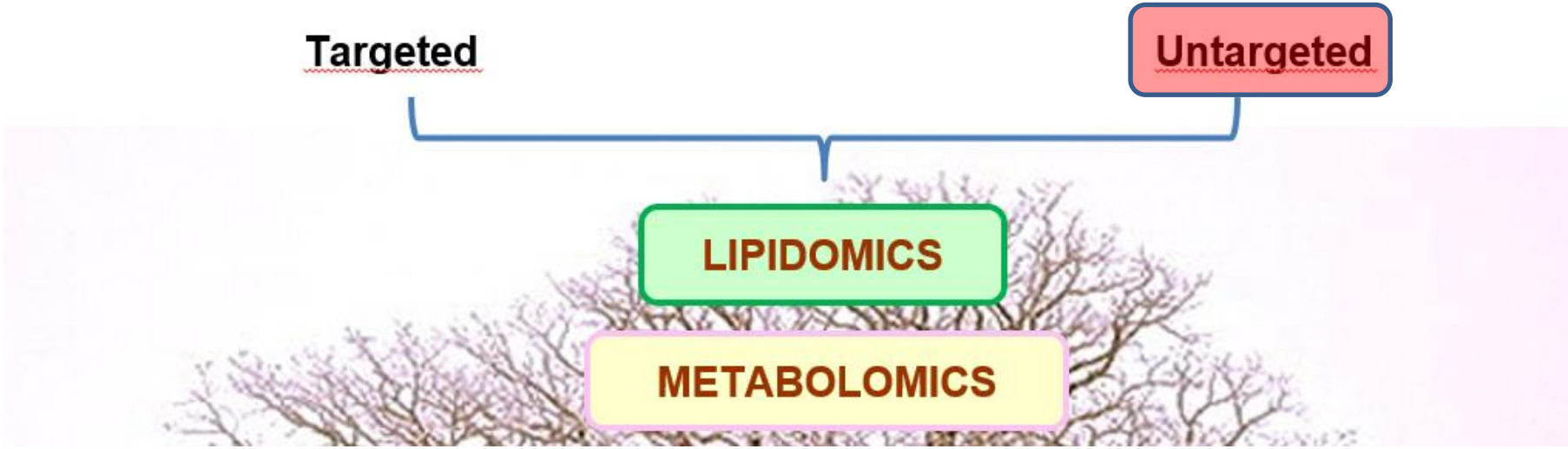


Any change/modification of a cellular function induced by drugs will be reflected in several modifications of lipid content, composition and distribution.

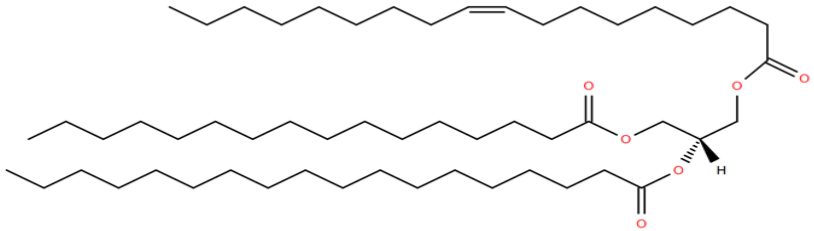




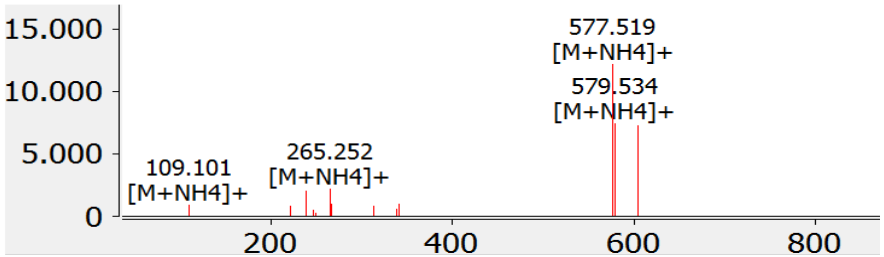
Several thousands of lipids must be identifyed (up to 1000000)

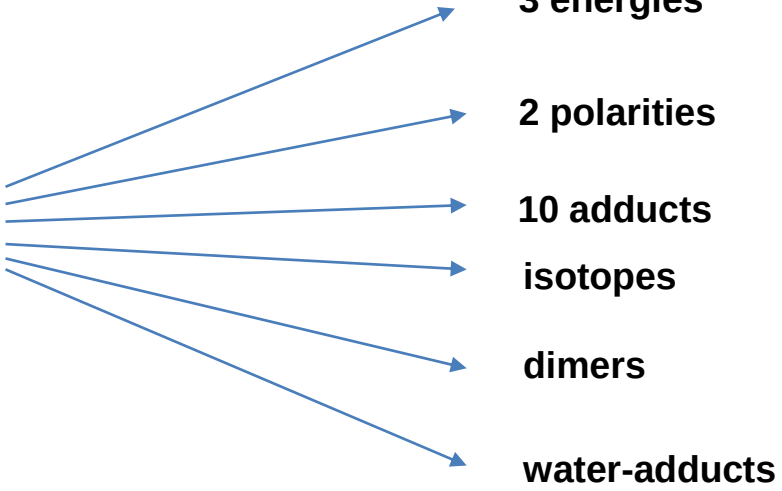


a)



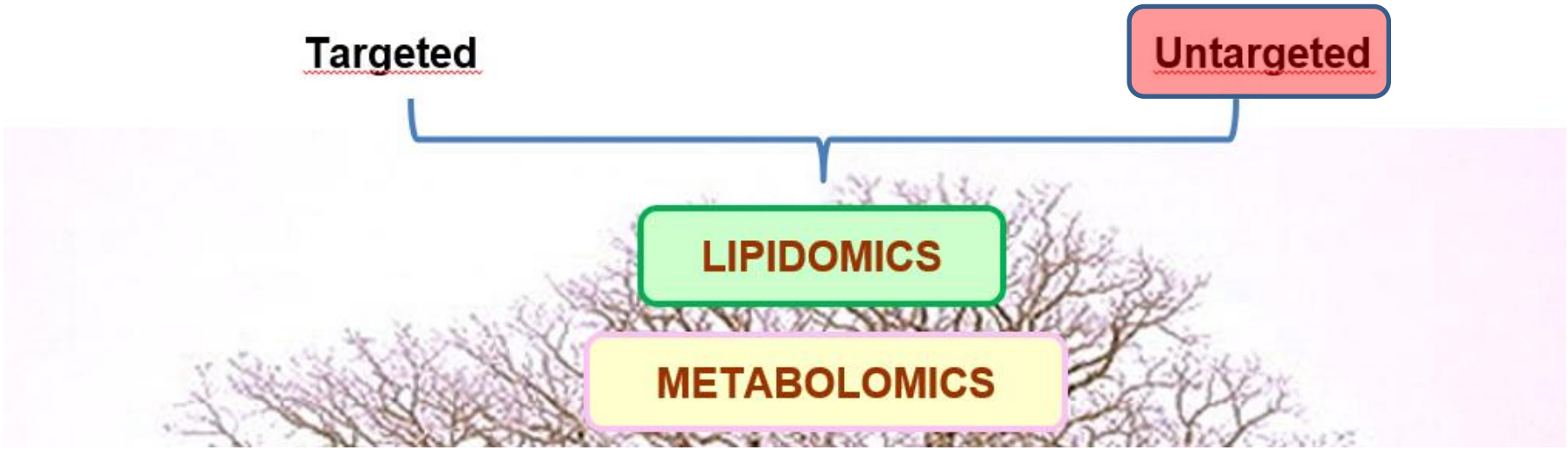
b)





An MS/MS spectra of the ion m/z=878.816 representing characteristic fragment ions to support for the identification of TAG 52:1

Several thousands of lipids must be identifyed (up to 1000000)



Identification of 10 lipids may require a days of work
For 2000 lipids one need 6-12 months time !!!

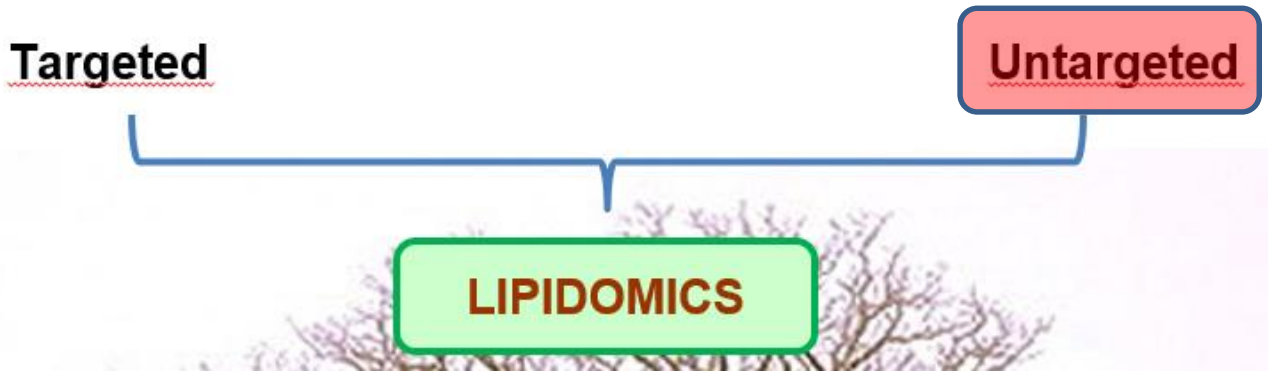
Proposed solution: database of commercial lipids

However: 1000 lipids availables = 0.1% potential lipids

very high cost (1 mg – 250 USD)

low stability (oxydation also at -80°C)

Several thousands of lipids must be identifyed (up to 1000000)



Identification of 10 lipids may require a days of work

For 2000 lipids one need 6-12 months time !!!

Proposed solution:

Article

Lipostar, a comprehensive platform-neutral cheminformatics tool for lipidomics

analytical chemistry

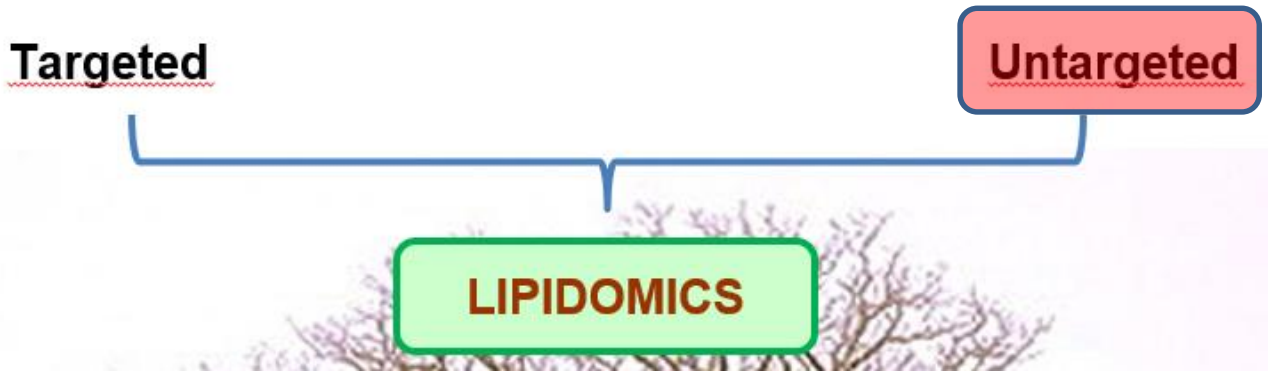
Laura Goracci, Sara Tortorella, Paolo Tiberi, Roberto Maria Pellegrino, Alessandra Di Veroli, Aurora Valeri, and Gabriele Cruciani

Anal. Chem., **Just Accepted Manuscript** • Publication Date (Web): 04 May 2017

Downloaded from http://pubs.acs.org on May 4, 2017

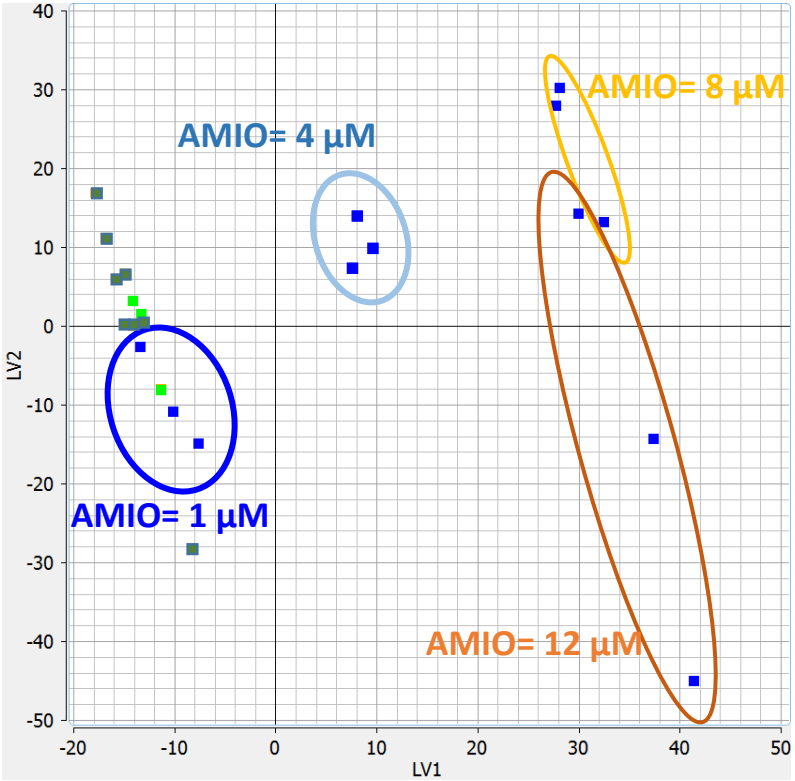
LipoStar is doing in less than a minute

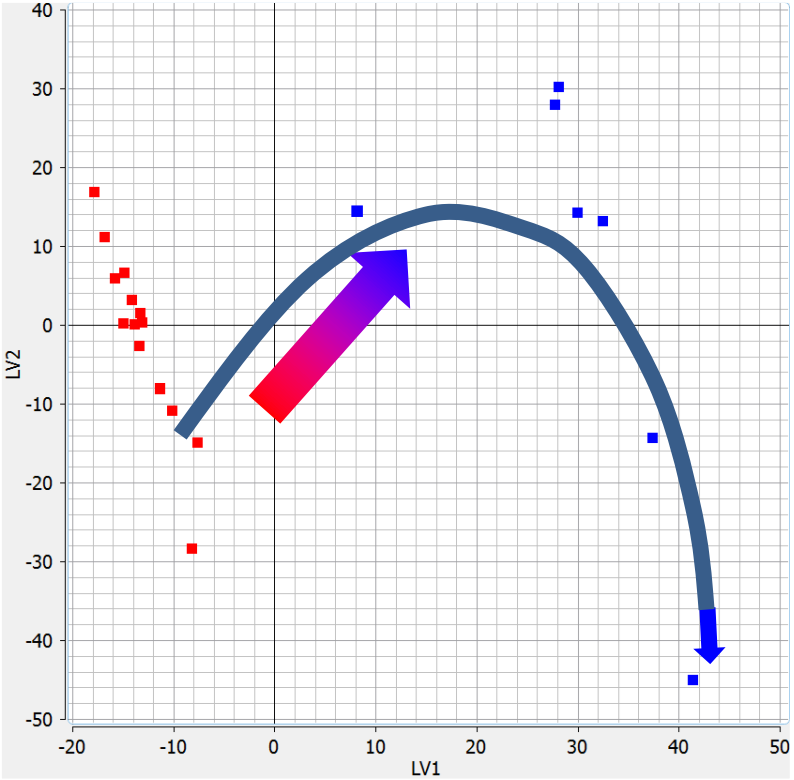
Several thousands of lipids must be identifyed (up to 1000000)



Lipidomic research in public and pharmaceutical industries

Lipids modification in liver Amiodarone (***Cholestasis***)



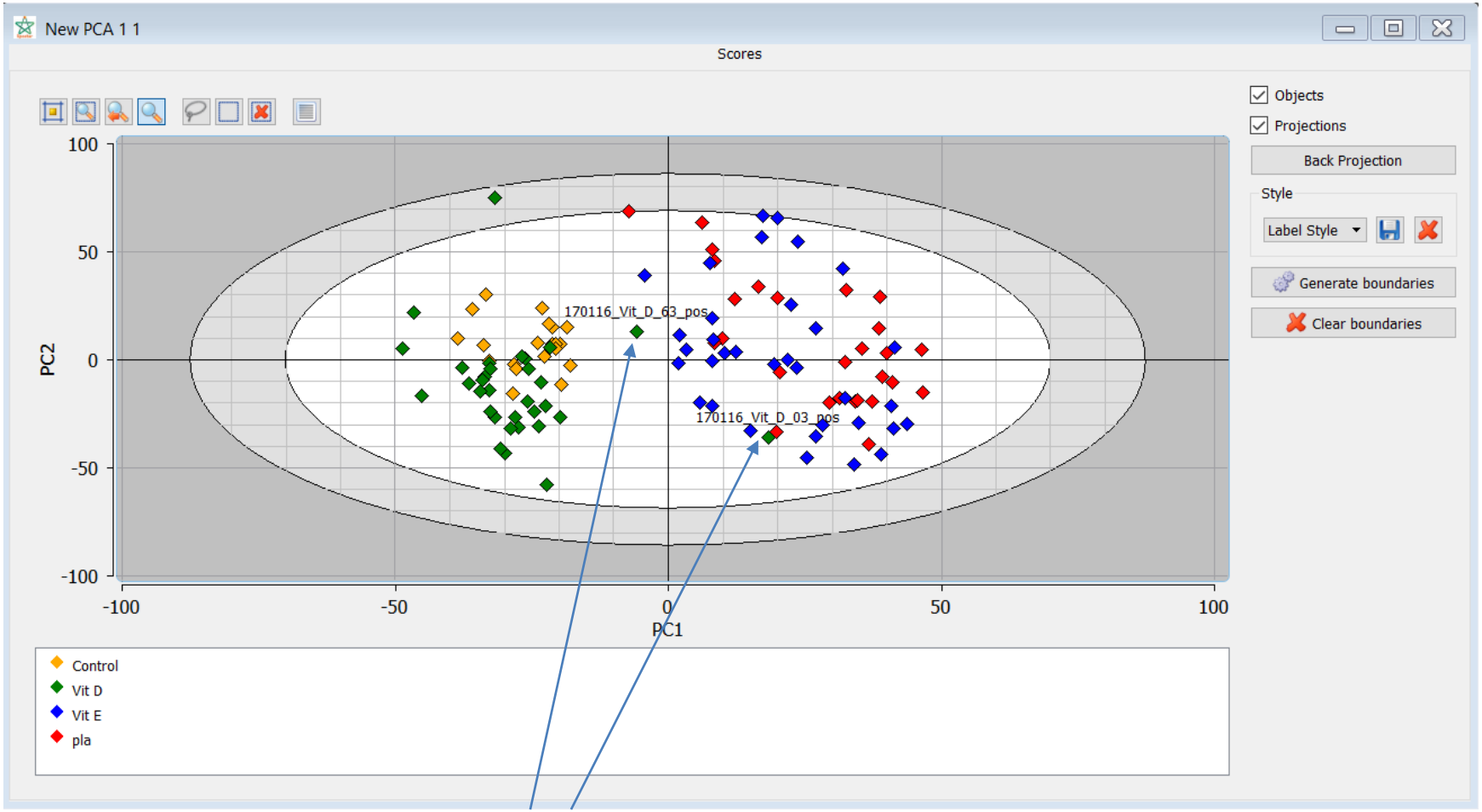


- Amiodarone (1, 4, 8, 12 µM)
- Cimetidine (4, 20, 40 µM)
- CTR-CTRV

Experiments show the tox-dose level

In collaboration with Roche

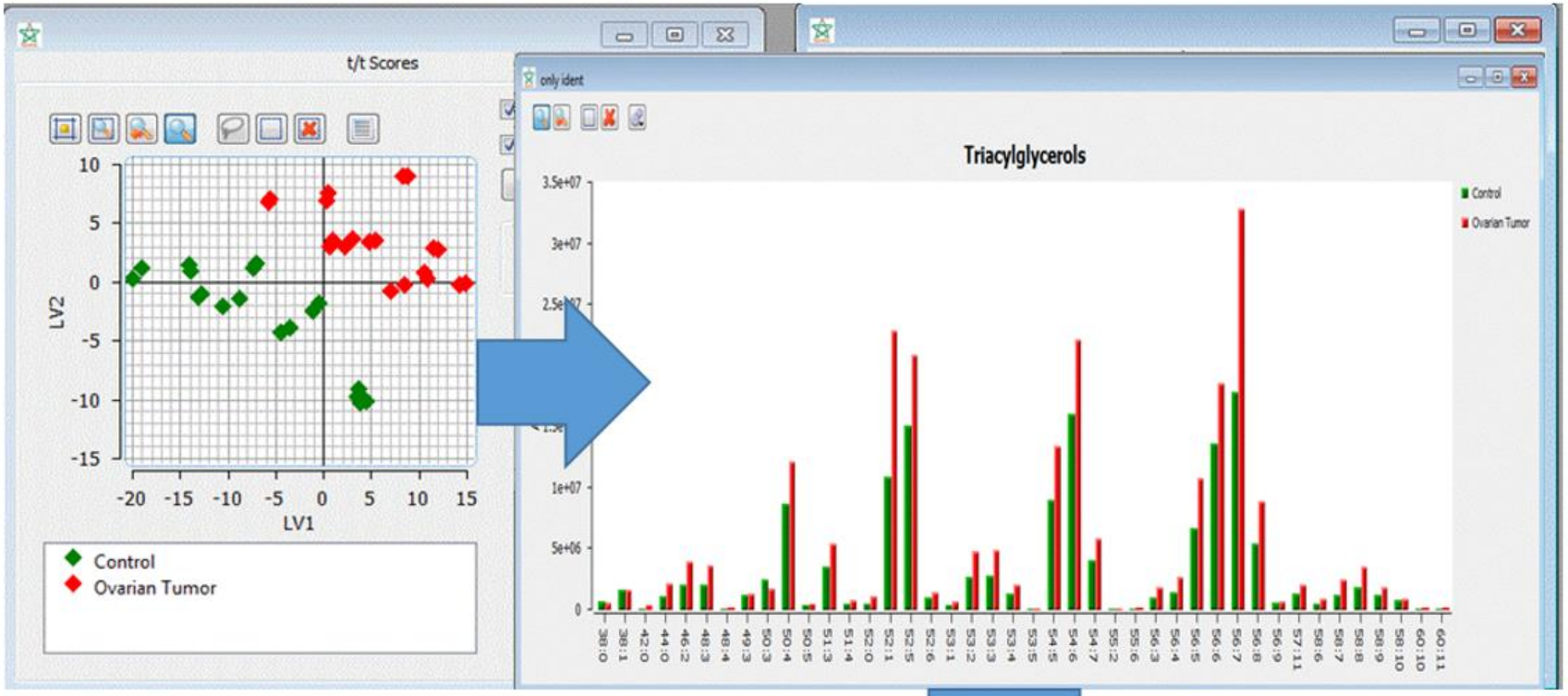
Steatosis: blood samples

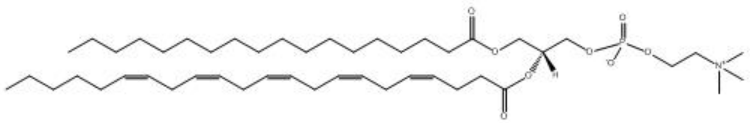


«Campioni anomali»: Vit_D_ 63 e Vit_D_3

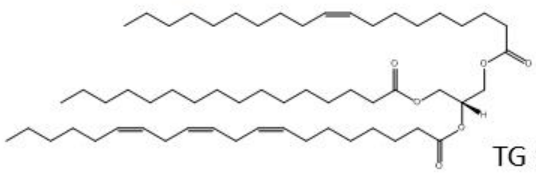
In collaboration with Bambino Gesù OSPEDALE PEDIATRICO

Ovarian cancer – serum samples





PC 22:5-ω6/18:0



TG 16:0/20:3-ω6/18:1

In collaboration with

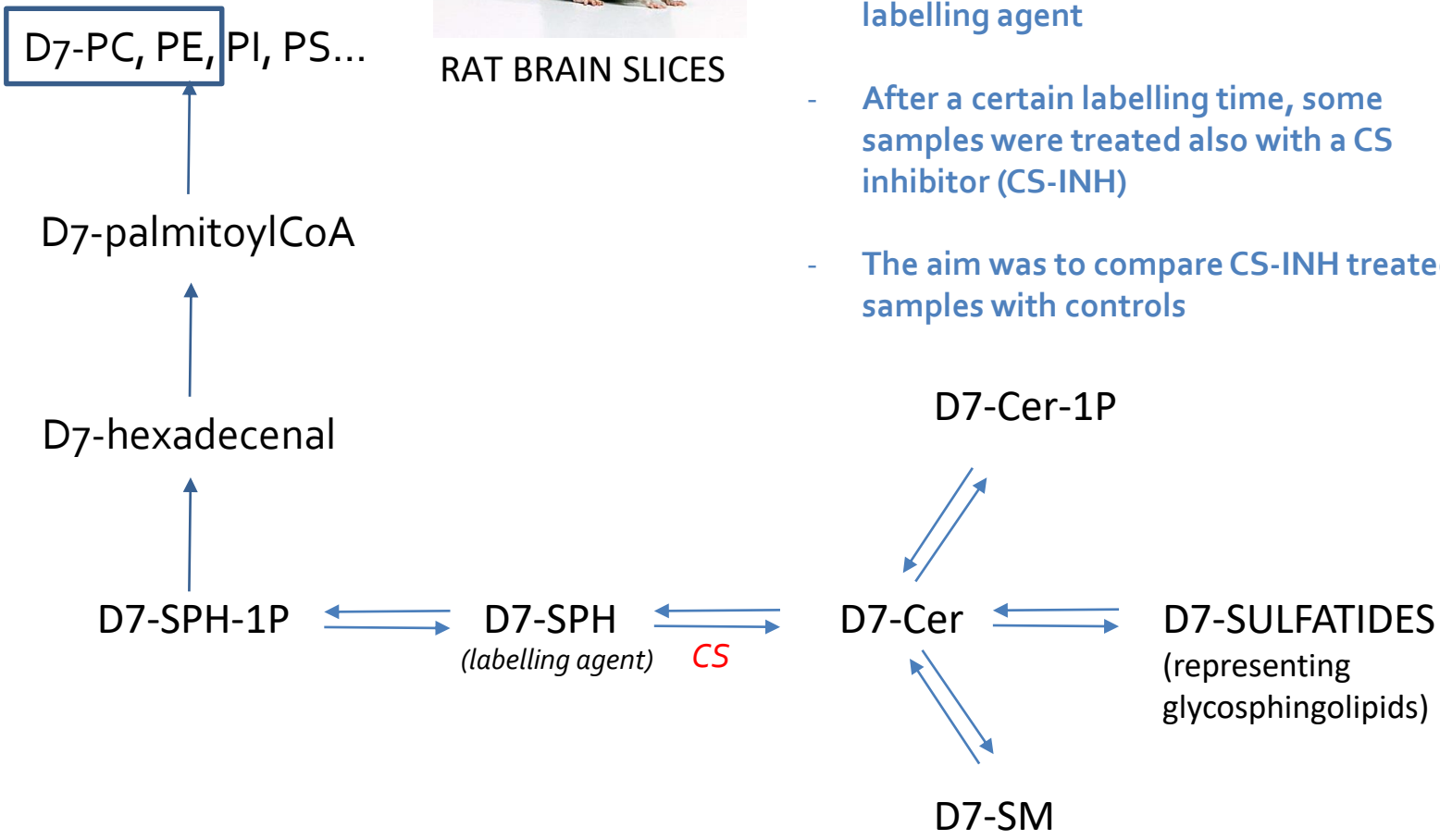
ISTITUTO NAZIONALE TUMORI
IRCCS - Fondazione Pascale

Anticancer compound - Ceramide Sinthase (CS) inhibitor

CS INH

RAT BRAIN SLICES

- Rat brain slices were treated with deuterated sphingosine (D7-SPH), as labelling agent
- After a certain labelling time, some samples were treated also with a CS inhibitor (CS-INH)
- The aim was to compare CS-INH treated samples with controls



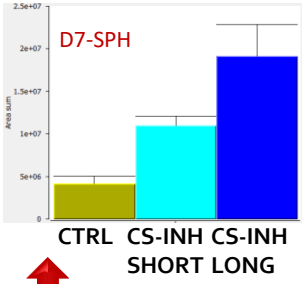
D7-PC, PE, PI, PS…

D7-palmitoylCoA

D7-hexadecenal

D7-SPH-1P

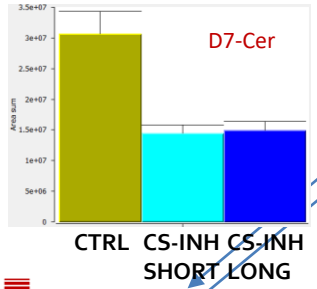
CTRL= controls
CS-INH SHORT= treated with CS-INH for a short time
CS-INH LONG = treated with CS-INH for a long time



D7-SPH
(labelling agent)

CS INH

CS

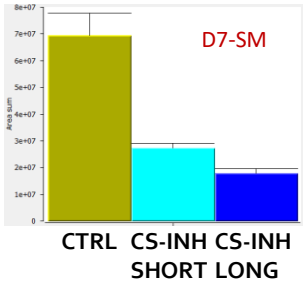


D7-Cer

D7-Cer-1P

D7-SULFATIDES
(representing glycosphingolipids)

D7-SM



The effect of the inhibitor on the CS is confirmed

LipoTox Technology Platform

LipoStar®
Fingerprinting System

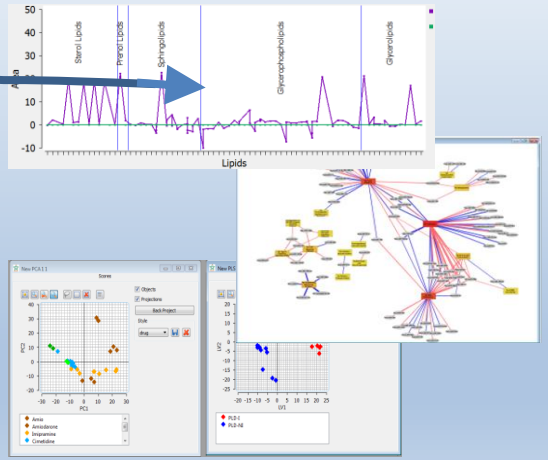
Lipidomics in 3D-Human microtissues (hepatocytes)

Reference
(DILI) Profile Database

Trovan	Most-DILI-concern
Mervan	Most-DILI-concern
Ananxyl	Most-DILI-concern
Survector	Most-DILI-concern
Symmetrel	Less -DILI-concern
Imuran	Less -DILI-concern
Paraplatin	Less -DILI-concern
Proventil	No -DILI-concern
Fosamax	No -DILI-concern
Ethyol	No -DILI-concern
Tracrium	No -DILI-concern

Biomarker responses to drugs are stored in the database >300 drugs

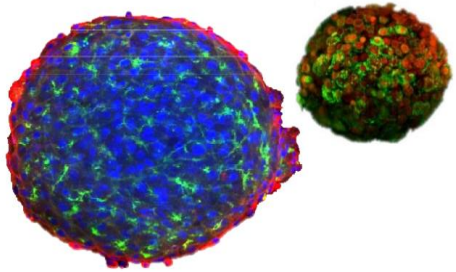
Predictive
Cheminformatic Tool



Informatics tools are used to predict clinical outcomes

High Throughput Human Lipidomics

lipostar



Confident decision making

- ✓ *In vivo*-like morphology using 3D co-cultures
- ✓ Relevant and robust functionality
- ✓ Long lifetime allows to capture long-term effects

… delivered in a multiwell standard format

- ✓ Scientists can fully focus or generating relevant data
- ✓ Seamless integration

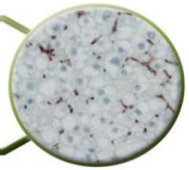
insphero

***In vivo* relevance meets *in vitro* convenience**

Portfolio of assay-ready microtissues

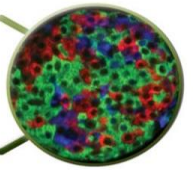
LIVER

- Human
- Rat
- Dog*
- Monkey*
- Minipig*
- HepG2



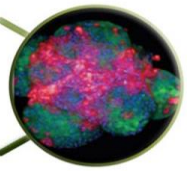
PANCREAS

- Human
- Rat*



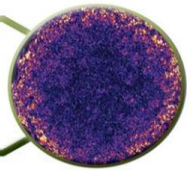
TUMOR

- Monoculture
- Co-culture
- Fluorescent



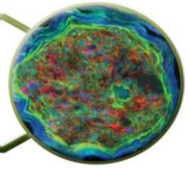
HEART

- Human (iPS)*



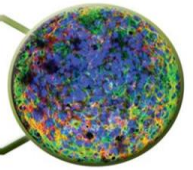
SKIN

- Dermal*
- Dermal-Epidermal co-culture*

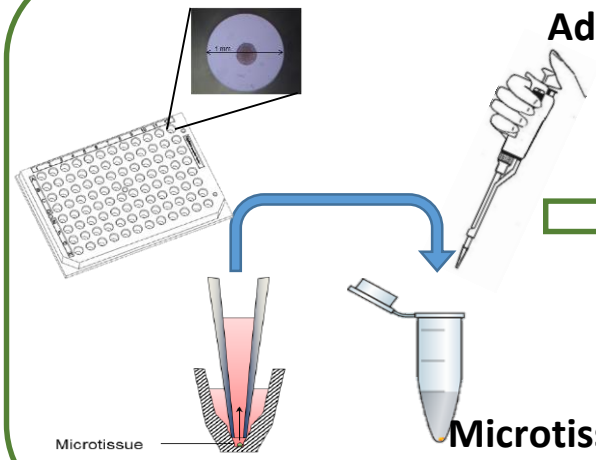


BRAIN

- Rat*



One pot Lipid extraction from 3D microtissues



Adding DCP

Microtissue retrival

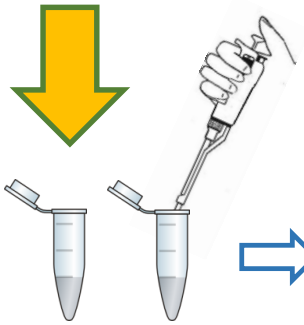
Centrifugation

Washing twice with PBS

Lipid extraction with MMC* (Methanol:MTBE:Chloroform) method on cells pellet

MetID

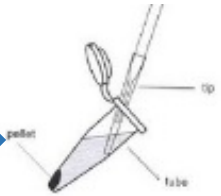
MMC extraction method



Adding of 150 µL of MMC mixture

Vortex and incubation

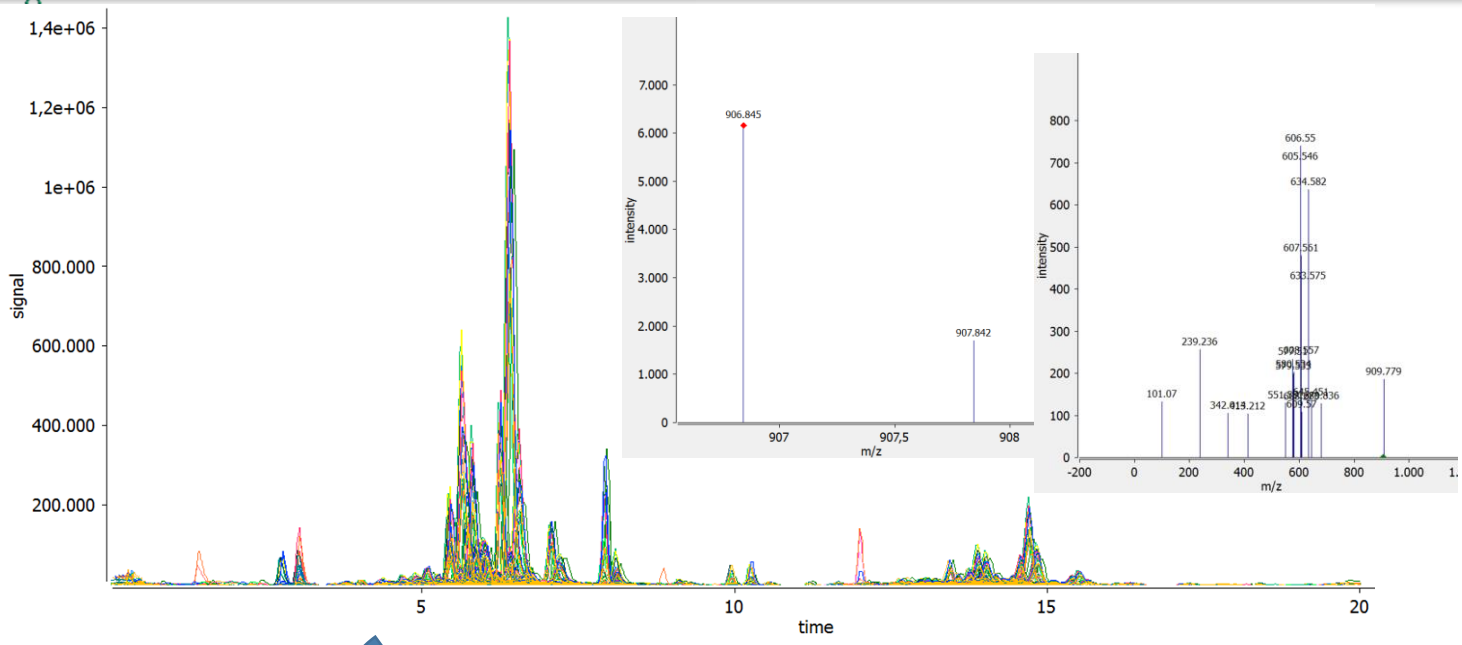
Centrifugation



Separation of supernatant

LC/MS Q TOF Analysis

* Anal Bioanal Chem. 2014 Dec;406(30), Pellegrino RM, Di Veroli A, Valeri A, Goracci L, Cruciani G.



lipostar

	750.4067@10.48	750.4069@10.50	870.4271@10.50	546.4879@10.50	647.4589@10.50	752.4067@10.50	1355.8301@10.50	1072.302
AMIO_150717_P_HEPG2_12_r1	15.3368 / 27	14.7236 / 18	13.4876 / 9	12.485 / 0	18.4137 / 35	14.07 / 0	12.3902 / 10	0 / 0
AMIO_150717_P_HEPG2_12_r2	15.3459 / 26	0 / 0	13.8561 / 17	12.3068 / 0	18.3132 / 34	13.8655 / 0	12.5033 / 11	0 / 0
AMIO_150717_P_HEPG2_12_r3	15.3869 / 25	14.6987 / 14	14.0833 / 5	11.9959 / 0	18.5292 / 51	14.2372 / 0	12.9484 / 11	8.24776 /
AMIO_150717_P_HEPG2_1_r1	15.6055 / 28	15.3904 / 26	13.7478 / 0	14.5564 / 8	18.4251 / 58	14.5766 / 0	12.9808 / 13	11.7484 /
AMIO_150717_P_HEPG2_1_r2	15.5152 / 36	15.2734 / 29	13.5487 / 14	13.5917 / 5	18.5793 / 47	14.5861 / 0	12.5329 / 11	11.2301 /
AMIO_150717_P_HEPG2_1_r3	15.6148 / 27	15.3392 / 21	13.7556 / 0	14.006 / 5	18.4776 / 40	14.3653 / 0	13.2623 / 13	12.1327 /
AMIO_150717_P_HEPG2_8_r1	15.4054 / 30	14.8846 / 22	13.783 / 11	13.6189 / 0	18.4224 / 41	14.3851 / 0	12.9361 / 11	10.356 /
AMIO_150717_P_HEPG2_8_r2	15.4025 / 30	14.8493 / 21	14.0566 / 19	13.2869 / 0	18.4619 / 35	14.3506 / 0	12.9602 / 12	0 / 0
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CIME_150717_P_HEPG2_20_r1	15.3651 / 24	15.0548 / 19	13.9319 / 16	11.2981 / 0	18.3762 / 38	14.3609 / 0	13.0447 / 13	12.0573 /
CIME_150717_P_HEPG2_20_r2	15.1801 / 26	14.6686 / 14	13.7485 / 6	11.4934 / 0	18.5461 / 41	14.2192 / 0	12.4838 / 11	10.9492 /
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CIME_150717_P_HEPG2_4_r3	15.3423 / 27	14.1101 / 17	13.9639 / 16	12.295 / 0	18.4929 / 39	13.2173 / 0	12.9168 / 14	12.5506 /
CtrlVeh_150717_P_HEPG2_r1	15.4114 / 31	14.9076 / 20	13.9225 / 11	11.933 / 0	18.9157 / 44	14.1761 / 0	13.1414 / 10	0 / 0
CtrlVeh_150717_P_HEPG2_r2	15.3953 / 26	14.9107 / 14	14.3844 / 11	12.6019 / 0	19.2063 / 53	14.2613 / 0	13.4877 / 14	0 / 0
Ctrl_150717_P_HEPG2_r1	15.287 / 25	14.7901 / 17	14.1314 / 15	12.1687 / 0	18.8884 / 49	14.0447 / 0	13.2163 / 10	0 / 0

Peak-detection
Signal/noise data pretreatment
Signal alignment
Structural assignment

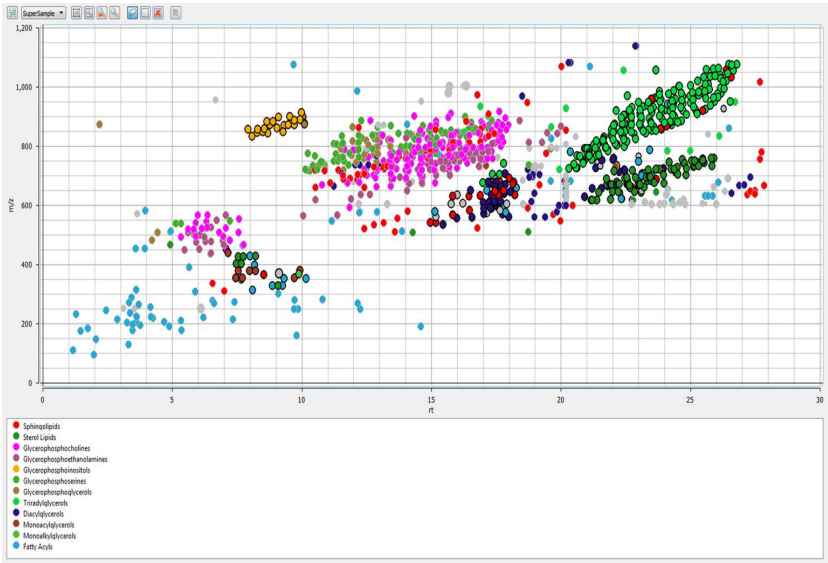
Identification, structural assignment and matrix building

Identification of 10 lipids may require days of work

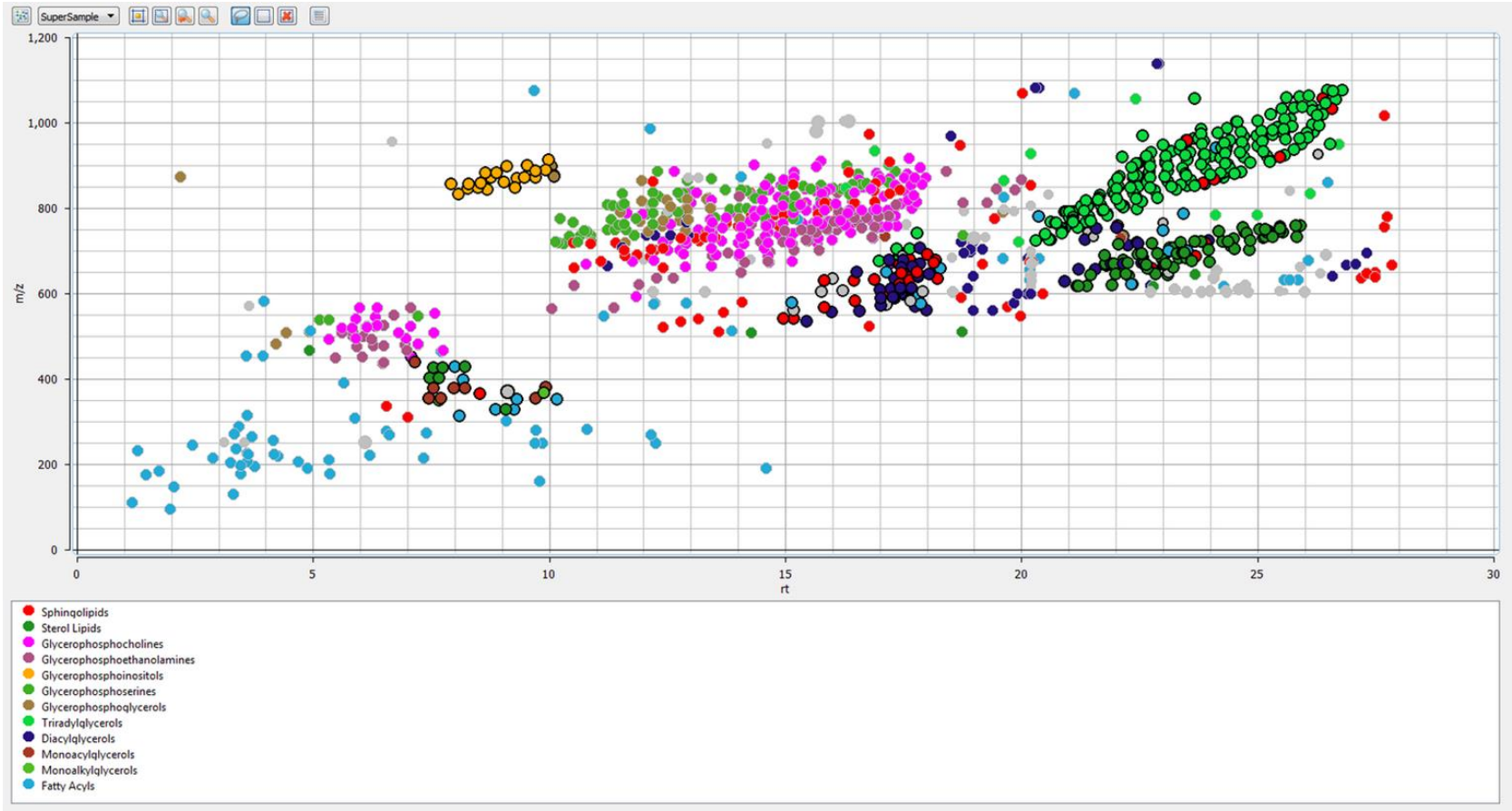
For 2000 lipids one need 6-12 months time !!!

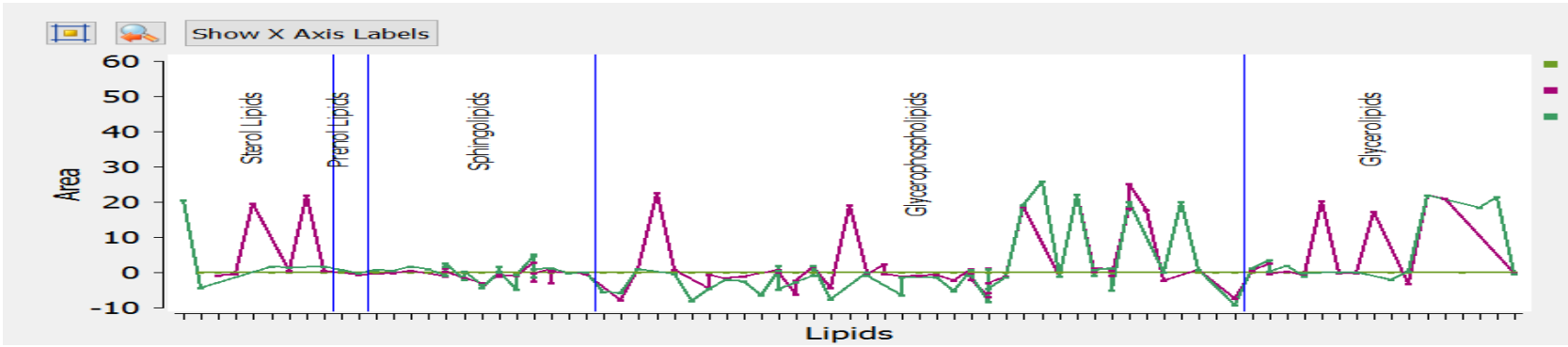
	750.4067@10.48	750.4069@10.50	870.4271@10.50	546.4879@10.50	647.4589@10.50	752.4067@10.50	1355.8301@10.50	1072.302
AMIO_150717_P_HEPG2_12_r1	15.3368 / 27	14.7236 / 18	13.4876 / 9	12.485 / 0	18.4137 / 35	14.07 / 0	12.3902 / 10	0 / 0
AMIO_150717_P_HEPG2_12_r2	15.3459 / 26	0 / 0	13.8561 / 17	12.3068 / 0	18.3132 / 34	13.8655 / 0	12.5033 / 11	0 / 0
AMIO_150717_P_HEPG2_12_r3	15.3869 / 25	14.6987 / 14	14.0833 / 5	11.9959 / 0	18.5292 / 51	14.2372 / 0	12.9484 / 11	8.24776 /
AMIO_150717_P_HEPG2_1_r1	15.6055 / 28	15.3904 / 26	13.7478 / 0	14.5564 / 8	18.4251 / 58	14.5766 / 0	12.9808 / 13	11.7484 /
AMIO_150717_P_HEPG2_1_r2	15.5152 / 36	15.2734 / 29	13.5487 / 14	13.5917 / 5	18.5793 / 47	14.5861 / 0	12.5329 / 11	11.2301 /
AMIO_150717_P_HEPG2_1_r3	15.6148 / 27	15.3392 / 21	13.7556 / 0	14.006 / 5	18.4776 / 40	14.3653 / 0	13.2623 / 13	12.1327 /
AMIO_150717_P_HEPG2_8_r1	15.4054 / 30	14.8846 / 22	13.783 / 11	13.6189 / 0	18.4224 / 41	14.3851 / 0	12.9361 / 11	10.356 /
AMIO_150717_P_HEPG2_8_r2	15.4025 / 30	14.8493 / 21	14.0566 / 19	13.2869 / 0	18.4619 / 35	14.3506 / 0	12.9602 / 12	0 / 0
AMIO_150717_P_HEPG2_8_r3	15.3563 / 28	0 / 0	13.8026 / 17	13.057 / 0	18.4723 / 41	14.2235 / 0	12.6554 / 12	9.74401 /
CIME_150717_P_HEPG2_20_r1	15.3651 / 24	15.0548 / 19	13.9319 / 16	11.2981 / 0	18.3762 / 38	14.3609 / 0	13.0447 / 13	12.0573 /
CIME_150717_P_HEPG2_20_r2	15.1801 / 26	14.6686 / 14	13.7485 / 6	11.4934 / 0	18.5461 / 41	14.2192 / 0	12.4838 / 11	10.9492 /
CIME_150717_P_HEPG2_20_r3	15.3078 / 21	14.7596 / 18	14.0168 / 17	12.1917 / 0	18.4176 / 31	13.7747 / 0	12.3232 / 11	12.3882 /
CIME_150717_P_HEPG2_40_r1	15.3595 / 33	14.9861 / 22	13.5181 / 14	12.1673 / 0	18.3477 / 48	14.089 / 0	12.7252 / 10	0 / 0
CIME_150717_P_HEPG2_40_r2	15.3927 / 22	14.9086 / 17	13.7314 / 10	12.1253 / 0	18.6289 / 43	14.105 / 0	12.7077 / 14	10.0178 /
CIME_150717_P_HEPG2_40_r3	15.2207 / 23	14.7107 / 16	13.8097 / 6	11.9175 / 0	18.5722 / 42	14.4094 / 0	12.0916 / 10	11.0267 /
CIME_150717_P_HEPG2_4_r1	15.3621 / 29	14.2811 / 17	13.8786 / 17	11.688 / 0	18.2823 / 56	14.289 / 0	12.5657 / 12	11.0957 /
CIME_150717_P_HEPG2_4_r2	15.2432 / 30	14.0426 / 15	13.4476 / 14	12.2778 / 0	17.7783 / 29	13.6723 / 0	11.7084 / 0	11.387 /
CIME_150717_P_HEPG2_4_r3	15.3423 / 27	14.1101 / 17	13.9639 / 16	12.295 / 0	18.4929 / 39	13.2173 / 0	12.9168 / 14	12.5506 /
CtrlVeh_150717_P_HEPG2_r1	15.4114 / 31	14.9076 / 20	13.9225 / 11	11.933 / 0	18.9157 / 44	14.1761 / 0	13.1414 / 10	0 / 0
CtrlVeh_150717_P_HEPG2_r2	15.3953 / 26	14.9107 / 14	14.3844 / 11	12.6019 / 0	19.2063 / 53	14.2613 / 0	13.4877 / 14	0 / 0
Ctrl_150717_P_HEPG2_r1	15.287 / 25	14.7901 / 17	14.1314 / 15	12.1687 / 0	18.8884 / 49	14.0447 / 0	13.2163 / 10	0 / 0

lipid01 lipid02 lipid03 lipid04

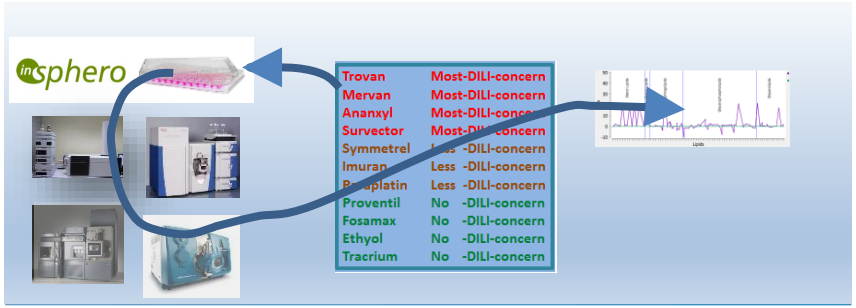


LipoStar is doing in less than a minute

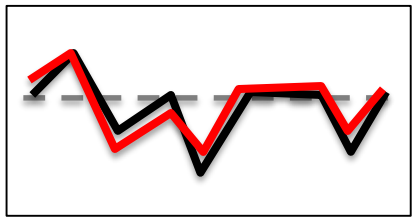

SuperSample
m/z
rt
Sphingolipids
Sterol Lipids
Glycerophosphocholines
Glycerophosphoethanolamines
Glycerophosphoinositols
Glycerophosphoserines
Glycerophosphoglycerols
Triradylglycerols
Diacylglycerols
Monoacylglycerols
Monoalkylglycerols
Fatty Acyls


Show X Axis Labels
Area
Sterol Lipids
Prenol Lipids
Sphingolipids
Glycerophospholipids
Glycerolipids
Lipids

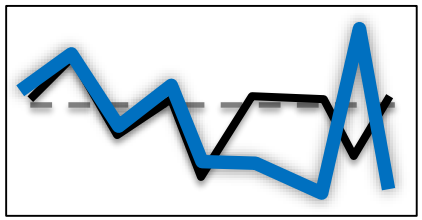
LipoTox Technology Platform



Similarity Analysis of Lipids Profiles



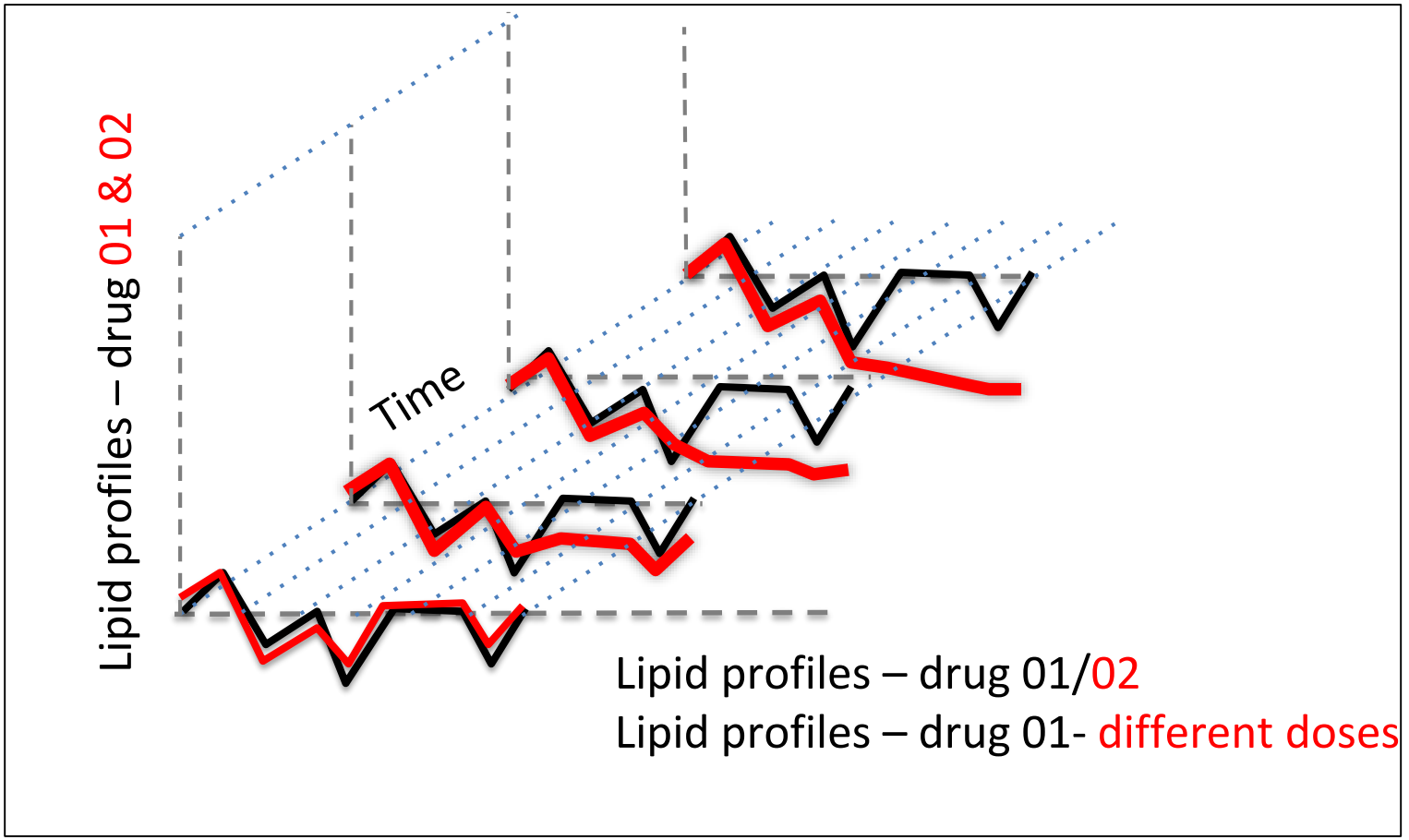
Highly correlated → Similar



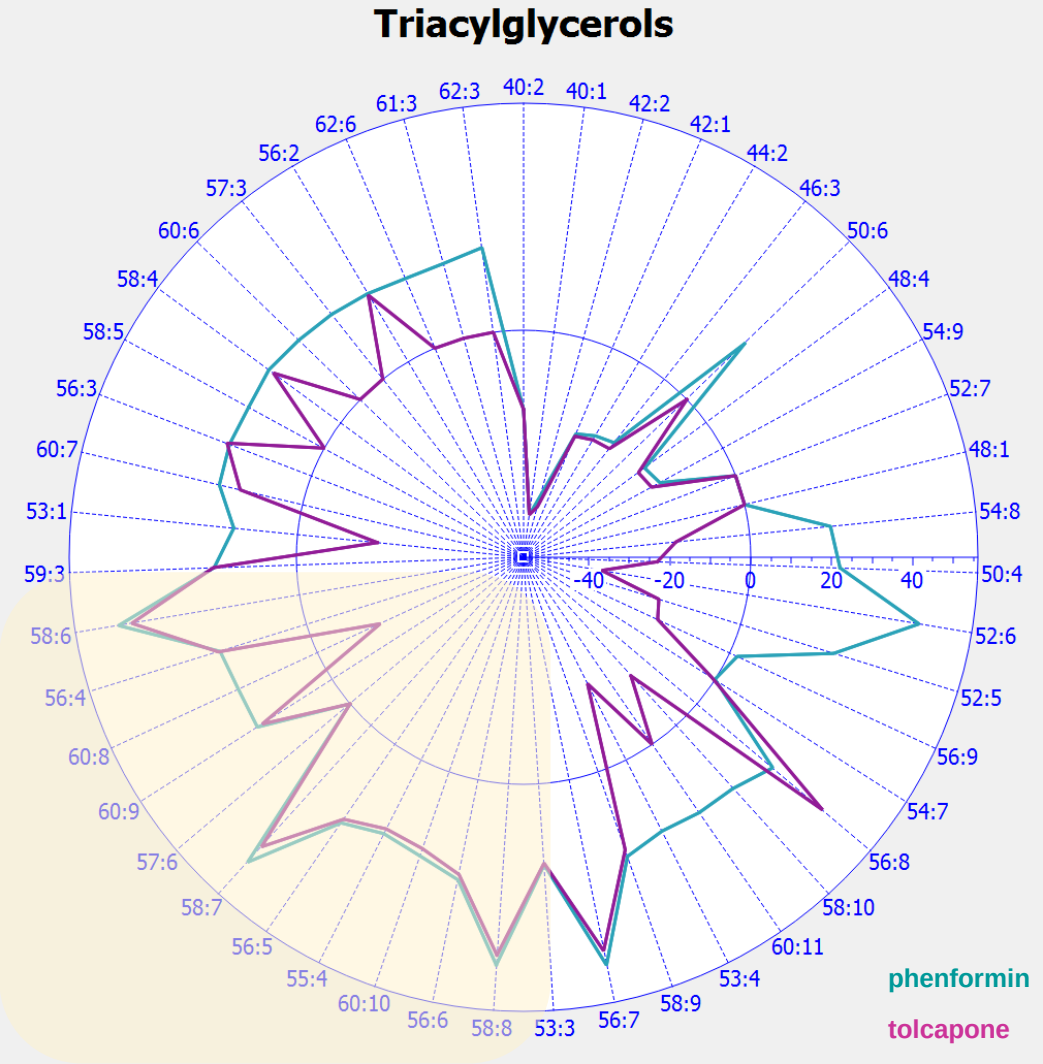
Medium correlated → Almost similar

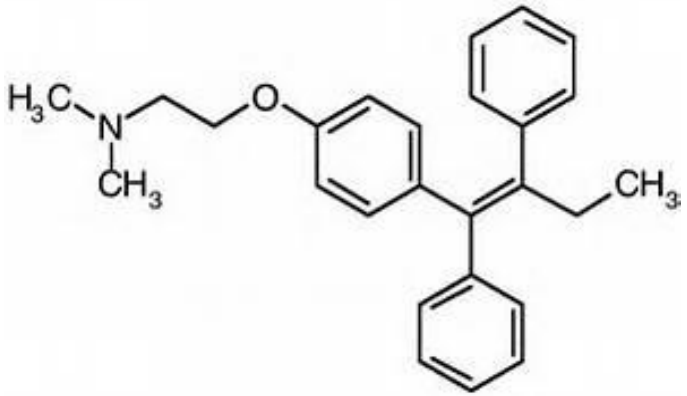
Low correlation → Not similar

Similarity Analysis of Profiles



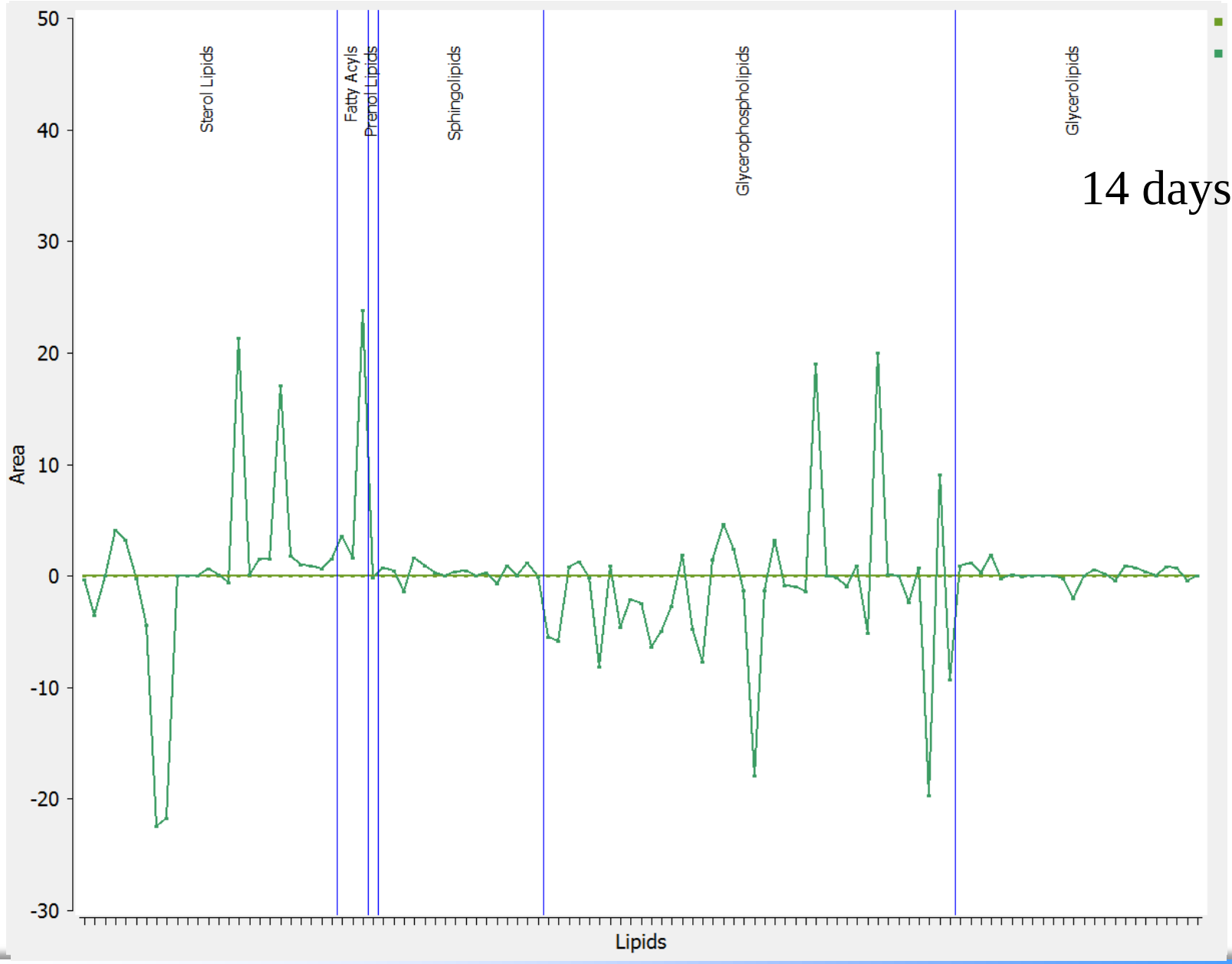
Triacylglycerols profiles for Drug 1 and Drug 2 (negative values are differences from control)

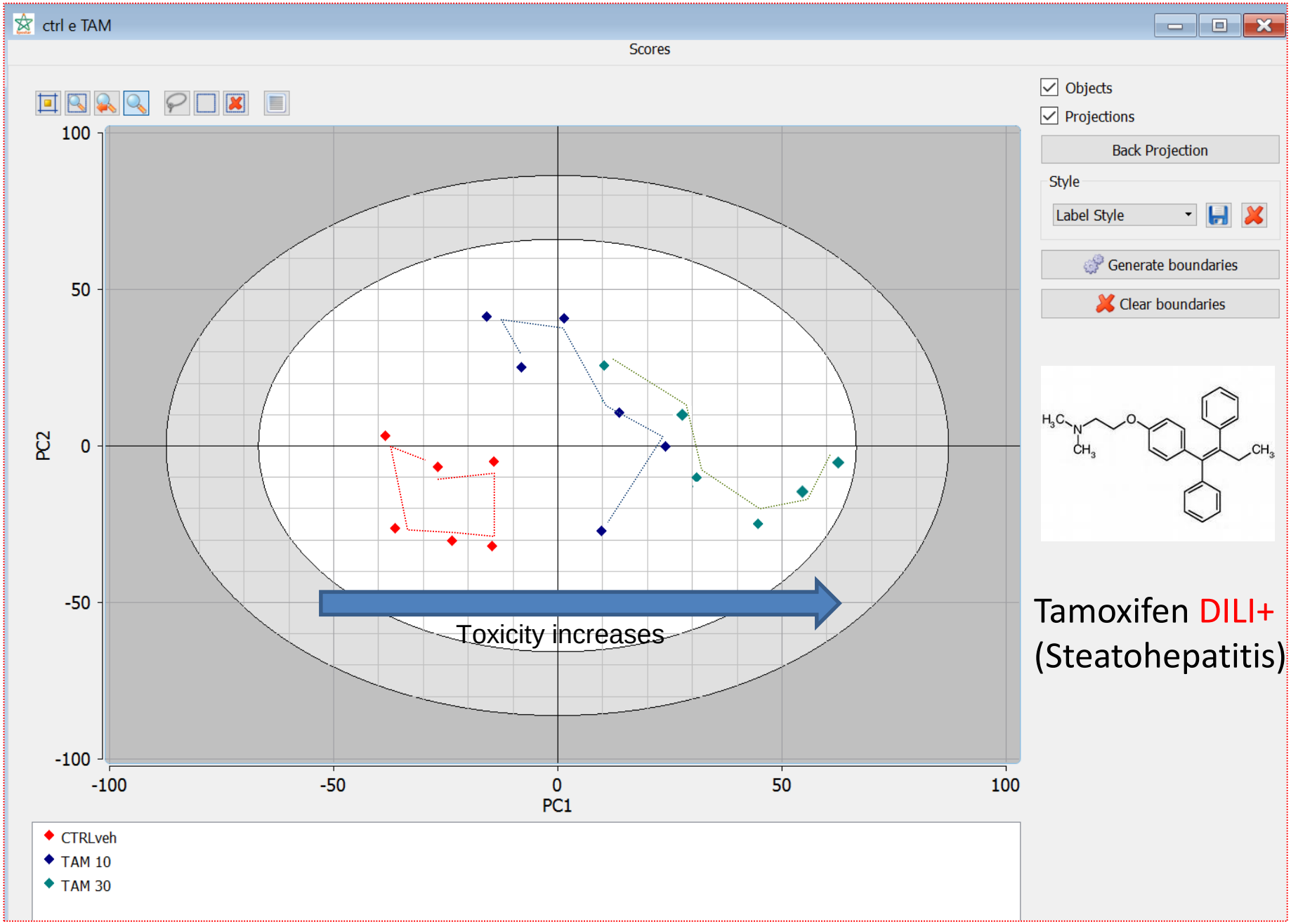




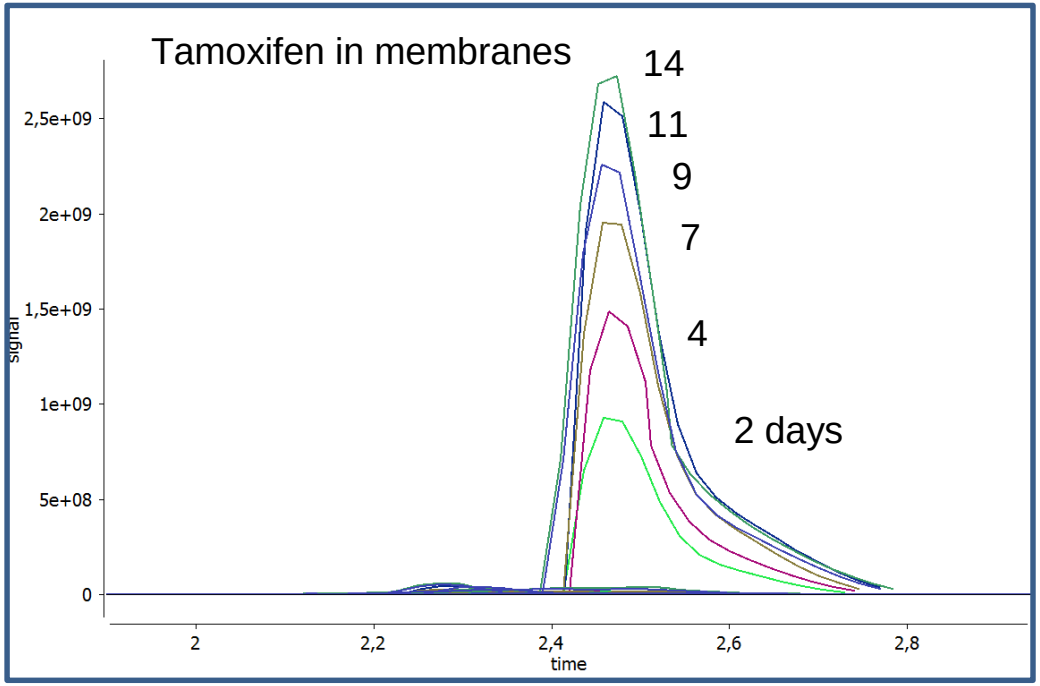
Tamoxifen DILI+
(Steatohepatitis)

LipoTox Technology Platform



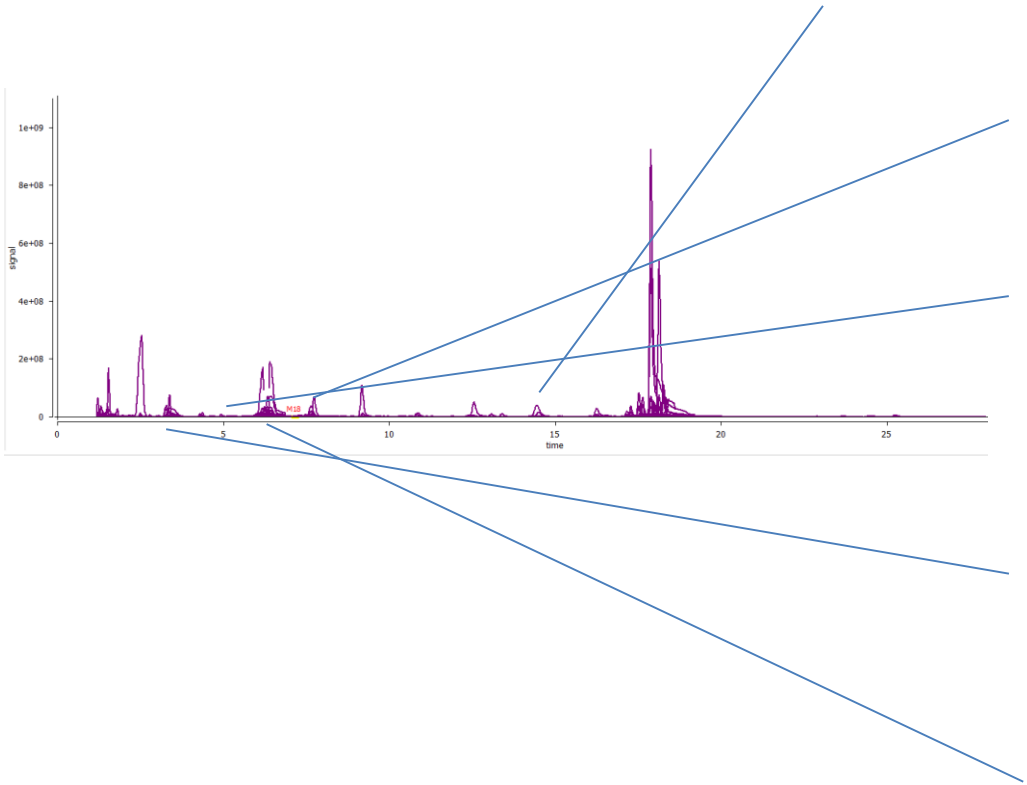

ctrl e TAM
Scores
Objects
Projections
Back Projection
Style
Label Style
Generate boundaries
Clear boundaries
100
50
0
-50
-100
PC2
-100
-50
0
50
100
PC1
Toxicity increases
CTRLveh
TAM 10
TAM 30
Tamoxifen DILI+
(Steatohepatitis)

Drug in membranes



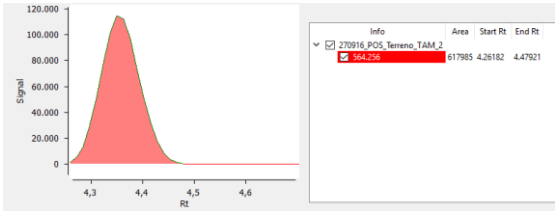
Drug in medium

Tamoxifen in medium



358,2353 in medium

564,2558 in medium



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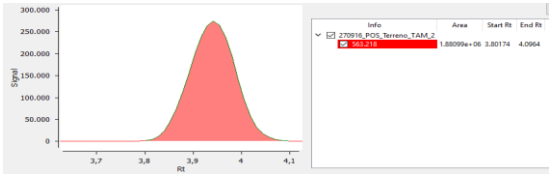
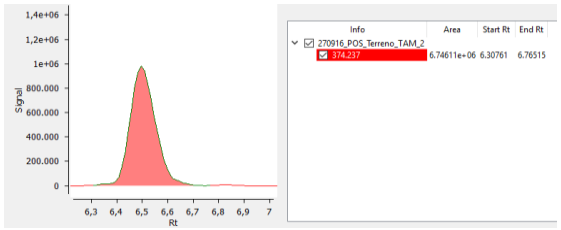
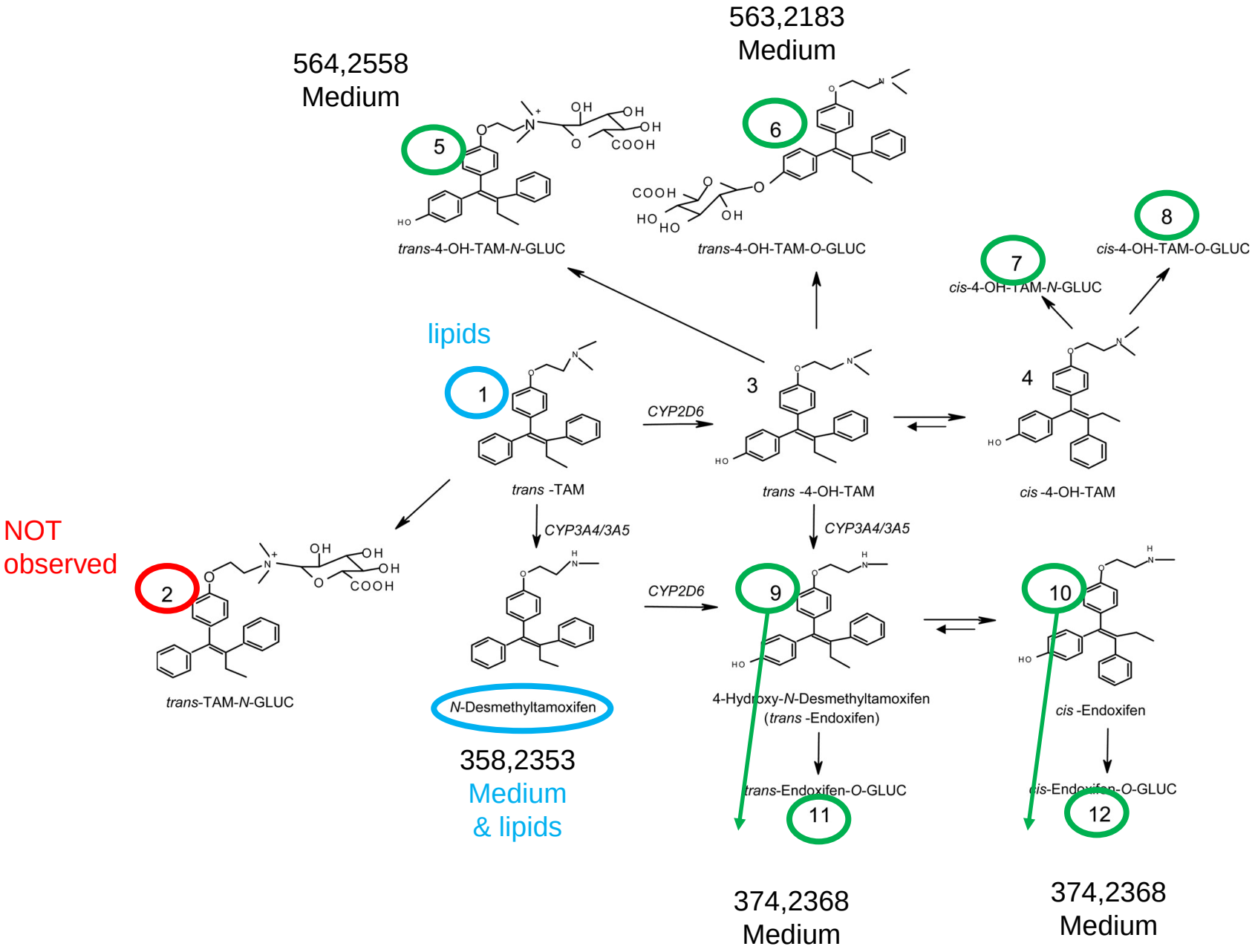


374,2368 in medium



Ultra fast automatic MetID



What Can We Do With LipoTox Profile Data?

- **Analyze overall toxicity profiles**
  - Profile characteristics
  - Unsupervised and supervised approaches to compare profiles
- **Focus on individual endpoints**
  - Correlate to external data
  - Build an understanding of clinical mechanisms

Applications

- **Compound characterization**
  - ADME Property profiles
  - Pathways, possible clinical indications
- **Mechanism of action**
  - Unexpected off-targets (toxicity)
- **Support therapeutic hypotheses**
  - Compare to competitor molecules, clinical standards of care
  - Identify translational biomarkers

Drug Combinations

- **Challenges for studying drug combinations:**
  - System may include more drugs
  - Suitably robust to capture combination effects

Summary

- Lipid profiling in human (or animal) 3D-microtissues generates property profiles (close to disease process) that can be used to:
  - Group chemicals into bio-activity classes
  - Generate mechanicistic hypotheses
  - Identify properties that may correlate with in vivo outcomes
- High throughput *in vitro* data is most informative when combined with available external information
  - Known drugs as reference
  - *In vivo* properties

LipoTox gives biological insight that may be crucial to improve drugs and to reduce time to market substantially.

Thanks to …

Alessandra Di Veroli
Aurora Valeri
Fabien Fontain
Laura Goracci
Massimo Baroni
Paolo Benedetti
Paolo Tiberi
Roberto Pellegrino
Sara Tortorella
Stefano di Bona

Robert Stanton
Simone Sciabola

Molecular Discovery

Rosso di sera .. bel tempo si spera !!
Red sky at night, shepherd's delight
Thanks all …