HelmholtzZentrum münchen
Deutsches Forschungszentrum für Gesundheit und Umwelt

Metabolomics

Gabi Kastenmüller

Helmholtz Zentrum München
Institute of Bioinformatics and Systems Biology

(g.kastenmueller@helmholtz-muenchen.de)

Munich, 17.02.16

HELMHOLTZ
ASSOCIATION

What is metabolomics?

Metabolomics

= analysis of **metabolomes**

Metabolome

= complete set

… of all small-molecules (<1000 Da)

… found within a biological system

(… at a specific time

… under specific conditions)

Metabonomics

= "the quantitative measurement of the dynamic multiparametric metabolic response of living systems to pathophysiological stimuli or genetic modification" (Wikipedia)

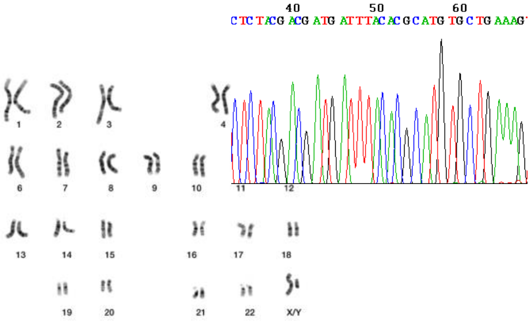
=> **Detection and quantitative measurement of (ideally) all small molecules (= metabolites) in a biological system**

What is metabolomics?

Gen*omics*

… complete set of “genes”

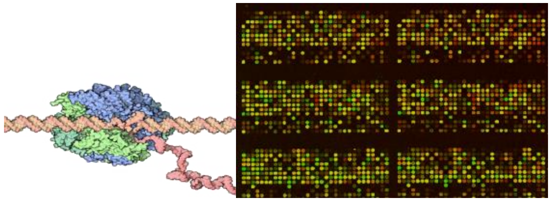
20000-30000 genes



Transcript*omics*

… complete set of transcripts

splicing variants*genes

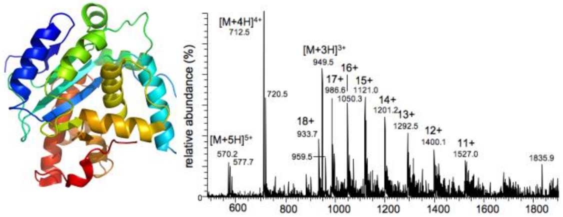


mRNA

Prote*omics*

… complete set of proteins

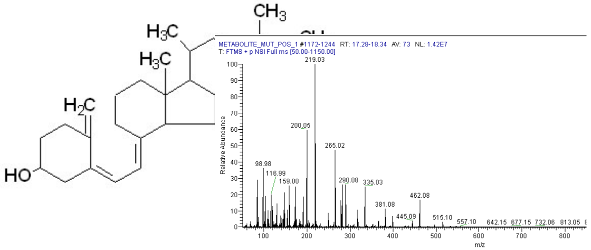
protein modifications*transcripts



Metabol*omics*

…. complete set of metabolites

~2500 (+~3500 food +~1200 drugs)



Why another *–omics*?

Factors

Genetic

Regulatory

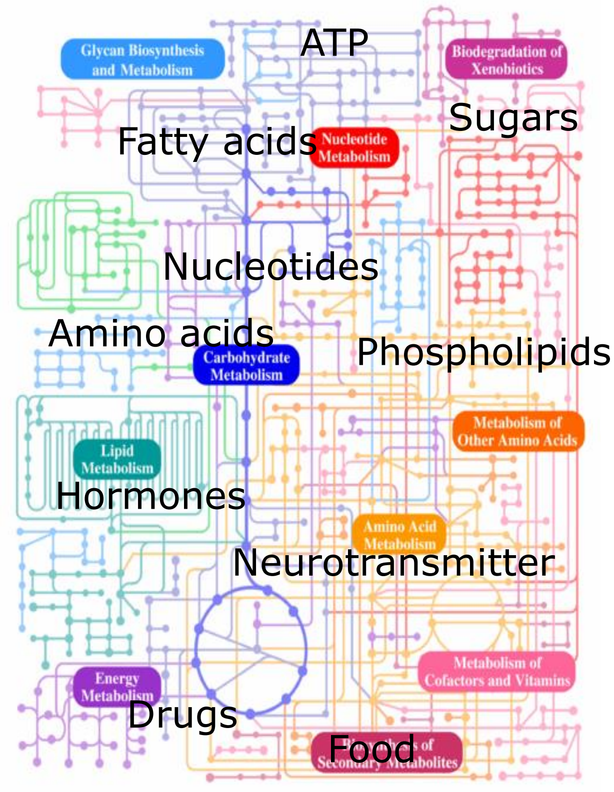
Environmental

Energy

Building Blocks

Signaling

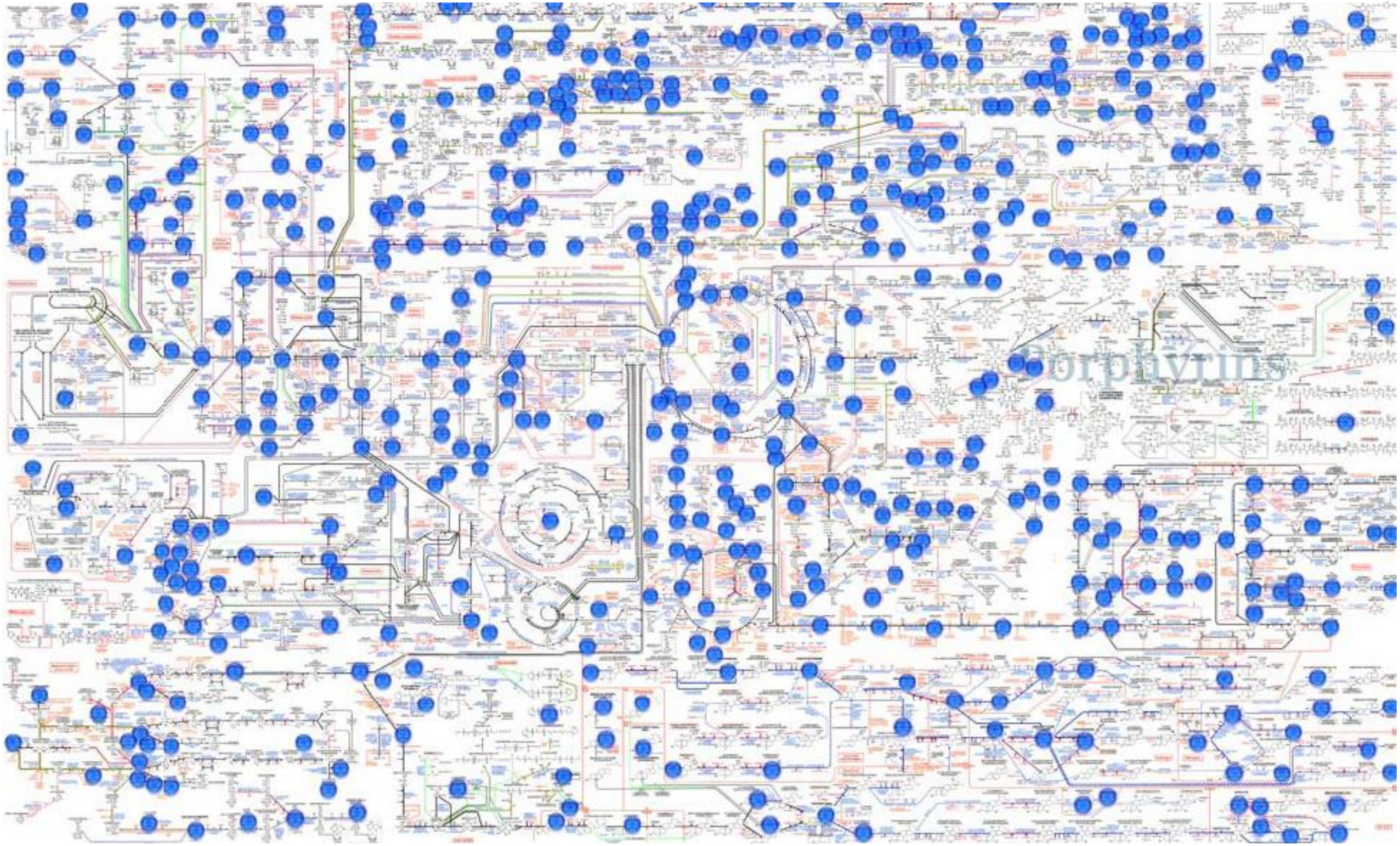
Xenobiotics



© KEGG

Metabolites are the true end points of most biological processes

How can we measure all these metabolites?



© Metabolon

EXPERIMENTAL BACKGROUND

SAMPLE COLLECTION

Sample types

- Blood
- Urine
- Further body fluids

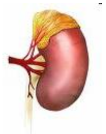
- Cerebrospinal Fluid
- Peritoneal Fluid
- Saliva
- Sweat
- Tears
- Feces
- Breath air/condensate

Sample types

- Blood
- Urine
- Further body fluids
- Tissue
- Cell cultures
- Plant extracts

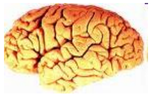
- Liver

- Kidney

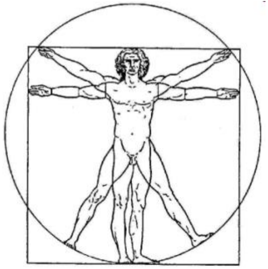


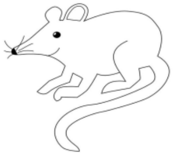
- Muscle

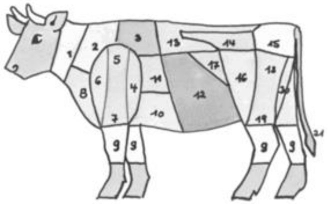
- Brain



- Fat







Sample collection

- Blood
  - Plasma
  - Serum
  - Spots
  - Additives (EDTA, Citrate, Heparin)
  - Storage (N_2, -80°C, -20°C, 4°C, RT)
  - Venous, Capillary, Arterious

Things to think about BEFORE sample collection

- Is there any established metabolomics method for the sample type („matrix)?
- Additives can disturb the measurement (e.g. DNA stabilisors).
- Reactions go on at room temperature

  => standard operating procedures (SOPs) to ensure comparability
- Lab differences might be large

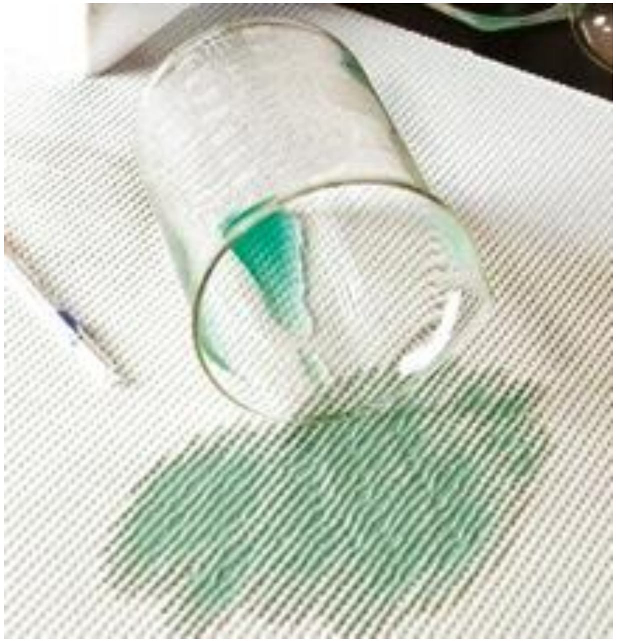
  => cases/controls from all sites

Discuss study design with collaborators for analytics and data analysis!

Sample collection …

… what you loose here

… you will never see (again)!



METABOLITE DETECTION & QUANTIFICATION

Targeted vs non-targeted approaches

Targeted	Non-targeted
Preselected set of metab. signals	All metab. signals that can be detected
• Pros: Known identity; better quant.;	• Pros: New/Unknown metabolites
• Cons: No new metabolites	• Cons: Difficult metabolite identification; less reliable quant
→ **Routine**	→ **Discovery**

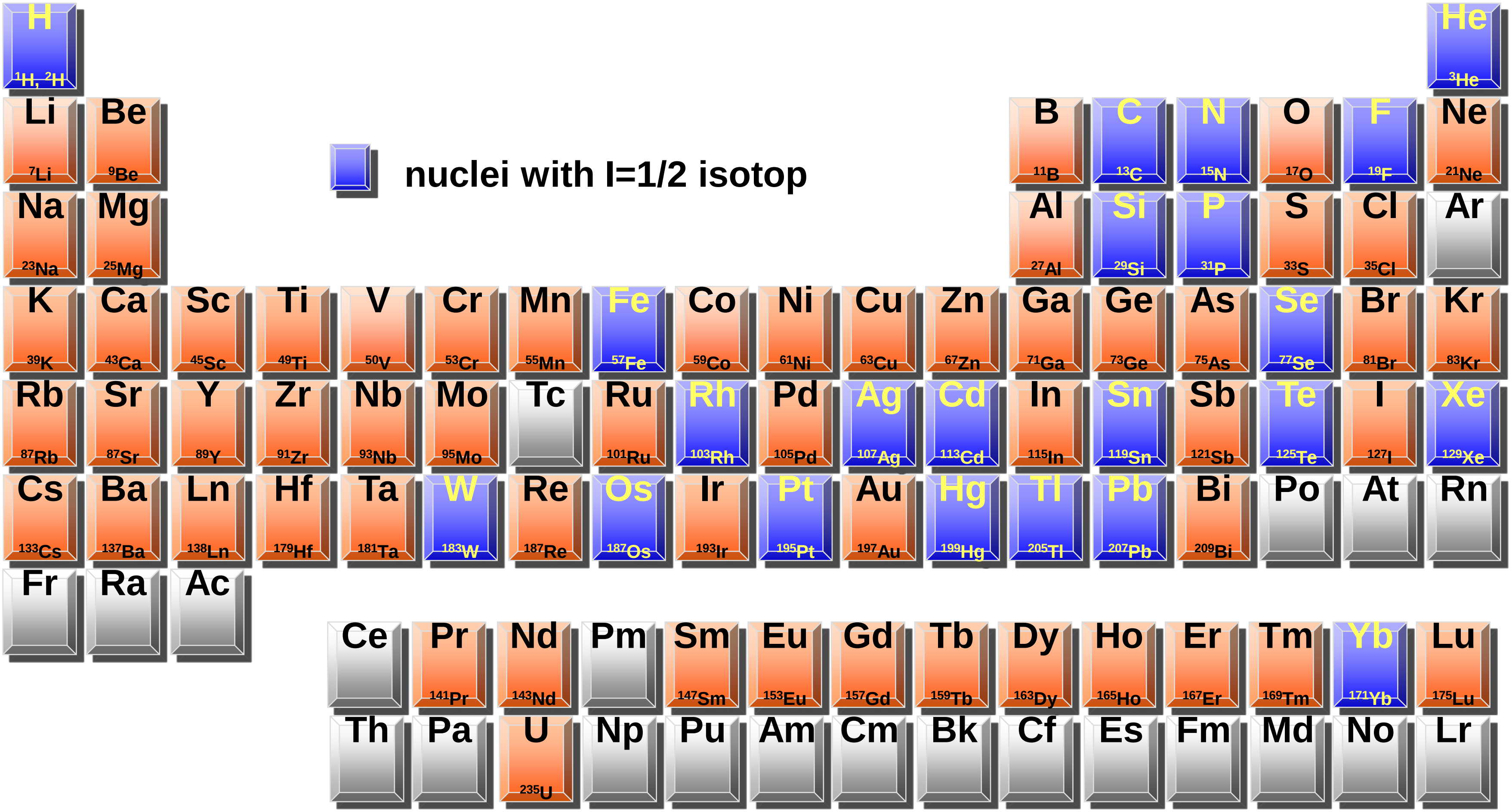
Technologies for high-throughput metabolomics

Mass spectrometry (MS)

Nuclear magnetic resonance (NMR)

NUCLEAR MAGNETIC RESONANCE (NMR)

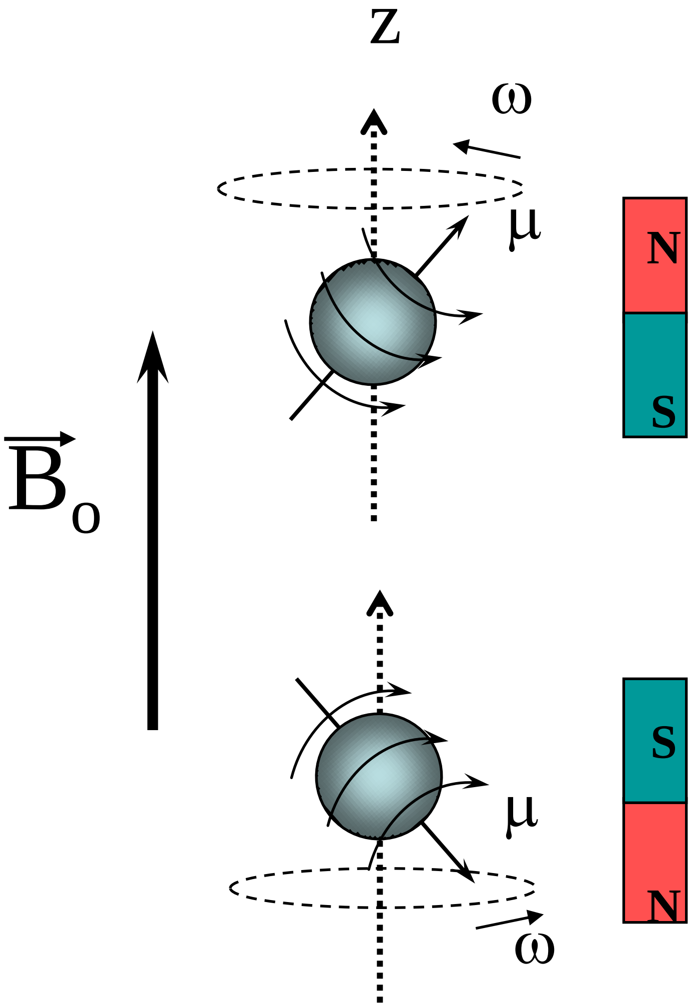
NMR



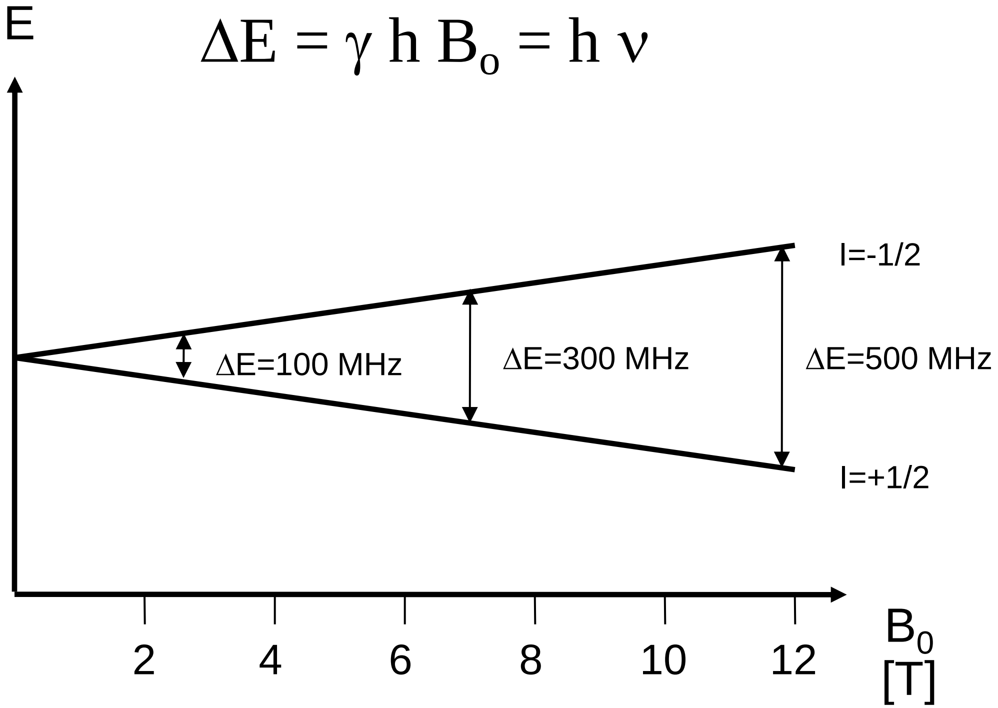
🟦 nuclei with I=1/2 isotop

H ^{1}H, ^{2}H																	He ^{3}He
Li ^{7}Li	Be ^{9}Be											B ^{11}B	C ^{13}C	N ^{15}N	O ^{17}O	F ^{19}F	Ne ^{21}Ne
Na ^{23}Na	Mg ^{25}Mg											Al ^{27}Al	Si ^{29}Si	P ^{31}P	S ^{33}S	Cl ^{35}Cl	Ar
K ^{39}K	Ca ^{43}Ca	Sc ^{45}Sc	Ti ^{49}Ti	V ^{50}V	Cr ^{53}Cr	Mn ^{55}Mn	Fe ^{57}Fe	Co ^{59}Co	Ni ^{61}Ni	Cu ^{63}Cu	Zn ^{67}Zn	Ga ^{71}Ga	Ge ^{73}Ge	As ^{75}As	Se ^{77}Se	Br ^{81}Br	Kr ^{83}Kr
Rb ^{87}Rb	Sr ^{87}Sr	Y ^{89}Y	Zr ^{91}Zr	Nb ^{93}Nb	Mo ^{95}Mo	Tc	Ru ^{101}Ru	Rh ^{103}Rh	Pd ^{105}Pd	Ag ^{107}Ag	Cd ^{113}Cd	In ^{115}In	Sn ^{119}Sn	Sb ^{121}Sb	Te ^{125}Te	I ^{127}I	Xe ^{129}Xe
Cs ^{133}Cs	Ba ^{137}Ba	Ln ^{138}Ln	Hf ^{179}Hf	Ta ^{181}Ta	W ^{183}W	Re ^{187}Re	Os ^{187}Os	Ir ^{193}Ir	Pt ^{195}Pt	Au ^{197}Au	Hg ^{199}Hg	Tl ^{205}Tl	Pb ^{207}Pb	Bi ^{209}Bi	Po	At	Rn
Fr	Ra	Ac															
				Ce	Pr ^{141}Pr	Nd ^{143}Nd	Pm	Sm ^{147}Sm	Eu ^{153}Eu	Gd ^{157}Gd	Tb ^{159}Tb	Dy ^{163}Dy	Ho ^{165}Ho	Er ^{167}Er	Tm ^{169}Tm	Yb ^{171}Yb	Lu ^{175}Lu
				Th	Pa	U ^{235}U	Np	Pu	Am	Cm	Bk	Cf	Es	Fm	Md	No	Lr

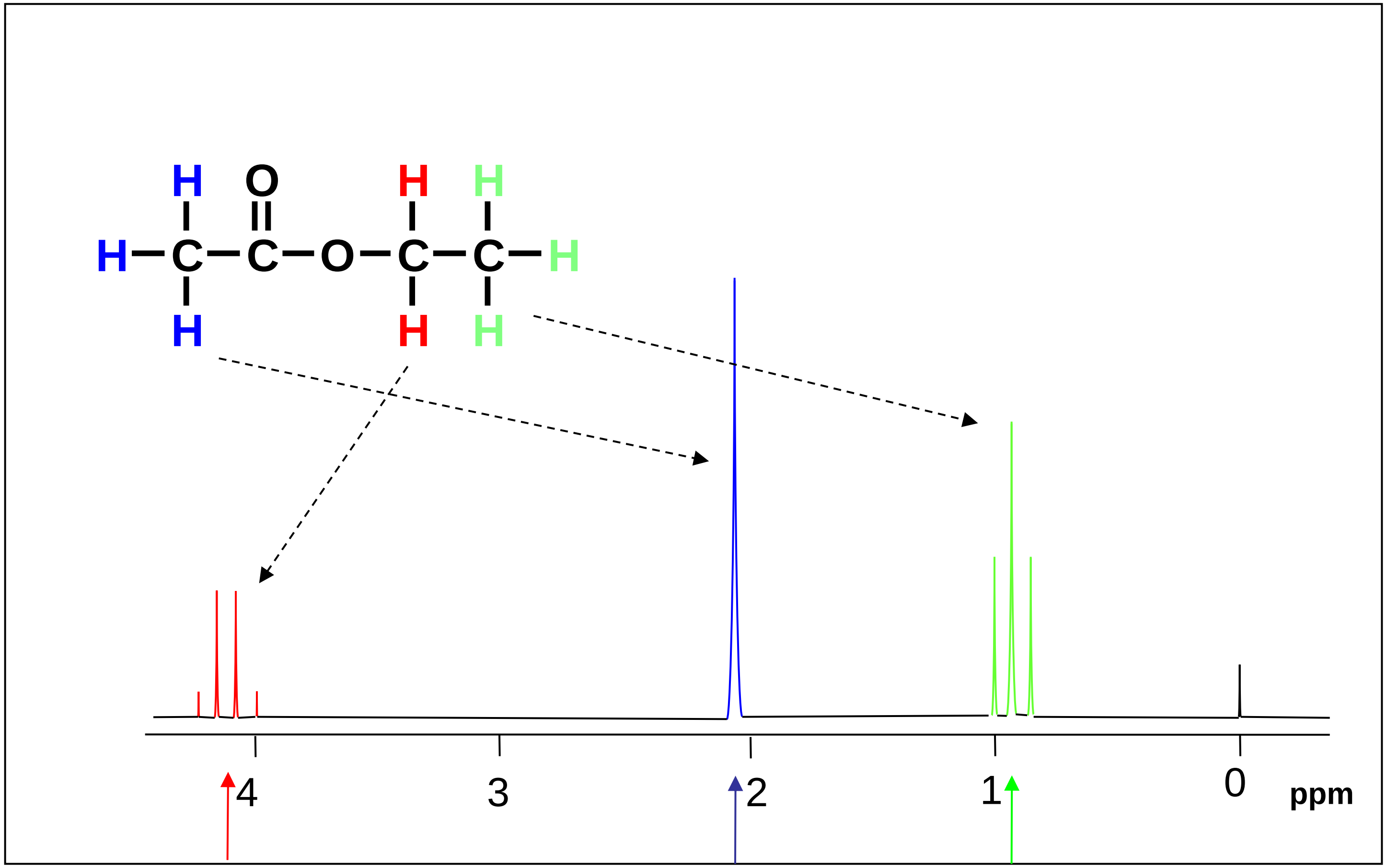
NMR



$$\Delta E = \gamma\, h\, B_o = h\, \nu$$



Metabolite fingerprint

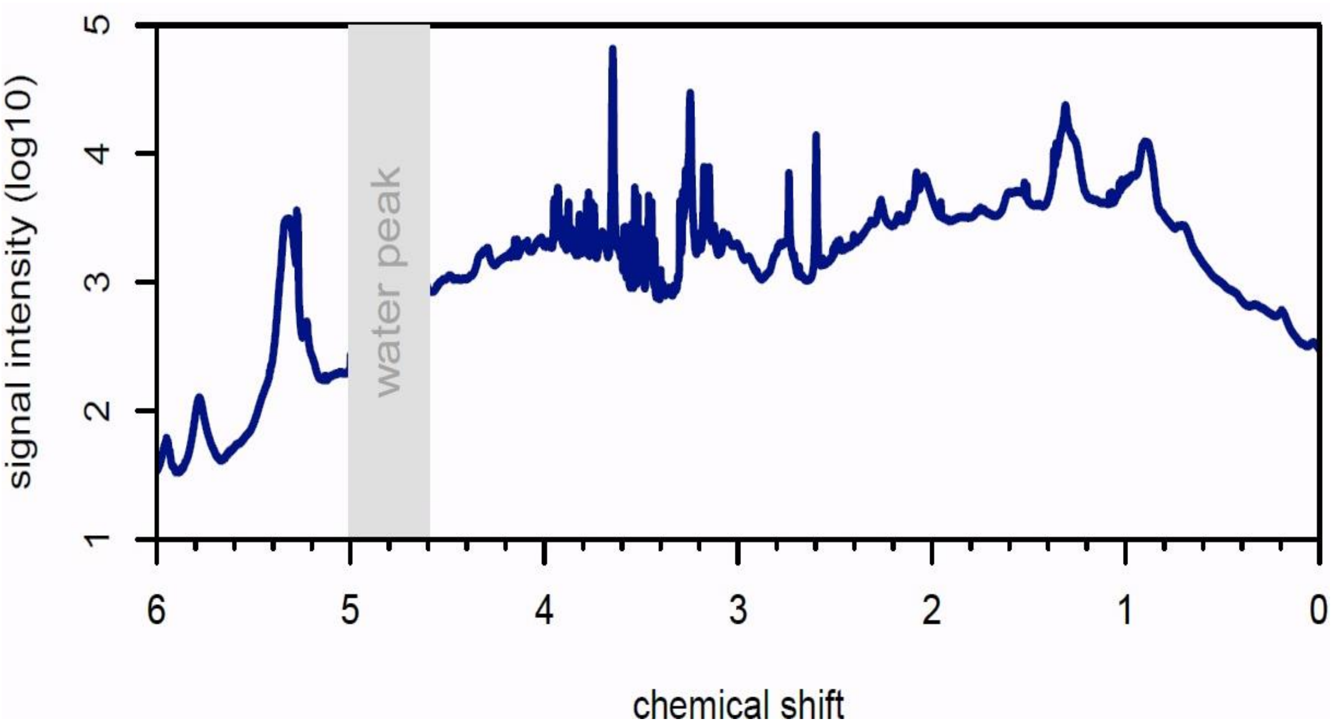


4.12 ppm

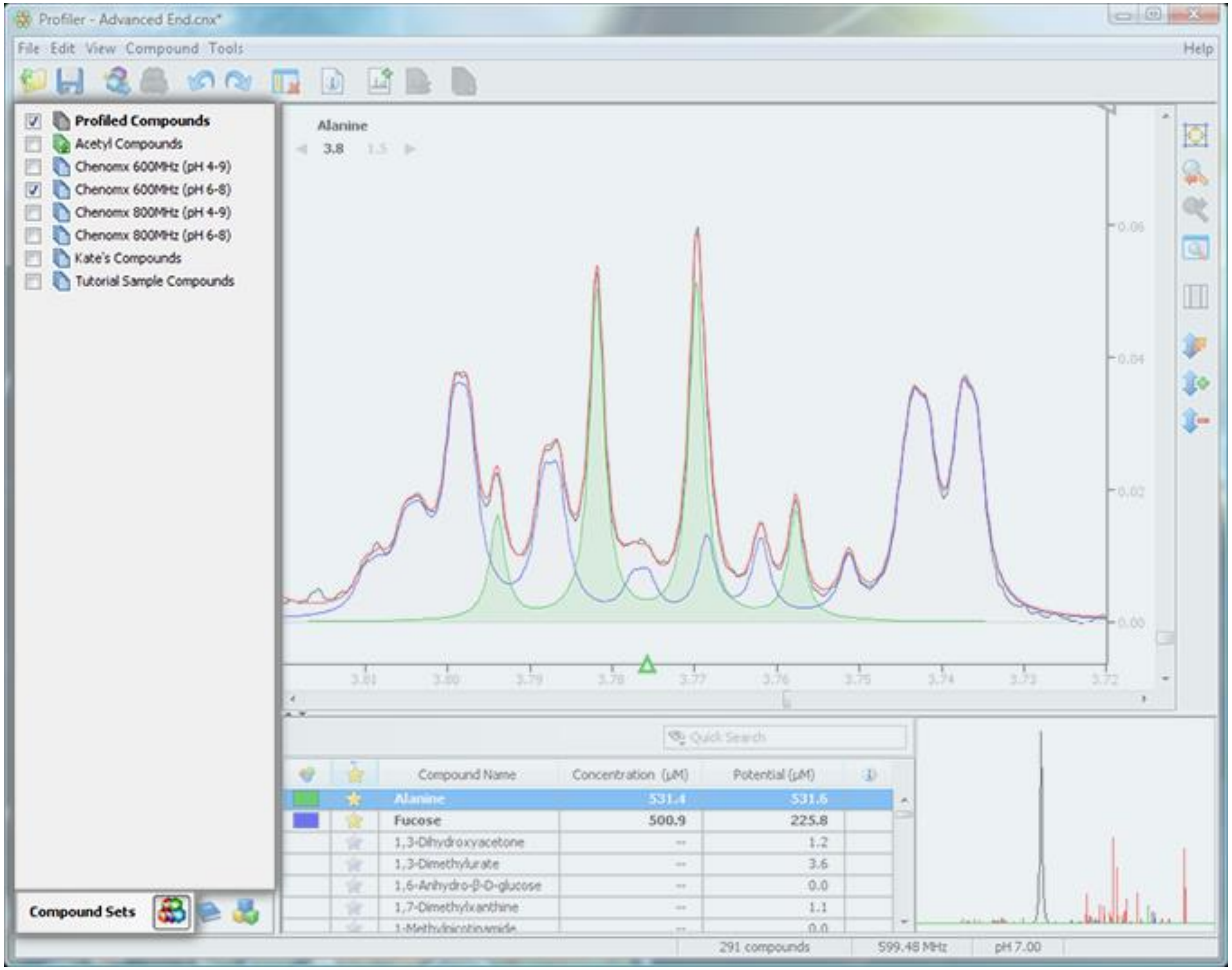
2.03 ppm

0.98 ppm

NMR spectrum for a plasma sample



Metabolite identification using spectra library

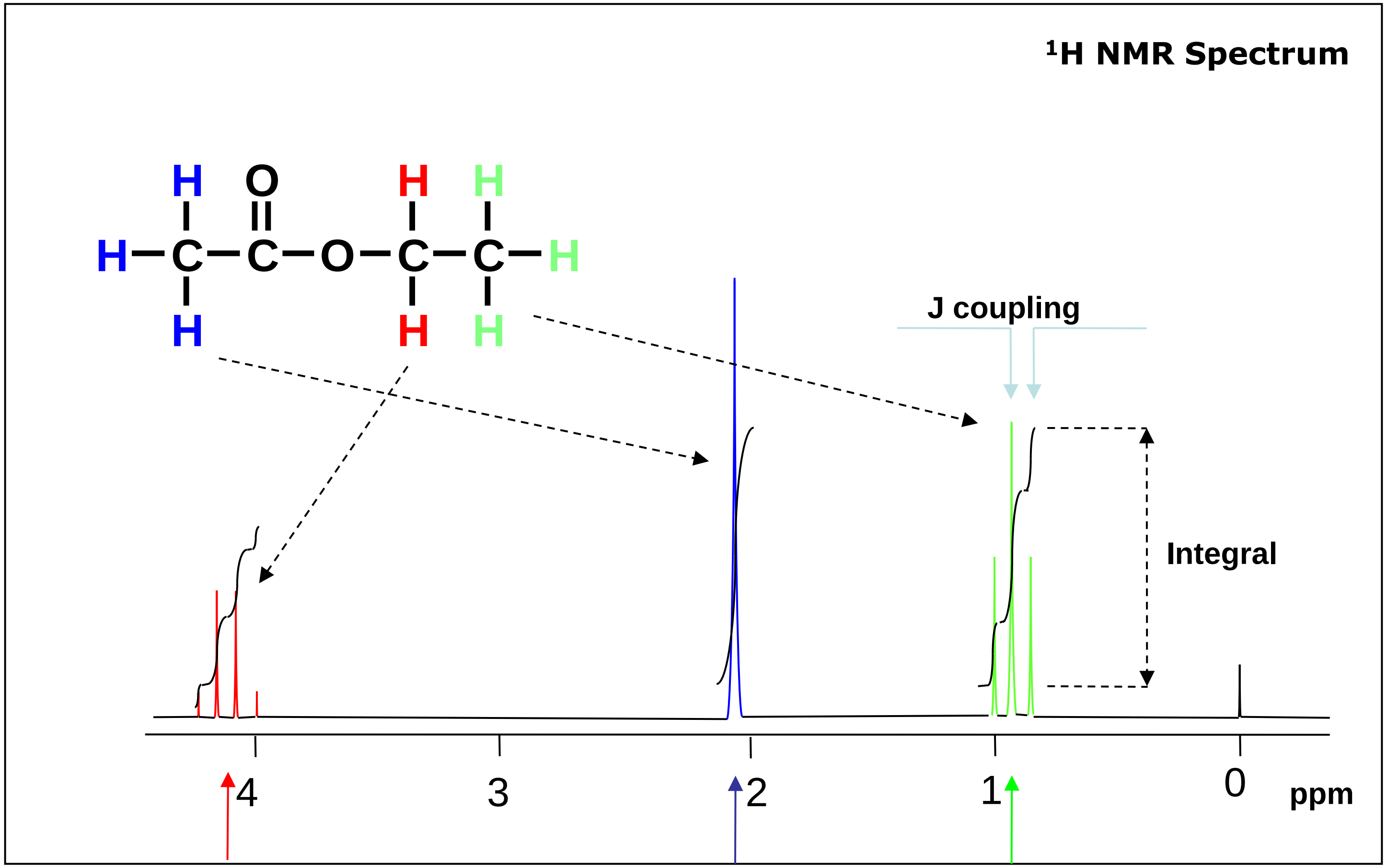


☑ **Profiled Compounds**
☐ Acetyl Compounds
☐ Chenomx 600MHz (pH 4-9)
☑ Chenomx 600MHz (pH 6-8)
☐ Chenomx 800MHz (pH 4-9)
☐ Chenomx 800MHz (pH 6-8)
☐ Kate's Compounds
☐ Tutorial Sample Compounds

		Compound Name	Concentration (μM)	Potential (μM)
🟩	★	Alanine	531.4	531.6
🟦	★	Fucose	500.9	225.8
	☆	1,3-Dihydroxyacetone	--	1.2
	☆	1,3-Dimethylurate	--	3.6
	☆	1,6-Anhydro-β-D-glucose	--	0.0
	☆	1,7-Dimethylxanthine	--	1.1
	☆	1-Methylnicotinamide	--	0.0

CHENOMX

Quantification



4.12 ppm

2.03 ppm

0.98 ppm

Technologies for high-throughput metabolomics

Sample preparation

Chromato-graphy

LC or GC-Mass spectrometry (MS)

Nuclear magnetic resonance (NMR)

Sample preparation

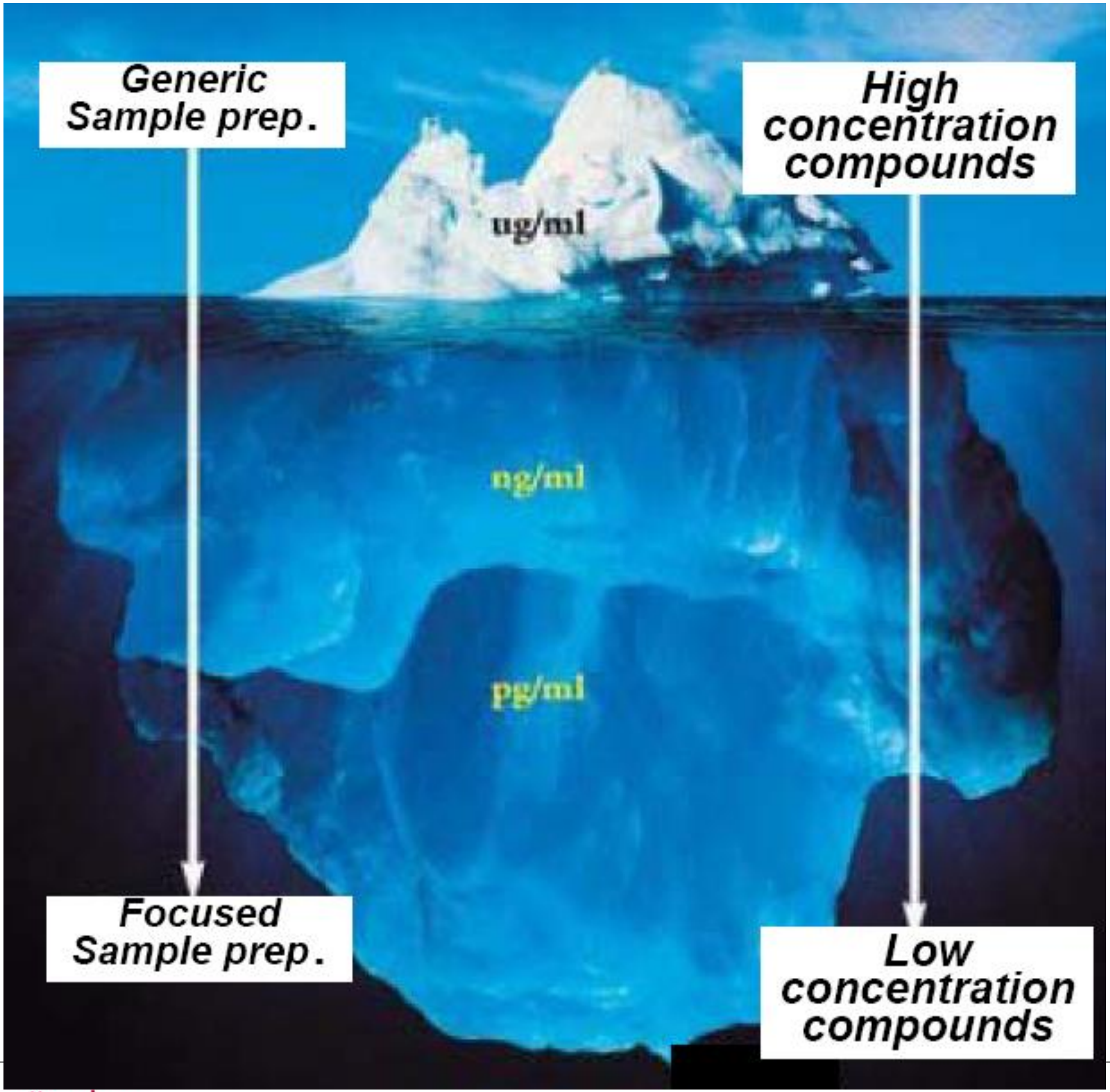
Homogenized sample

- Extraction (e.g. solvent extraction with MeOH)

  => Depending on the extraction method, different metabolite classes may be analyzed

- Addition of standards (for QC and quantification)
- Derivatisation

Sample preparation



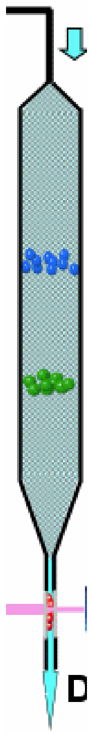
Generic Sample prep.

High concentration compounds

Focused Sample prep.

Low concentration compounds

Separation techniques



- Liquid chromatography

liquid mobile phase; solid stationary phase

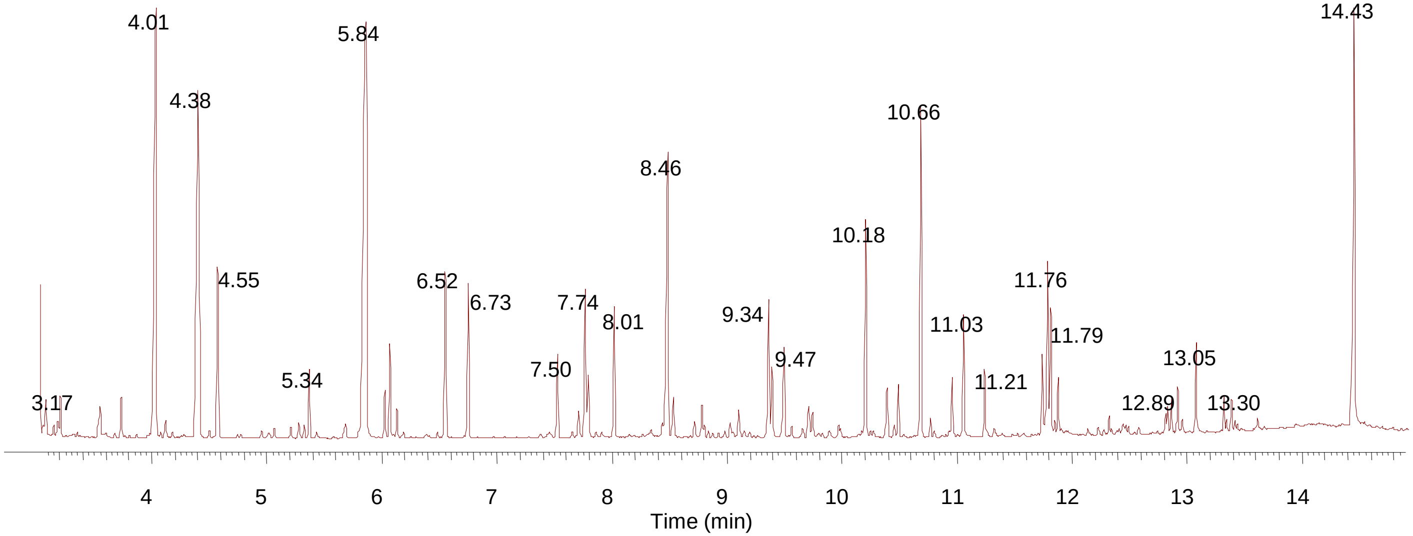
- Gas chromatography

carrier gas mobile phase; liquid stationary phase

- Capillary Electrophoresis

=> Reduces complexity by introducing a temporal dimension (elution/retention time)

Separation techniques

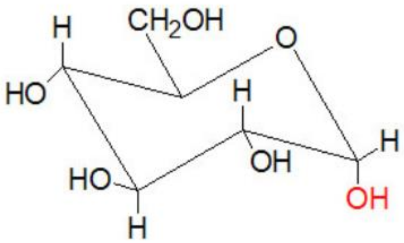


Chromatogram

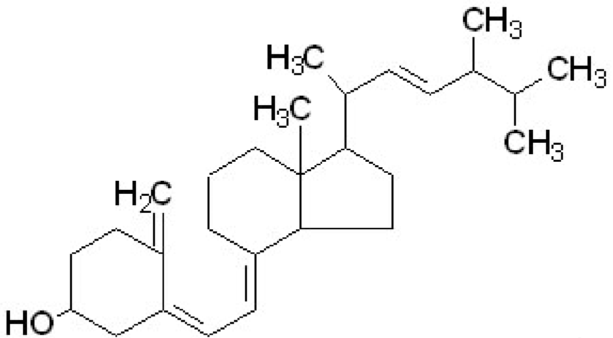
MASS SPECTROMETRY (MS)

Mass spectrometry

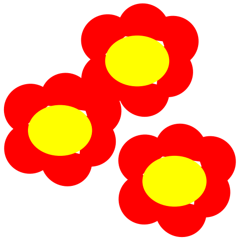
Principle: Separation/Identification of molecules by mass (precisely: mass/charge)



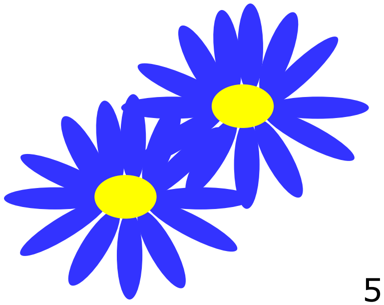
180.1 g/mol



396.7 g/mol



3.1 g

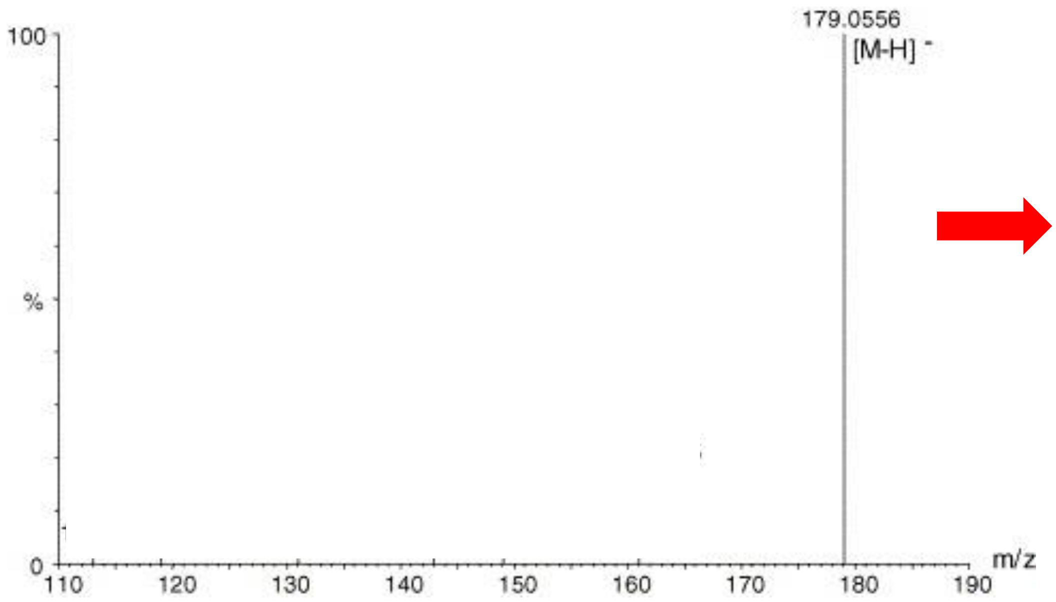


5.4 g

Don’t be afraid of abbreviations

Separation	Ionisation	Mass detection
HPLC	EI	FTICR
UPLC	ESI	Triple Quad
GC	MALDI	TOF
FIA	APCI	Ion Trap
		Orbitrap

Mass spectrometry



Taylor et al., 2005

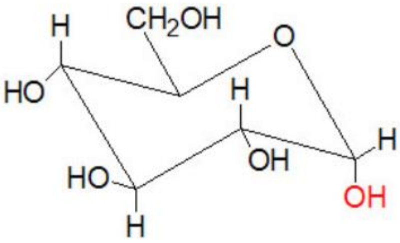
Possible molecular formulas:

C6H12O6

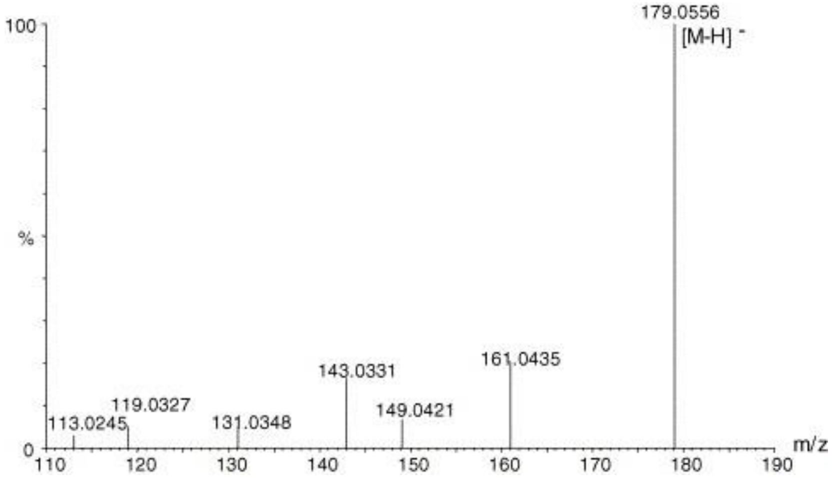
Which molecule is it?

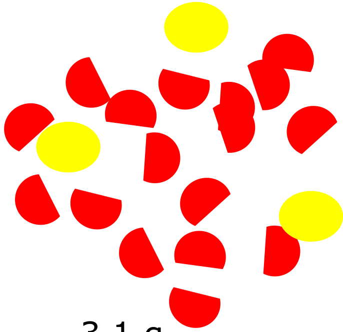
Fragmentation

e.g. by collision with gas molecules



180.1 g/mol

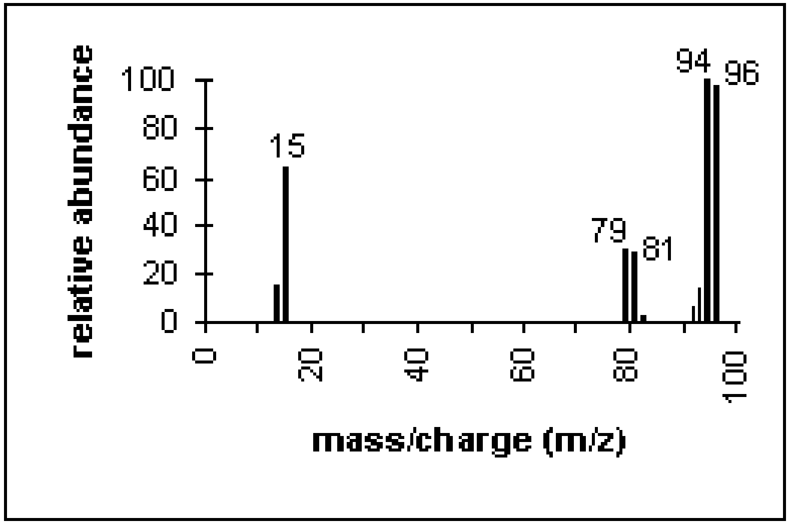


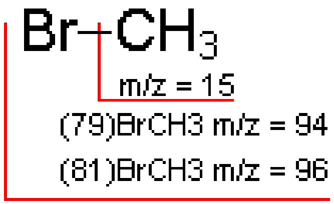


3.1 g

5.4 g

Relative abundance of isotopes





$Br-CH_3$

m/z = 15

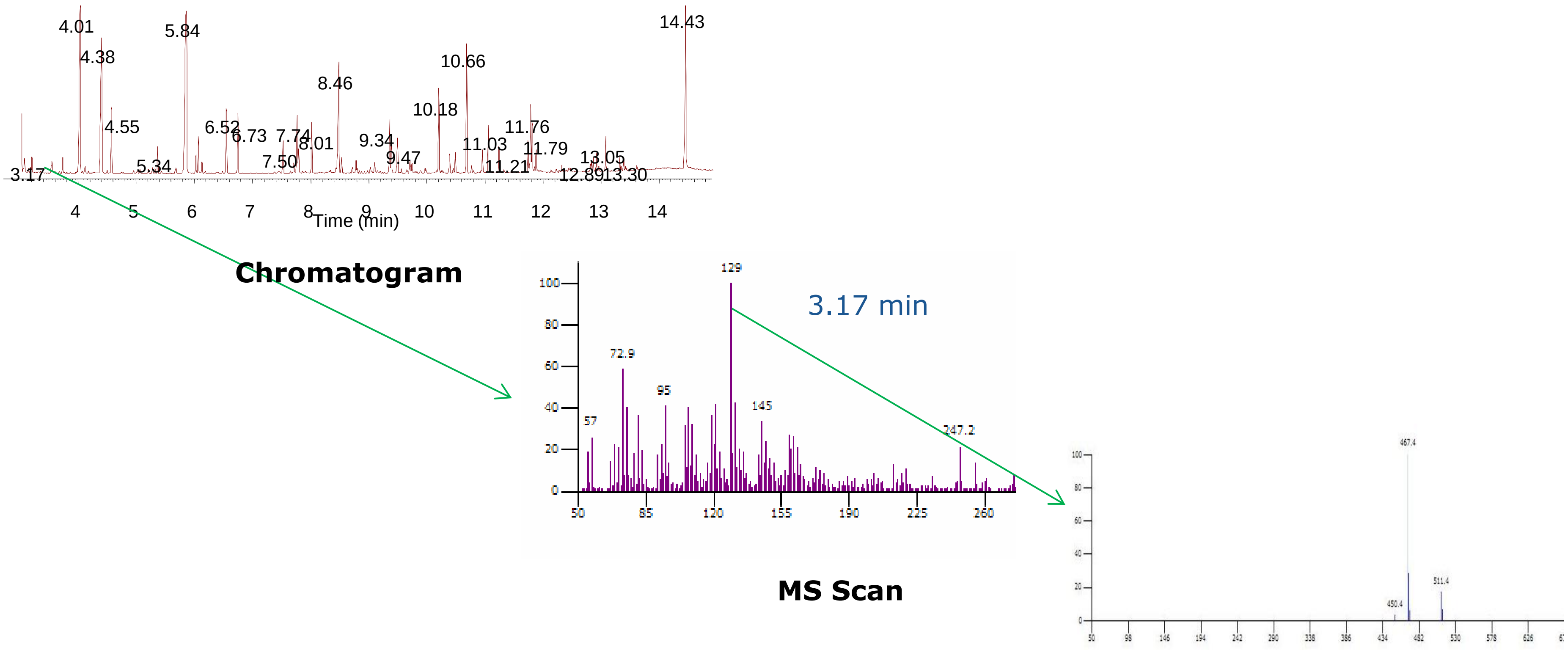
(79)BrCH3 m/z = 94

(81)BrCH3 m/z = 96

The ratio of peaks containing ^{79}Br and its isotope ^{81}Br (100/98) confirms the presence of bromine in the compound.

http://www.chem.arizona.edu/massspec/intro_html/intro.html

LC-MS spectra for a sample



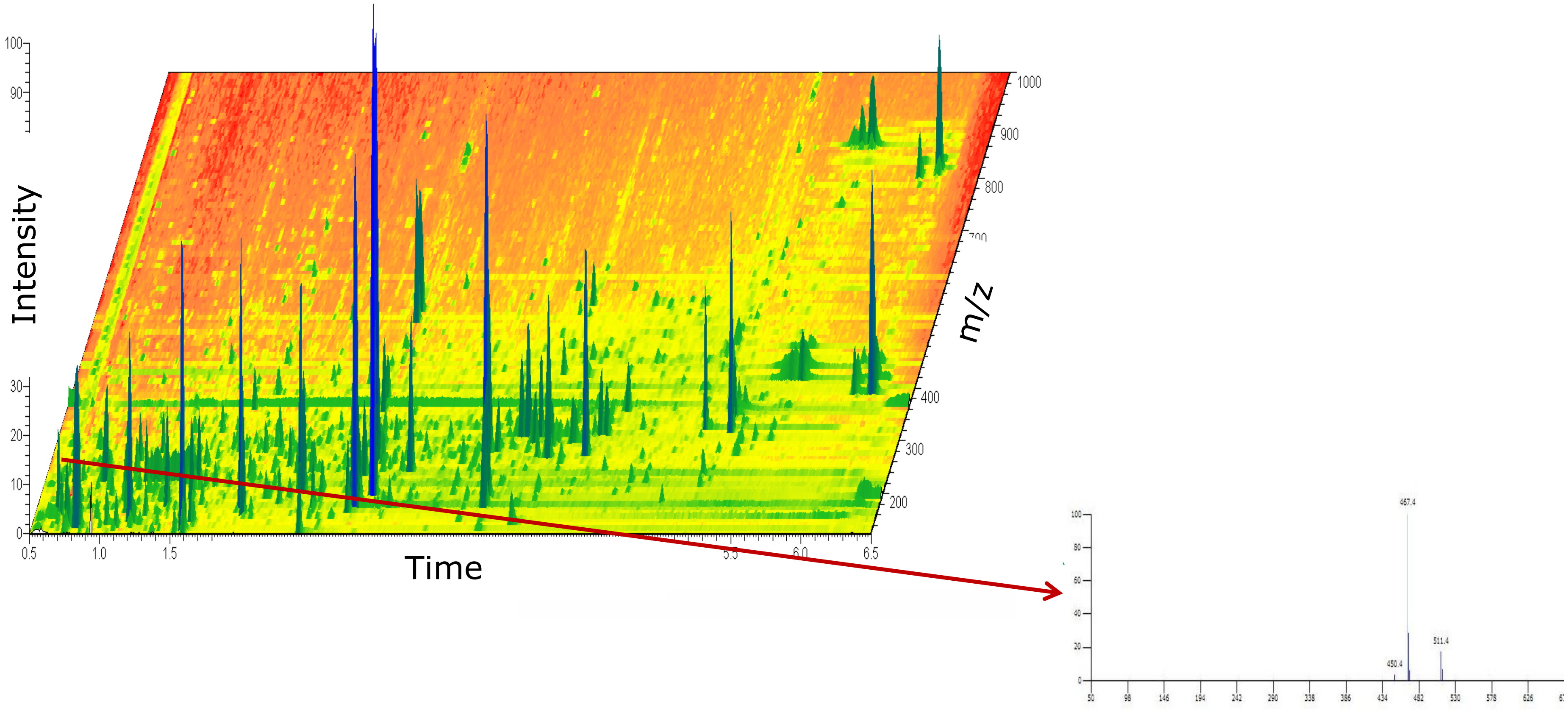
Chromatogram

3.17 min

MS Scan

MS/MS Fragmentation

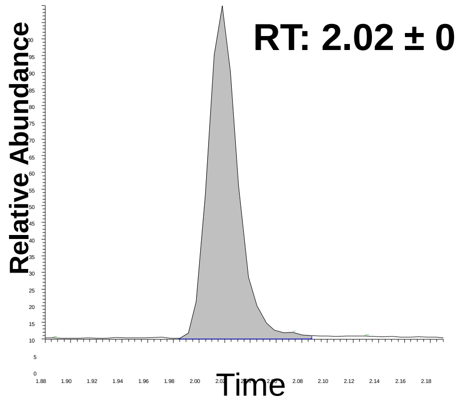
LC-MS spectra for a sample



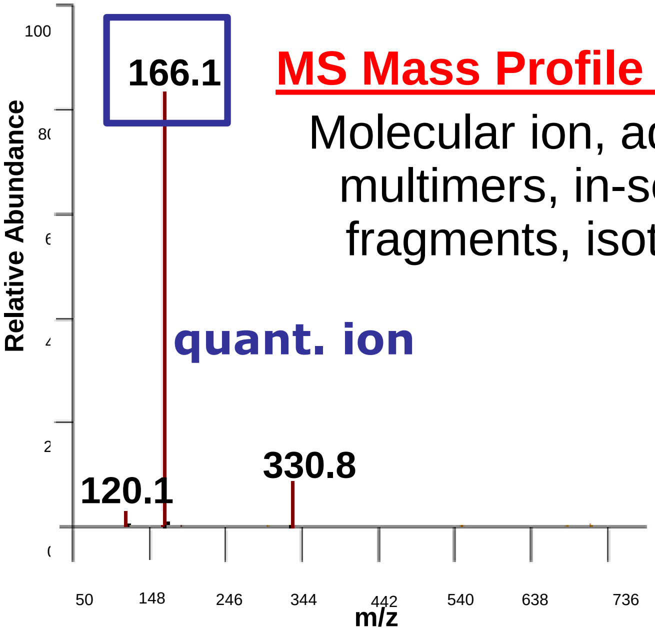
MS/MS Fragmentation

Metabolite fingerprint

Retention



RT: 2.02 ± 0.05 min

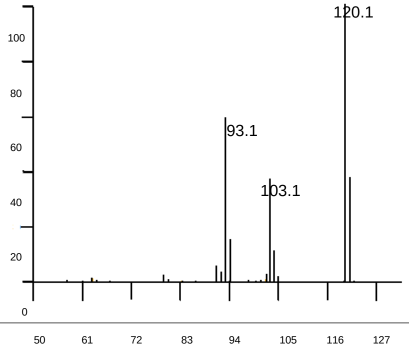


MS Mass Profile (m/z)

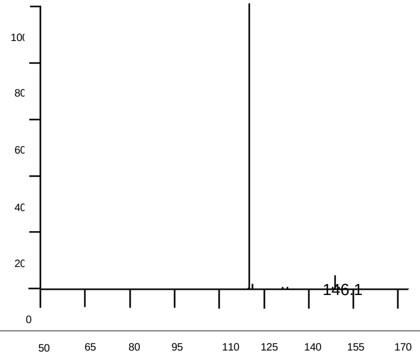
Molecular ion, adducts, multimers, in-source fragments, isotopes

Fragmentation Spectra (EI or MS/MS)

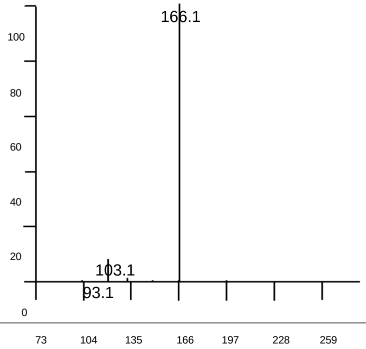
MS/MS 120.1



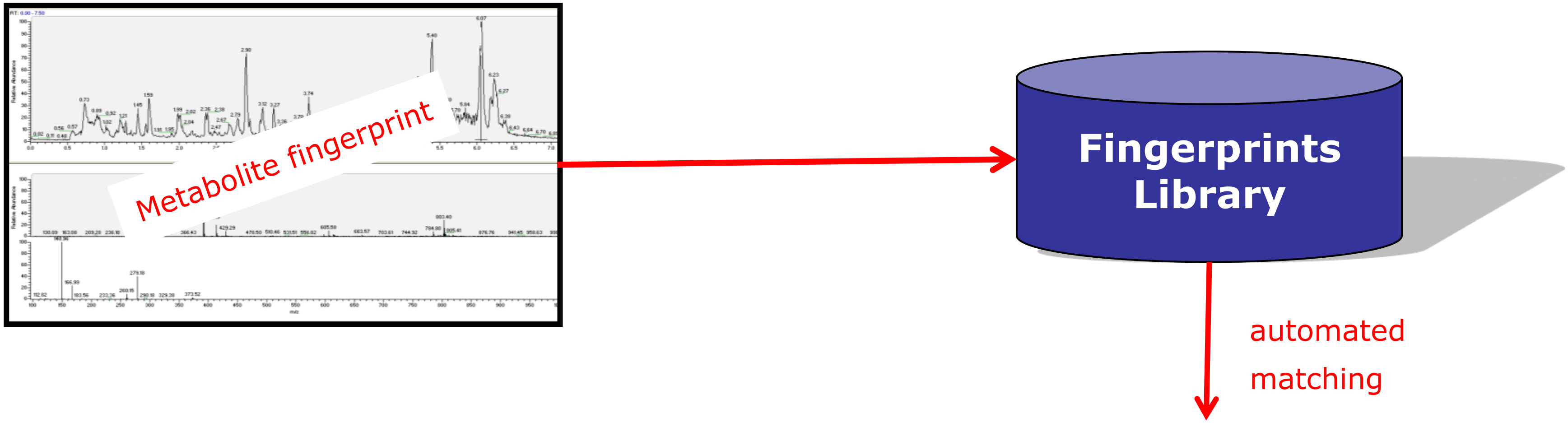
MS/MS 166.1



MS/MS 330.8



Metabolite identification with spectra library



Metabolite fingerprint

Fingerprints Library

automated matching

Suggested matching metabolites

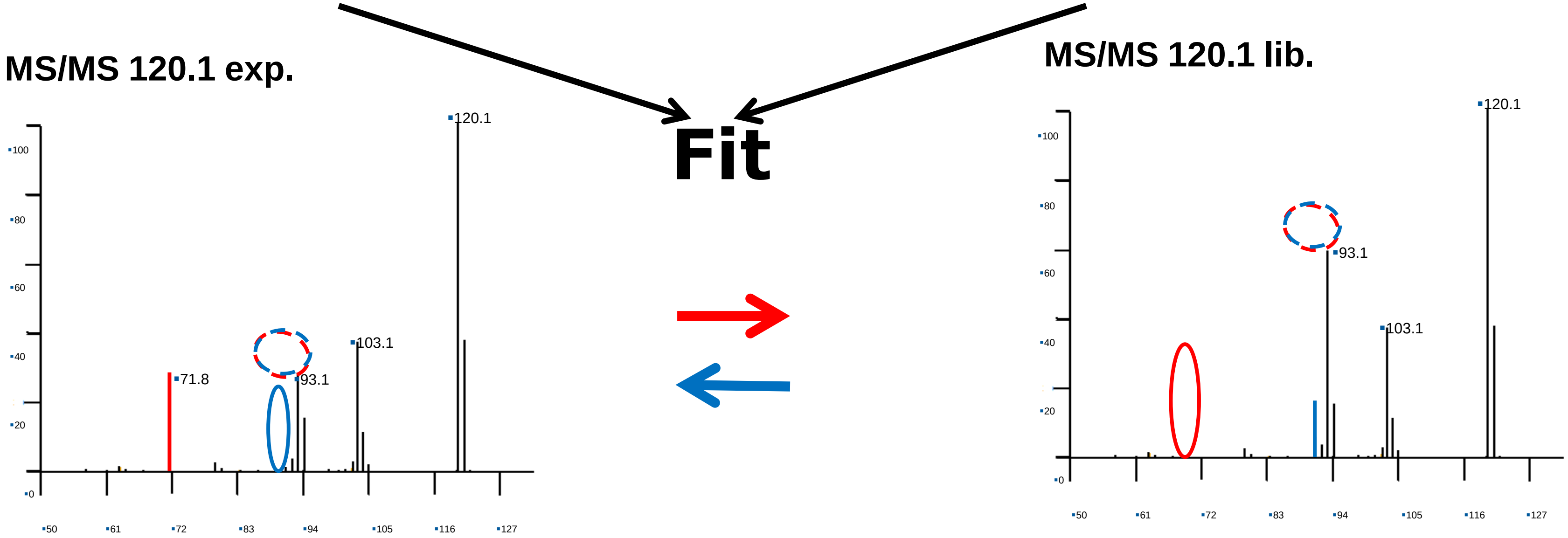
Automated matching

Forward-Fit

Reverse-Fit

Matches everything in the **component** to the library

Matches the **library** entry to the component

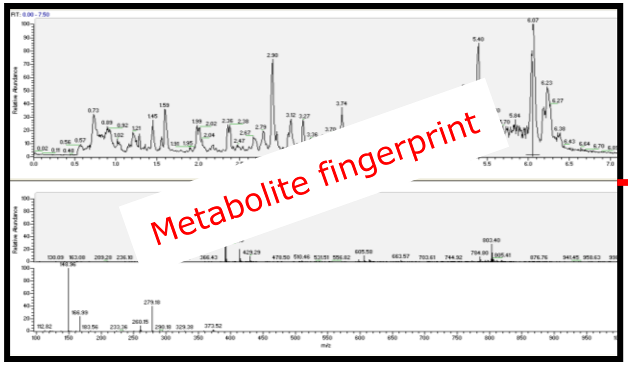


MS/MS 120.1 exp.

Fit

MS/MS 120.1 lib.

Metabolite identification with spectra library



Fingerprints Library

?

automated matching

Suggested matching metabolites

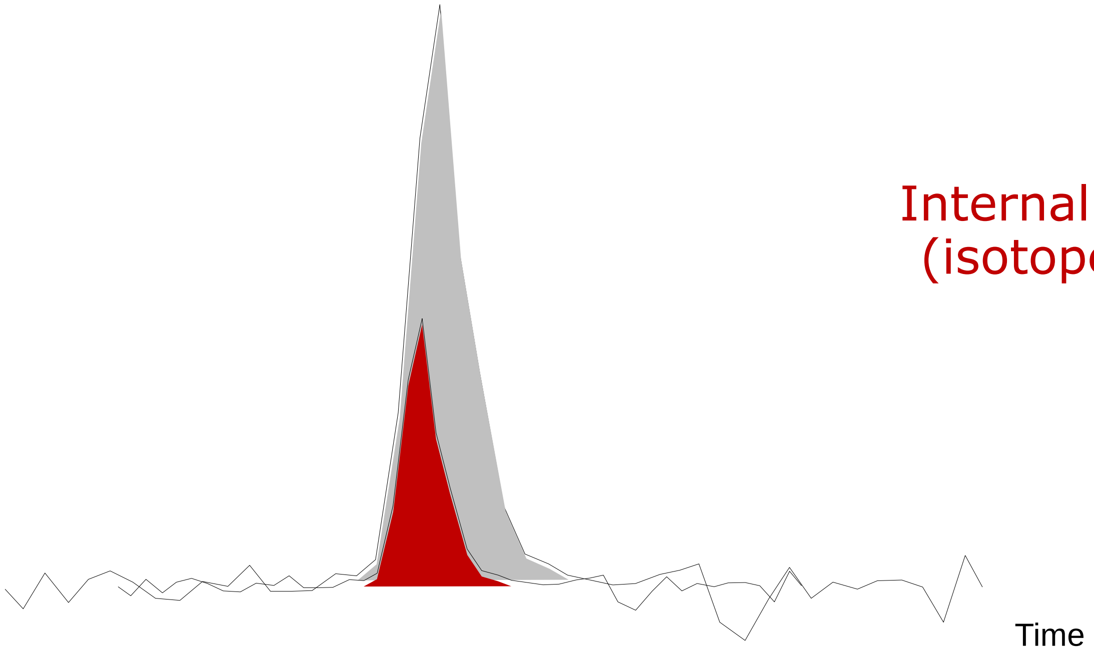
manual curation

manual curation

“Knowns”

“Known unknowns”

Quantification



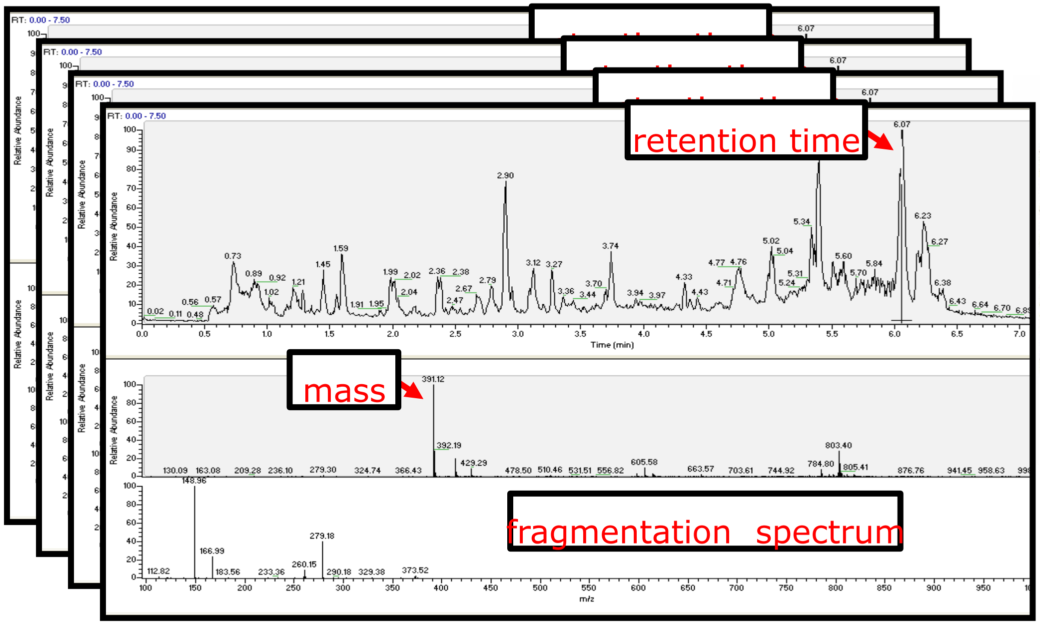
Internal standard
(isotope labled)

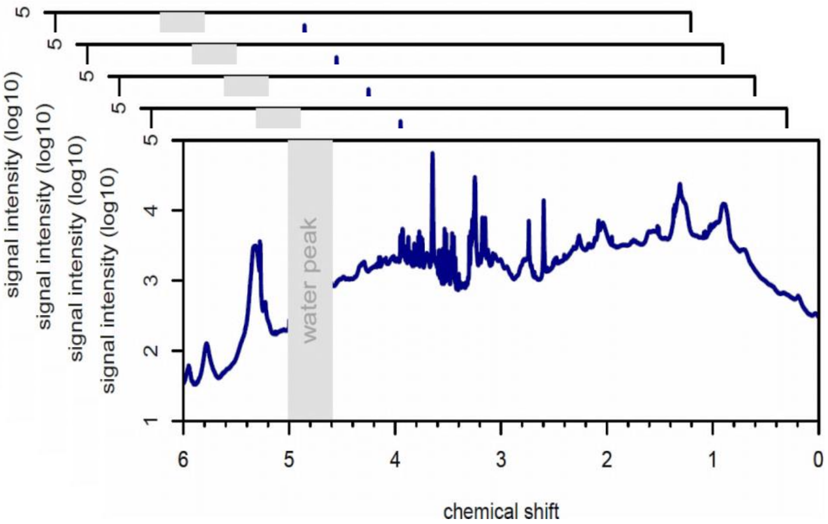
RT: 2.02
m/z: 120.14
Area: 120089

RT: 2.02
m/z: 121.15
Area: 40112

DATA ANALYSIS

Raw metabolomics data





LC or GC-MS

NMR

Four steps

- Raw data processing

peak detection, peak alignment, peak integration, identification of metabolites, …

- Primary data analysis (QC):

outlier detection, normalization (batch effects, dilution), missing value handling/imputing…

- Statistical analysis:

univariate/multivariate hypothesis tests, supervised/unsupervised machine learning (classification/clustering), …

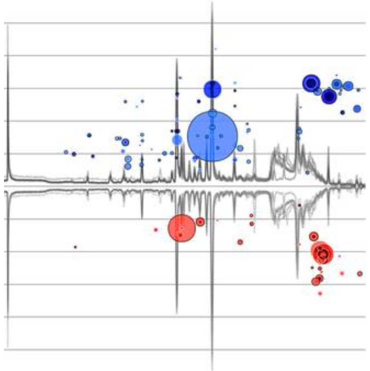
- Bioinformatic analysis:

biological context, network analyses, data integration

Raw data analysis tools

Scripps Center For Metabolomics
METLIN: Metabolite and Tandem MS Database

XCMS Online



metlin.scripps.edu/xcms/

MetaboAnalyst 2.0
-- a comprehensive tool suite for metabolomic data analysis

www.metaboanalyst.ca

Batman (NMR)

batman.r-forge.r-project.org/

Raw data analysis

Targeted

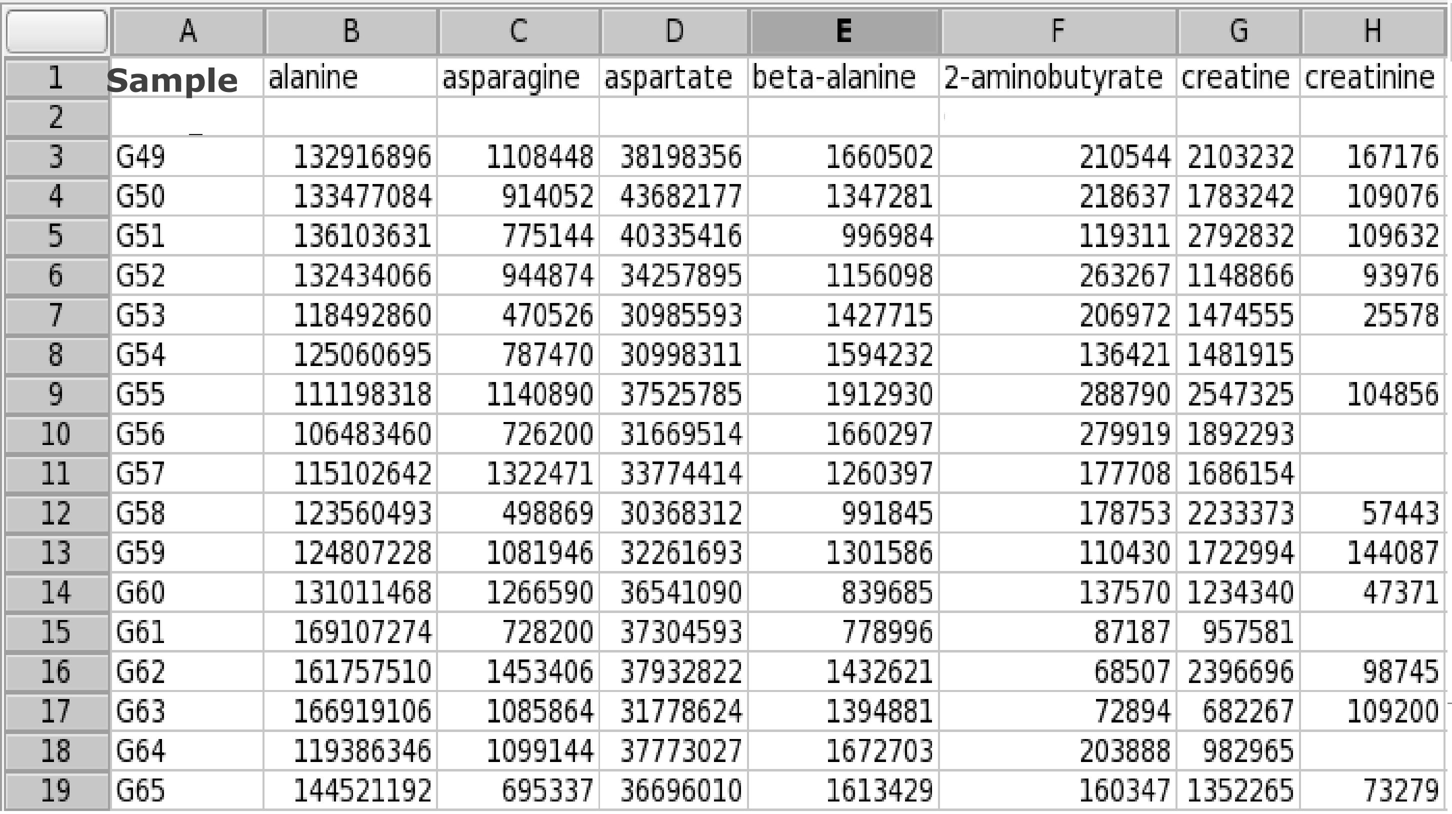
Non-targeted

Preselected set of metab. signals

All metab. signals that can be detected

Ident. metabolites

Peak list



	A	B	C	D	E	F	G	H
1	**Sample**	alanine	asparagine	aspartate	beta-alanine	2-aminobutyrate	creatine	creatinine
2	_							
3	G49	132916896	1108448	38198356	1660502	210544	2103232	167176
4	G50	133477084	914052	43682177	1347281	218637	1783242	109076
5	G51	136103631	775144	40335416	996984	119311	2792832	109632
6	G52	132434066	944874	34257895	1156098	263267	1148866	93976
7	G53	118492860	470526	30985593	1427715	206972	1474555	25578
8	G54	125060695	787470	30998311	1594232	136421	1481915	
9	G55	111198318	1140890	37525785	1912930	288790	2547325	104856
10	G56	106483460	726200	31669514	1660297	279919	1892293	
11	G57	115102642	1322471	33774414	1260397	177708	1686154	
12	G58	123560493	498869	30368312	991845	178753	2233373	57443
13	G59	124807228	1081946	32261693	1301586	110430	1722994	144087
14	G60	131011468	1266590	36541090	839685	137570	1234340	47371
15	G61	169107274	728200	37304593	778996	87187	957581	
16	G62	161757510	1453406	37932822	1432621	68507	2396696	98745
17	G63	166919106	1085864	31778624	1394881	72894	682267	109200
18	G64	119386346	1099144	37773027	1672703	203888	982965	
19	G65	144521192	695337	36696010	1613429	160347	1352265	73279

...

...

...

...

...

...

...

...

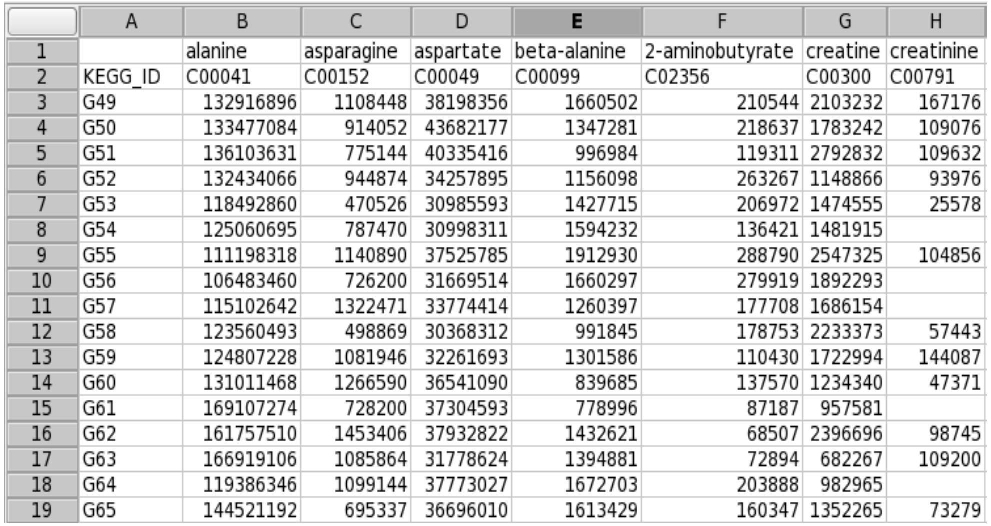
...

...

...

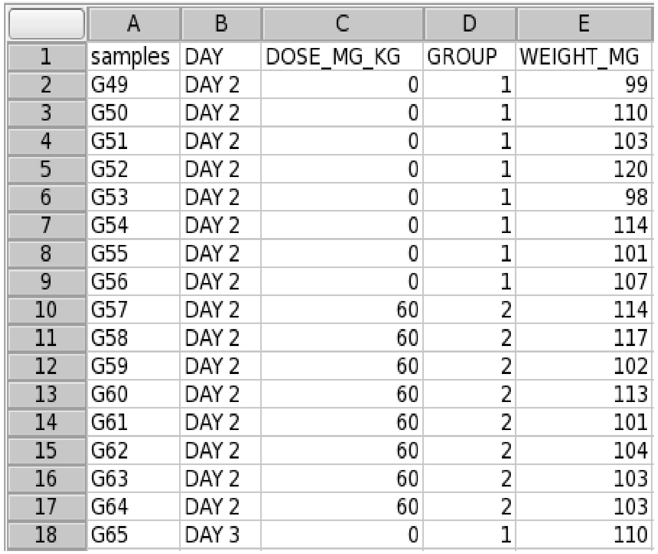
Metabolomics Data

Metabolite concentrations



	A	B	C	D	E	F	G	H
1		alanine	asparagine	aspartate	beta-alanine	2-aminobutyrate	creatine	creatinine
2	KEGG_ID	C00041	C00152	C00049	C00099	C02356	C00300	C00791
3	G49	132916896	1108448	38198356	1660502	210544	2103232	167176
4	G50	133477084	914052	43682177	1347281	218637	1783242	109076
5	G51	136103631	775144	40335416	996984	119311	2792832	109632
6	G52	132434066	944874	34257895	1156098	263267	1148866	93976
7	G53	118492860	470526	30985593	1427715	206972	1474555	25578
8	G54	125060695	787470	30998311	1594232	136421	1481915	
9	G55	111198318	1140890	37525785	1912930	288790	2547325	104856
10	G56	106483460	726200	31669514	1660297	279919	1892293	
11	G57	115102642	1322471	33774414	1260397	177708	1686154	
12	G58	123560493	498869	30368312	991845	178753	2233373	57443
13	G59	124807228	1081946	32261693	1301586	110430	1722994	144087
14	G60	131011468	1266590	36541090	839685	137570	1234340	47371
15	G61	169107274	728200	37304593	778996	87187	957581	
16	G62	161757510	1453406	37932822	1432621	68507	2396696	98745
17	G63	166919106	1085864	31778624	1394881	72894	682267	109200
18	G64	119386346	1099144	37773027	1672703	203888	982965	
19	G65	144521192	695337	36696010	1613429	160347	1352265	73279

Sample phenotypes



	A	B	C	D	E
1	samples	DAY	DOSE_MG_KG	GROUP	WEIGHT_MG
2	G49	DAY 2	0	1	99
3	G50	DAY 2	0	1	110
4	G51	DAY 2	0	1	103
5	G52	DAY 2	0	1	120
6	G53	DAY 2	0	1	98
7	G54	DAY 2	0	1	114
8	G55	DAY 2	0	1	101
9	G56	DAY 2	0	1	107
10	G57	DAY 2	60	2	114
11	G58	DAY 2	60	2	117
12	G59	DAY 2	60	2	102
13	G60	DAY 2	60	2	113
14	G61	DAY 2	60	2	101
15	G62	DAY 2	60	2	104
16	G63	DAY 2	60	2	103
17	G64	DAY 2	60	2	103
18	G65	DAY 3	0	1	110

… including batch, collection date, etc

Four steps

- Raw data processing

peak detection, peak alignment, peak integration, identification of metabolites, …

- Primary data analysis (QC):

outlier detection, normalization (batch effects, dilution), missing value handling/imputing…

- Statistical analysis:

univariate/multivariate hypothesis tests,

supervised/unsupervised machine learning (classification/clustering), …

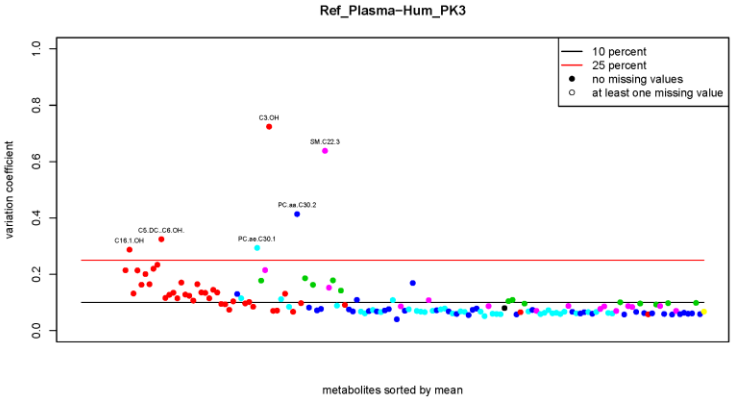
- Bioinformatic analysis:

biological context, network analyses, data integration

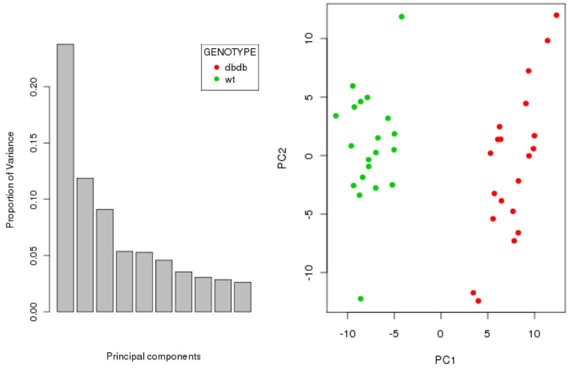
Primary data analysis (exploratory)

metaP server

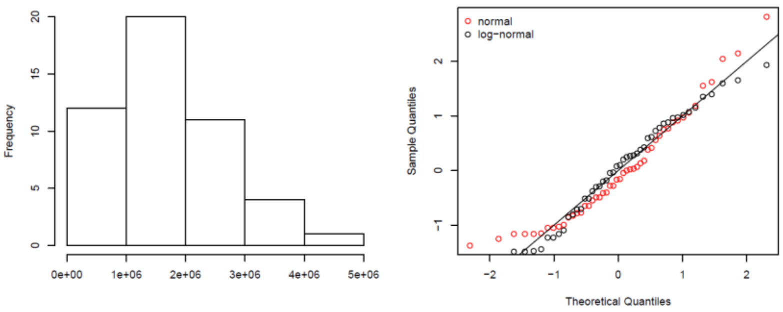
metap.helmholtz-muenchen.de/metap2



Quality control



PCA



Distributions

HelmholtzZentrum münchen
Deutsches Forschungszentrum für Gesundheit und Umwelt

Four steps

- Raw data processing

peak detection, peak alignment, peak integration, identification of metabolites, …

- Primary data analysis (QC):

outlier detection, normalization (batch effects, dilution), missing value handling/imputing…

- Statistical analysis:

univariate/multivariate hypothesis tests,

supervised/unsupervised machine learning (classification/clustering), …

- Bioinformatic analysis:

biological context, network analyses, data integration

Results from statistical analysis

e.g. cases vs. control:

Fumarate	↑
Arginine	↓
Citrulline	↓
Ornithine	↓
Glutamine	↑
Urea	↑
Aspartate	↓
N-acetylglutamate	→
…	

Four steps

- Raw data processing

peak detection, peak alignment, peak integration, identification of metabolites, …

- Primary data analysis (QC):

outlier detection, normalization (batch effects, dilution), missing value handling/imputing…

- Statistical analysis:

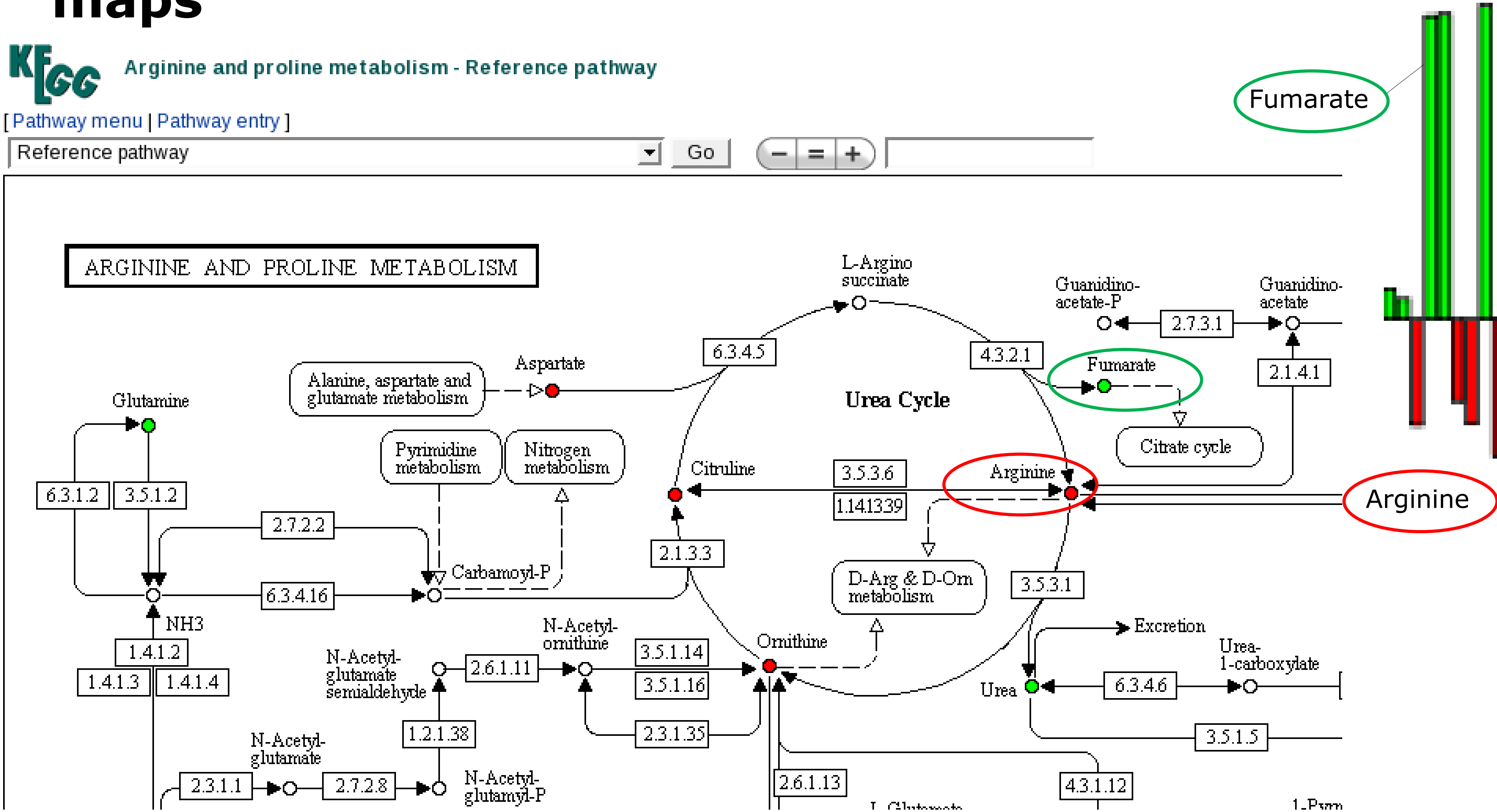
univariate/multivariate hypothesis tests,

supervised/unsupervised machine learning (classification/clustering), …

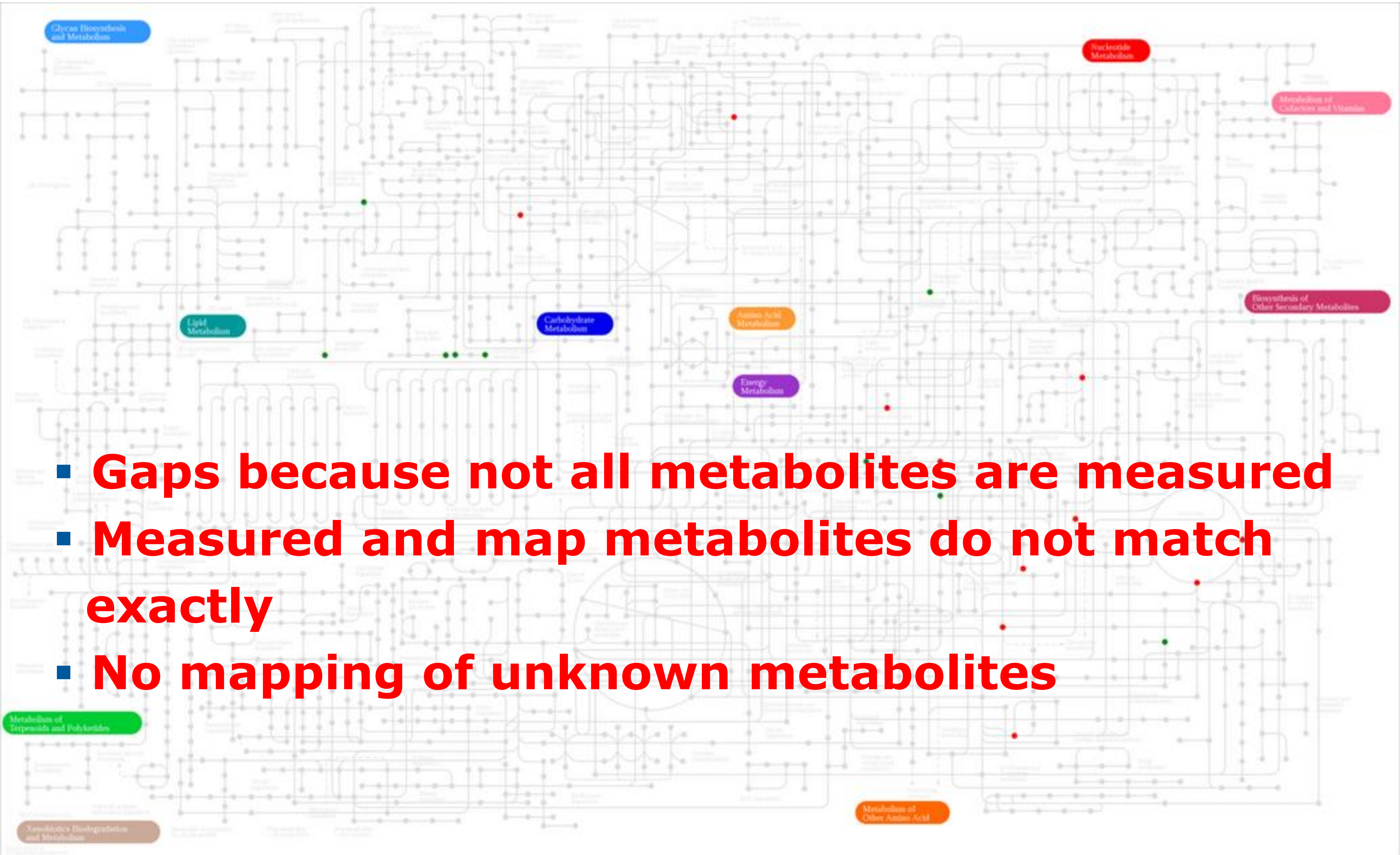
- Bioinformatic analysis:

biological context, network analyses, data integration

Approach 1: Mapping results onto pathway maps



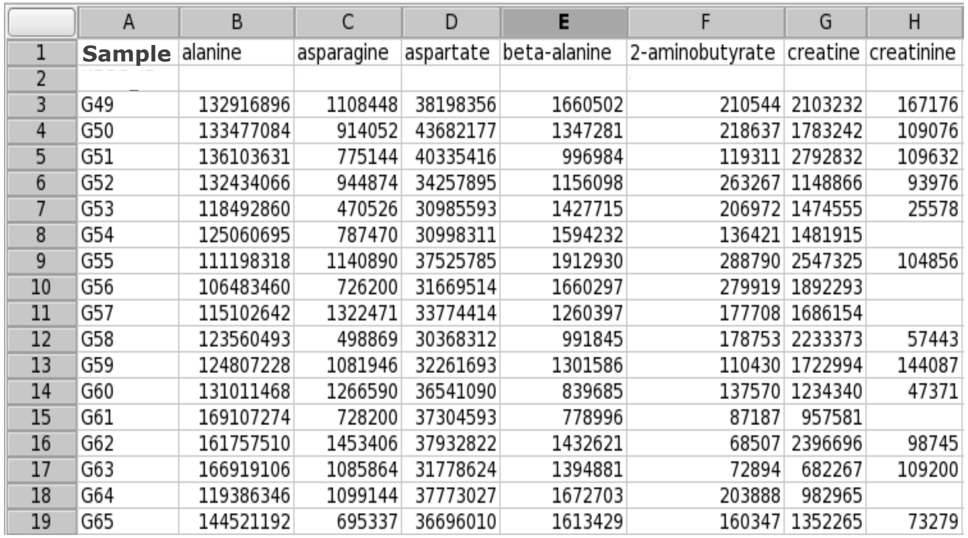
But: mapping problem



- Gaps because not all metabolites are measured
- Measured and map metabolites do not match exactly
- No mapping of unknown metabolites

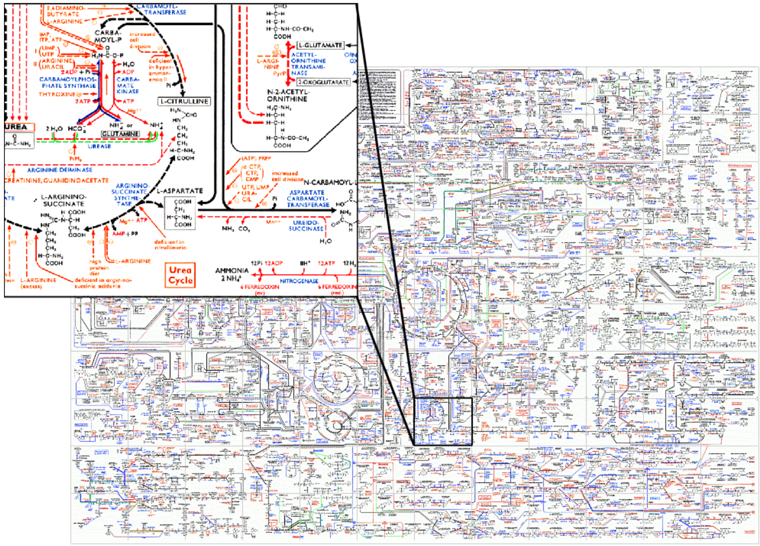
Approach 2: Reconstruction of networks from data

Metabolomics data

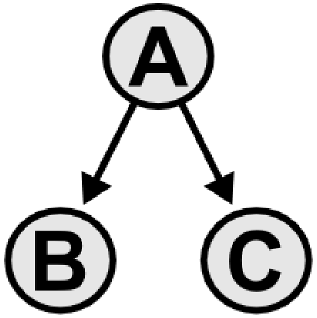


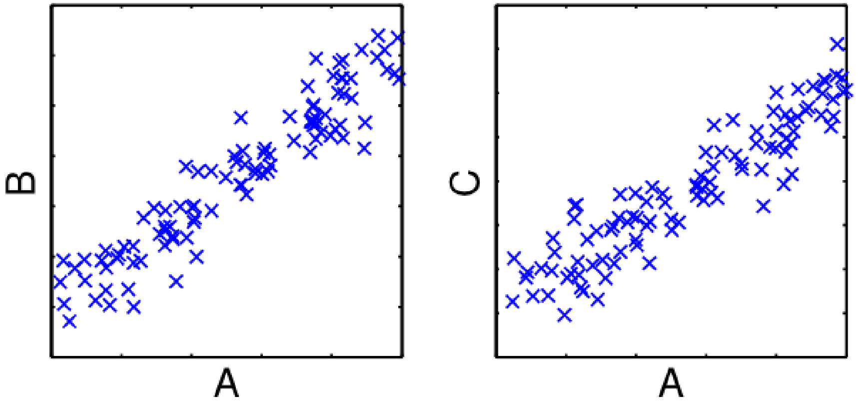
	A	B	C	D	E	F	G	H
1	**Sample**	alanine	asparagine	aspartate	beta-alanine	2-aminobutyrate	creatine	creatinine
2								
3	G49	132916896	1108448	38198356	1660502	210544	2103232	167176
4	G50	133477084	914052	43682177	1347281	218637	1783242	109076
5	G51	136103631	775144	40335416	996984	119311	2792832	109632
6	G52	132434066	944874	34257895	1156098	263267	1148866	93976
7	G53	118492860	470526	30985593	1427715	206972	1474555	25578
8	G54	125060695	787470	30998311	1594232	136421	1481915	
9	G55	111198318	1140890	37525785	1912930	288790	2547325	104856
10	G56	106483460	726200	31669514	1660297	279919	1892293	
11	G57	115102642	1322471	33774414	1260397	177708	1686154	
12	G58	123560493	498869	30368312	991845	178753	2233373	57443
13	G59	124807228	1081946	32261693	1301586	110430	1722994	144087
14	G60	131011468	1266590	36541090	839685	137570	1234340	47371
15	G61	169107274	728200	37304593	778996	87187	957581	
16	G62	161757510	1453406	37932822	1432621	68507	2396696	98745
17	G63	166919106	1085864	31778624	1394881	72894	682267	109200
18	G64	119386346	1099144	37773027	1672703	203888	982965	
19	G65	144521192	695337	36696010	1613429	160347	1352265	73279

Metabolic network



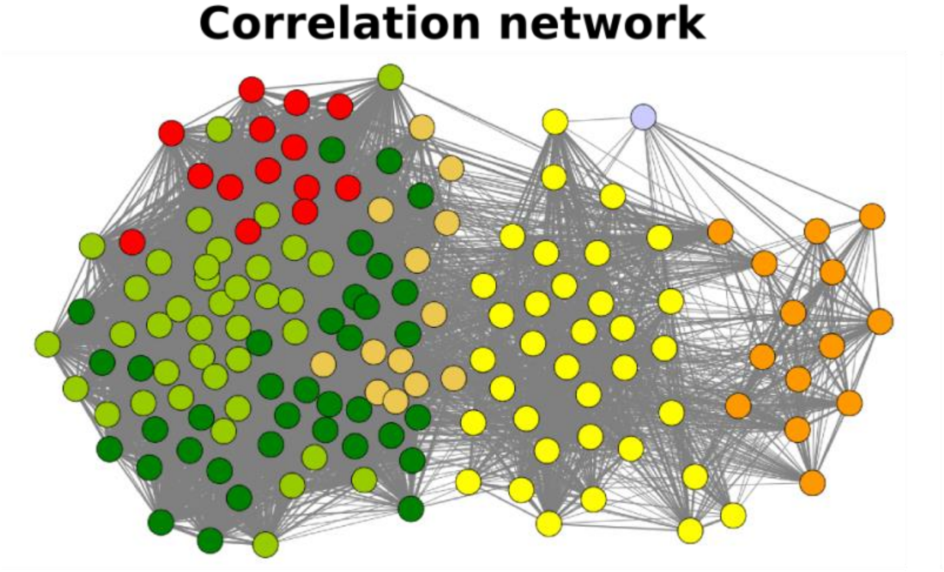
Reconstruction of metabolic networks using correlations





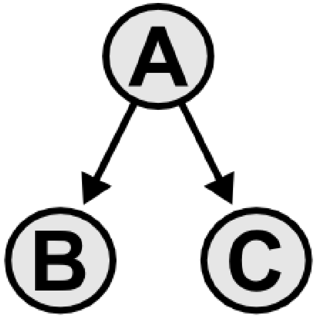
$\rho_{AB} = 0.95$ $\rho_{AC} = 0.92$

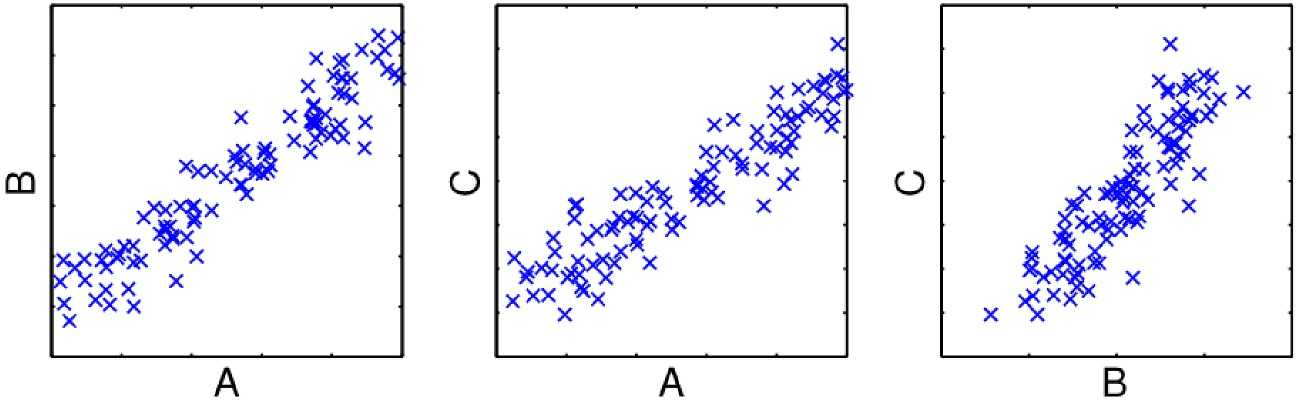
Problem: indirect effects


Correlation network

Krumsiek et al.,
BMC Systems Biology, 2011

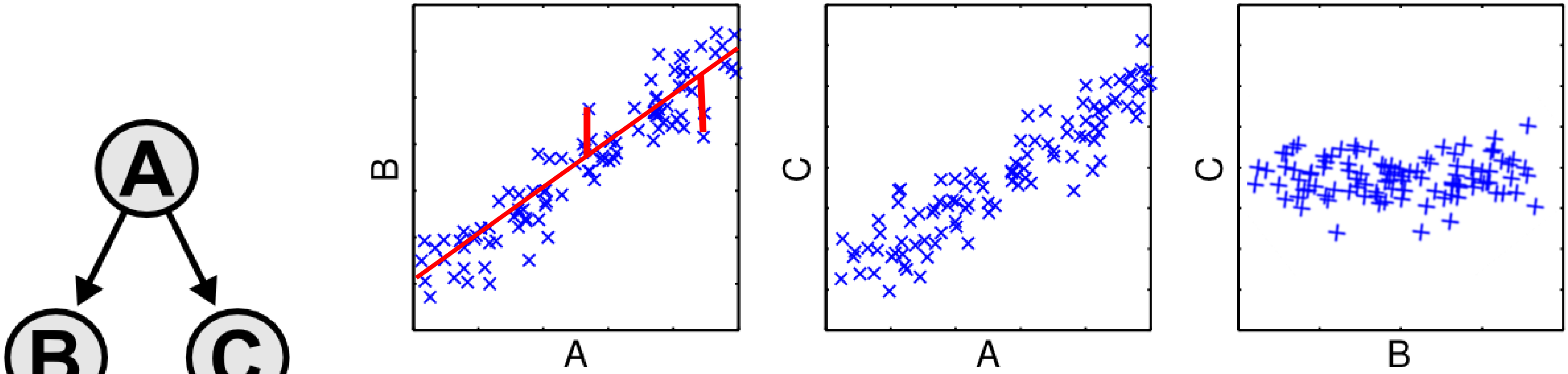
Problem: indirect effects





$\rho_{AB} = 0.95$ $\rho_{AC} = 0.92$ $\rho_{BC} = 0.87$

Eliminating indirect effects: partial correlation



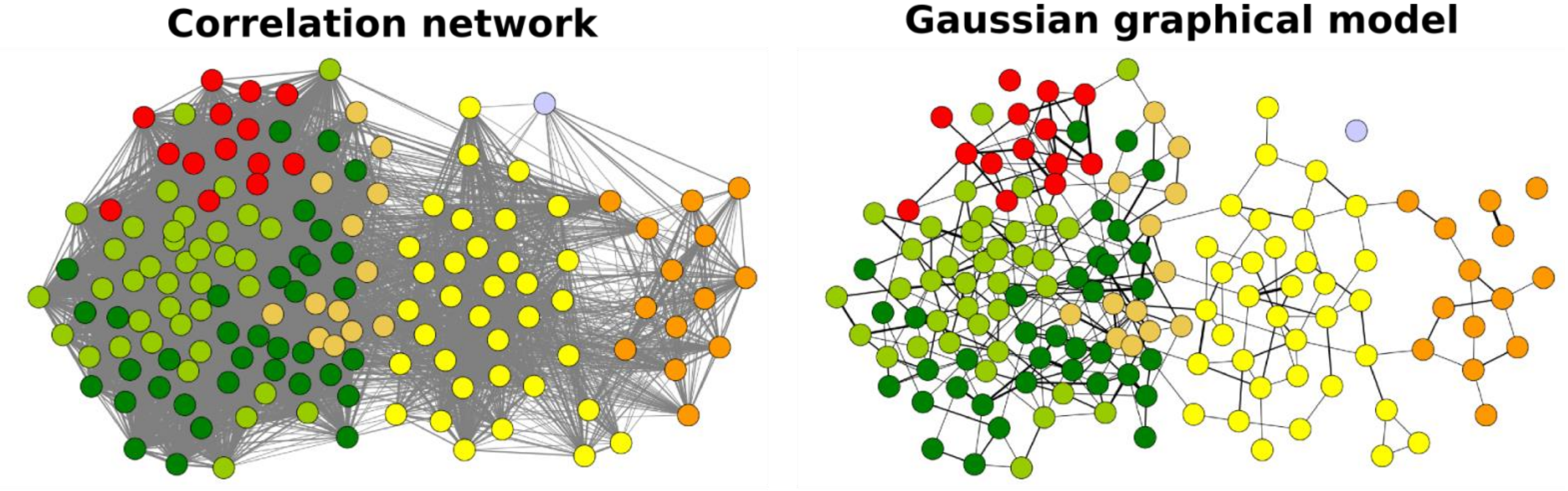
$\rho_{AB} = 0.95$
$\zeta_{AB} = 0.65$

$\rho_{AC} = 0.92$
$\zeta_{AC} = 0.68$

$\rho_{BC} = 0.87$
$\zeta_{BC} = 0.04$

Krumsiek et al.,
BMC Systems Biology, 2011

Reconstruction of metabolic networks using partial correlation networks (=GGMs)

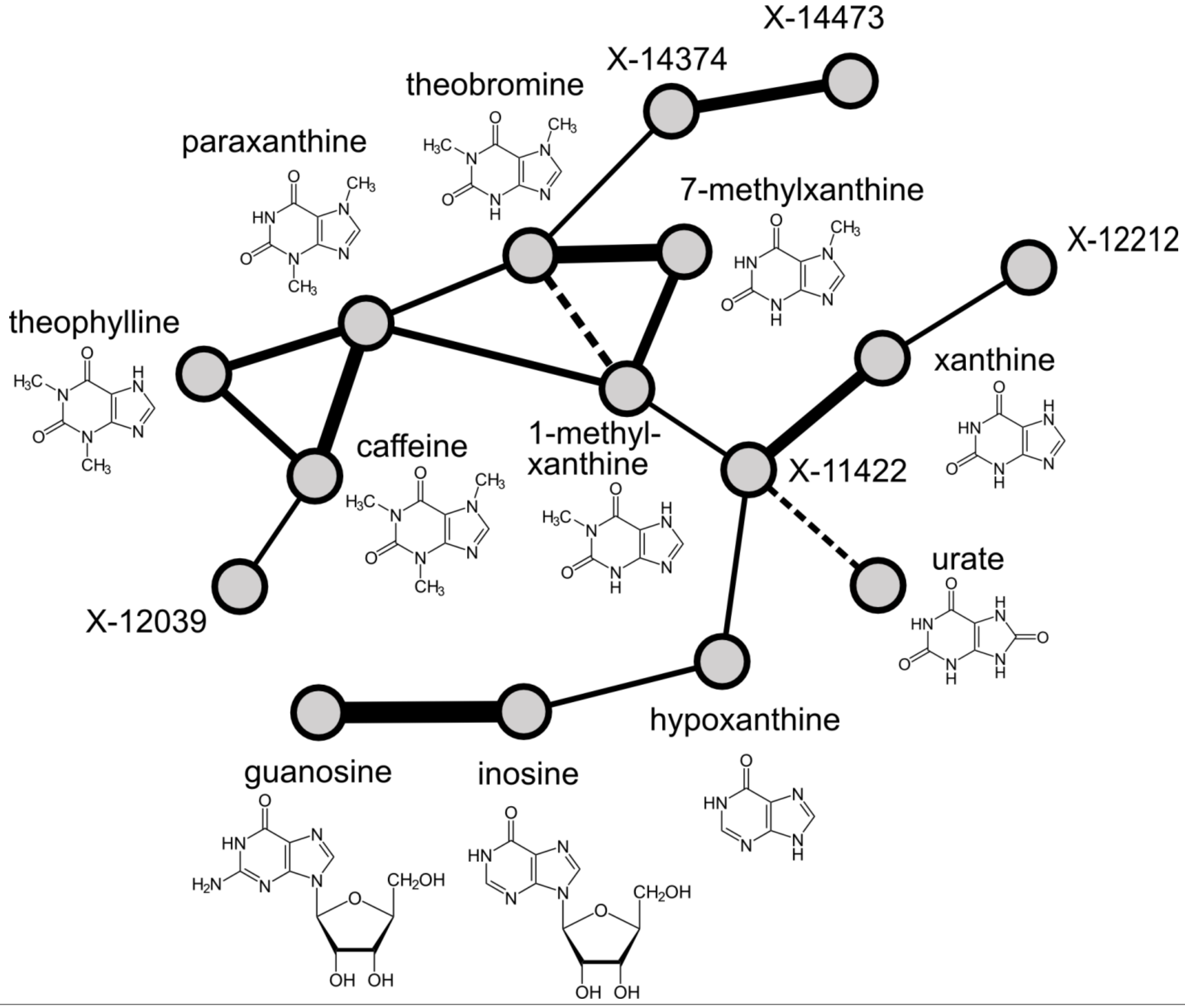


Correlation network

Gaussian graphical model

Krumsiek et al.,
BMC Systems Biology, 2011

Reconstructions by GGMs: Closer inspection

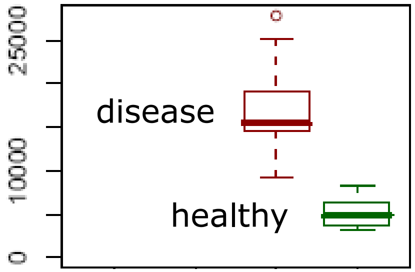


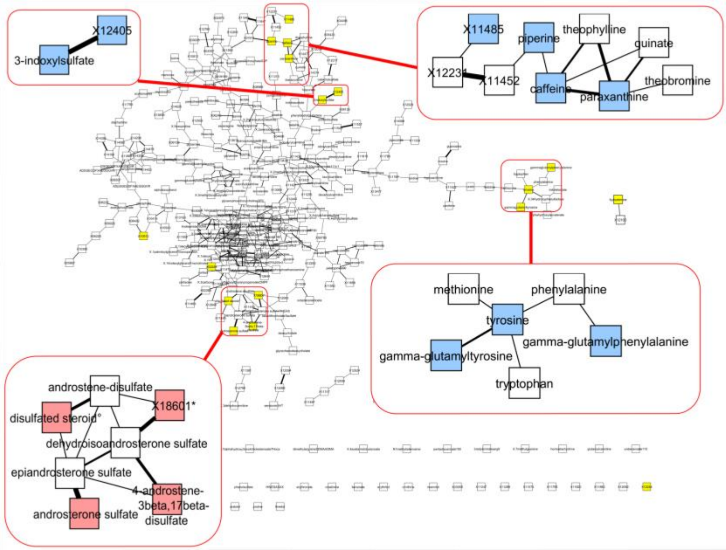
Krumsiek et al.,
PLoS Genet., 2012

APPLICATIONS & AIMS

Applications & Aims of Metabolomics

- „Biomarkers“ discovery
  - Diagnosis
  - Response on therapy
  - Stratification => Personalized Medicine
- Pathomechanistic insights
- Preclinical drug testing

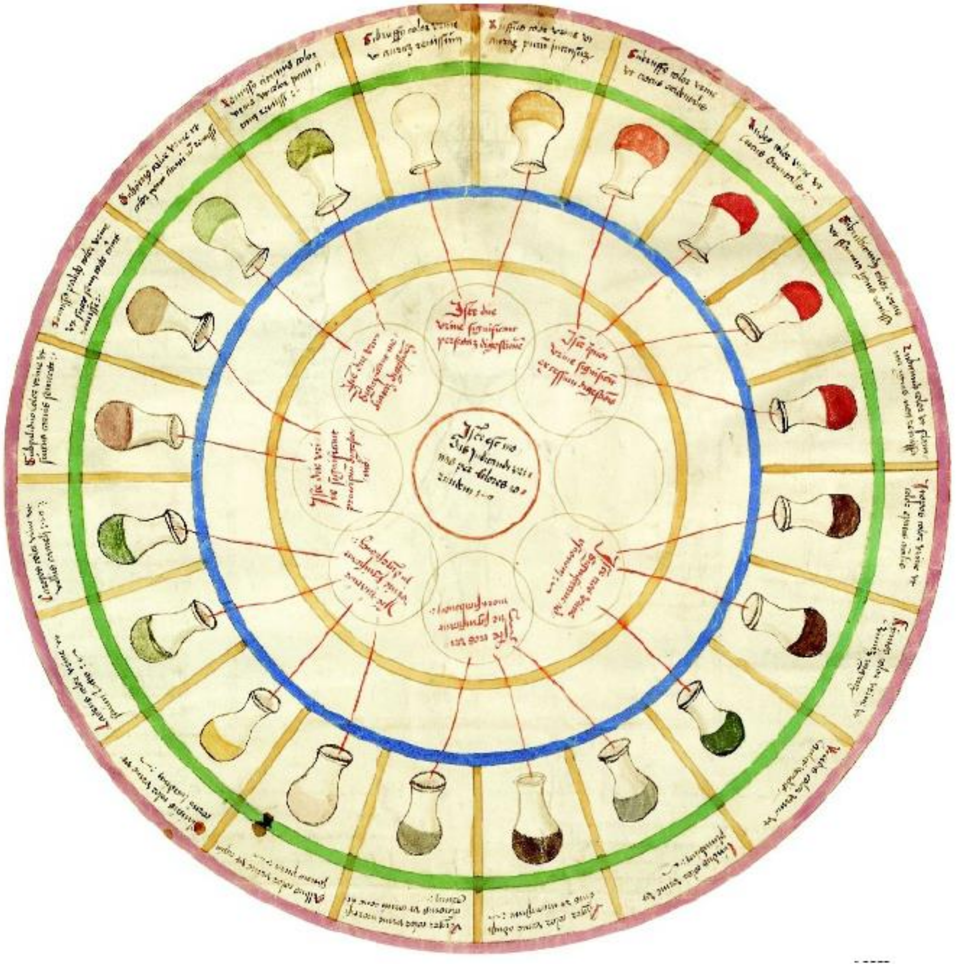




Applications & Aims of Metabolomics

- Biomarkers discovery
  - **Diagnosis**
  - Response on therapy
  - Stratification => Personalized Medicine
- Pathomechanistic insights
- Preclinical drug testing

Metabolites as Diagnostic Biomarkers



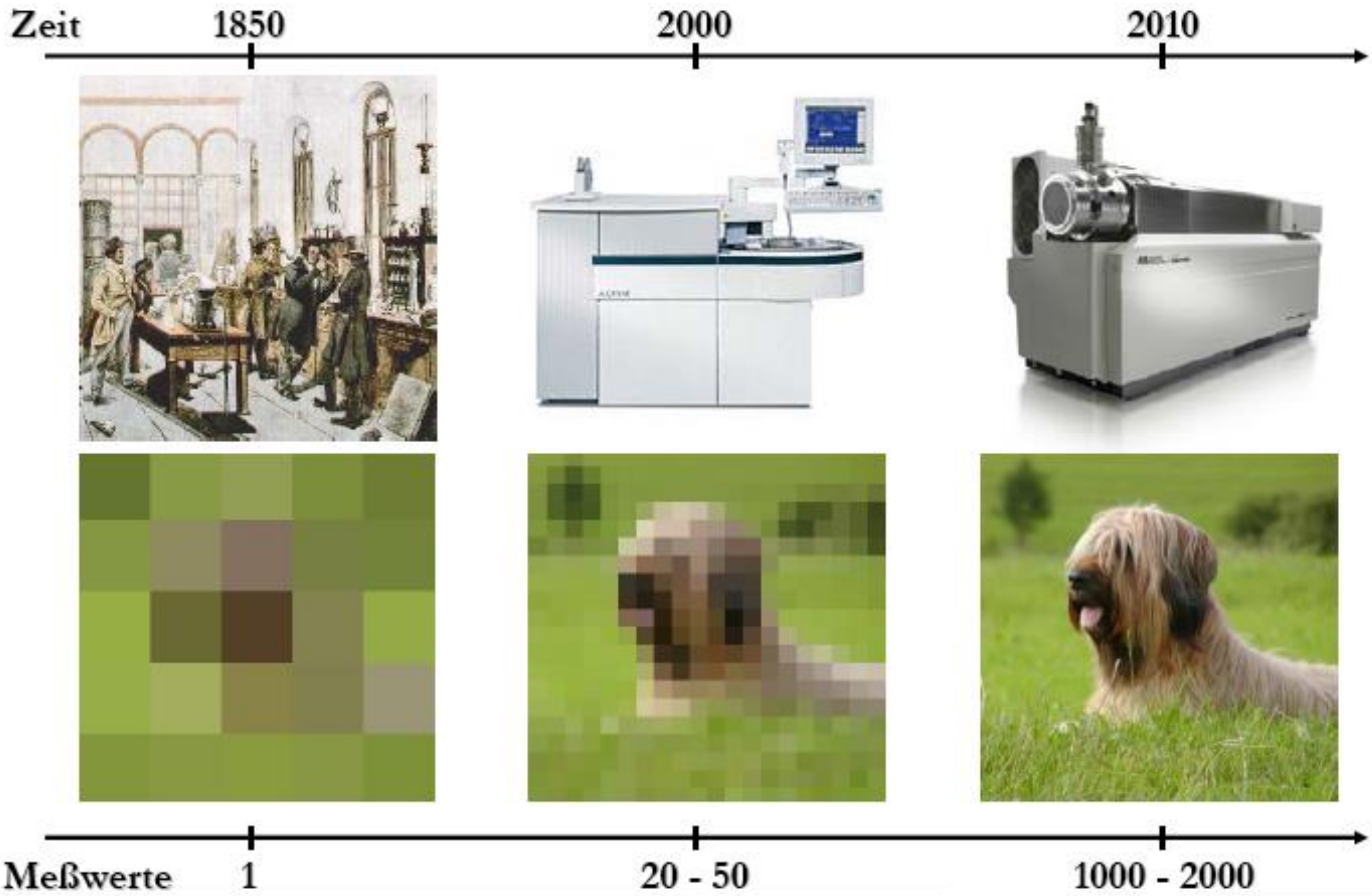
Diagnostic **‘urine charts’** were widely used from the Middle Ages onwards

These charts linked the **colors**, **smells** and **tastes** of urine to various medical conditions.

So what is new?

Pinder, Epiphanie medicorum (1506), Universitätsbibliothek München

Increase of “molecular resolution”



© www.biocrates.at

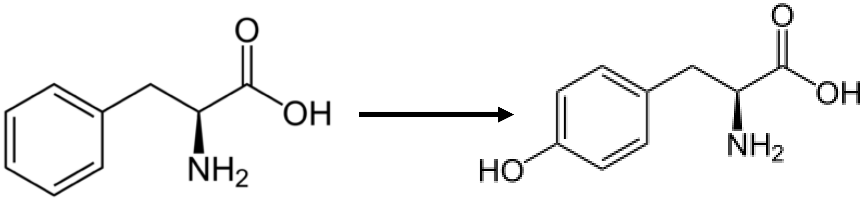
Example: Newborn screening

~40 metabolites
(amino acids, carnitines)

tested to identify

inborn errors of metabolism

Phenylketonuria (>1 in 25,000)



phenylalanine ↑

tyrosine

[Inborn errors of metabolism]

… are merely ***extreme examples of variations of chemical behavior***

which are probably everywhere

present in minor degrees.

A.E. Garrod, Lancet, 1902

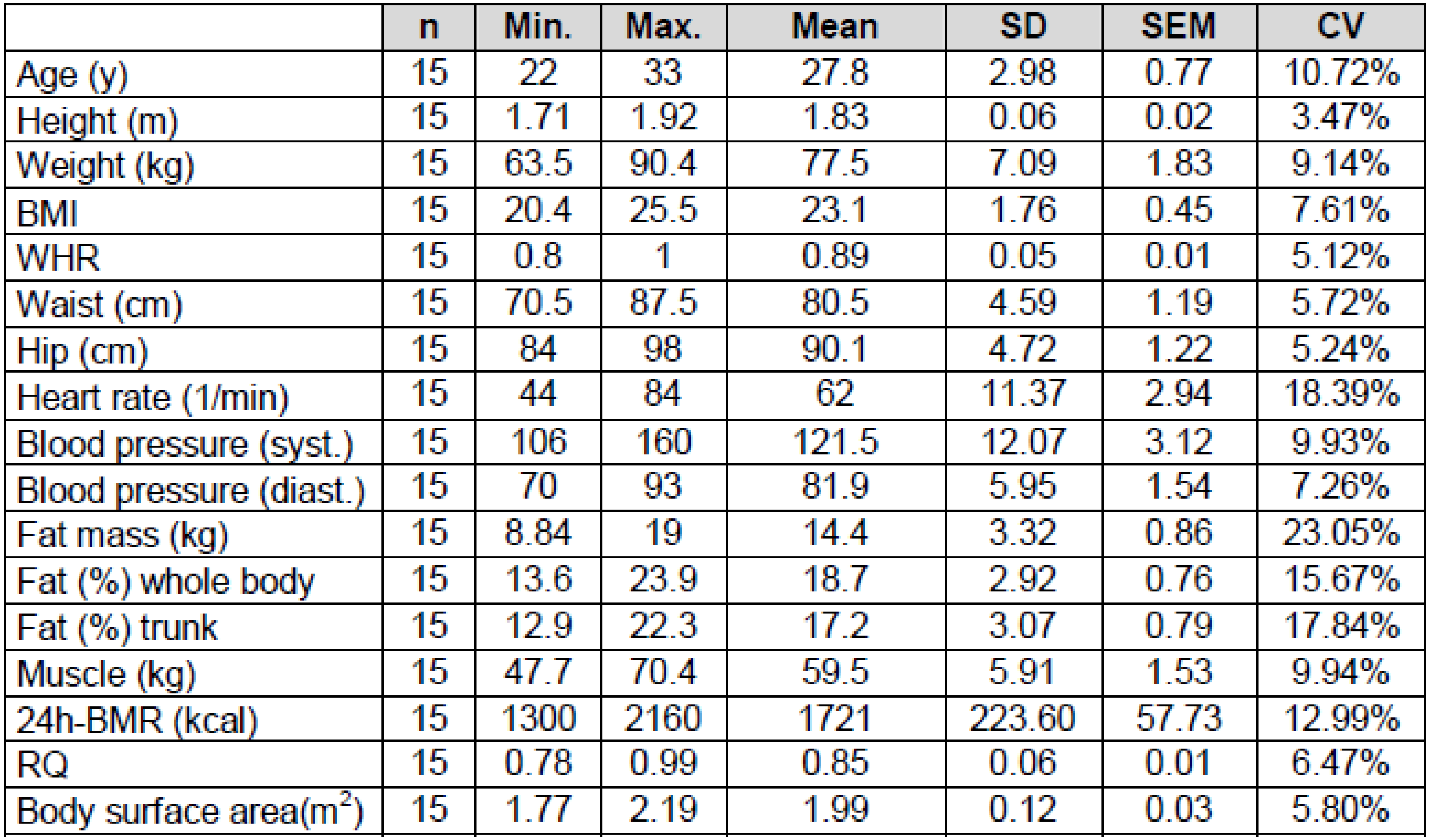
Garrod suggested a link between ***chemical individuality*** and ***predisposition to disease.***

THE “NORMAL” HUMAN METABOLOME (HUMET)

Krug et al., FASEB, 2012

HuMet: Studying the “normal” human metabolome

- 15 young healthy men:



	n	Min.	Max.	Mean	SD	SEM	CV
Age (y)	15	22	33	27.8	2.98	0.77	10.72%
Height (m)	15	1.71	1.92	1.83	0.06	0.02	3.47%
Weight (kg)	15	63.5	90.4	77.5	7.09	1.83	9.14%
BMI	15	20.4	25.5	23.1	1.76	0.45	7.61%
WHR	15	0.8	1	0.89	0.05	0.01	5.12%
Waist (cm)	15	70.5	87.5	80.5	4.59	1.19	5.72%
Hip (cm)	15	84	98	90.1	4.72	1.22	5.24%
Heart rate (1/min)	15	44	84	62	11.37	2.94	18.39%
Blood pressure (syst.)	15	106	160	121.5	12.07	3.12	9.93%
Blood pressure (diast.)	15	70	93	81.9	5.95	1.54	7.26%
Fat mass (kg)	15	8.84	19	14.4	3.32	0.86	23.05%
Fat (%) whole body	15	13.6	23.9	18.7	2.92	0.76	15.67%
Fat (%) trunk	15	12.9	22.3	17.2	3.07	0.79	17.84%
Muscle (kg)	15	47.7	70.4	59.5	5.91	1.53	9.94%
24h-BMR (kcal)	15	1300	2160	1721	223.60	57.73	12.99%
RQ	15	0.78	0.99	0.85	0.06	0.01	6.47%
Body surface area(m^2)	15	1.77	2.19	1.99	0.12	0.03	5.80%

Metabolome – a snapshot of biochemical state

8 am fasting

↓ ↓

Metabolome 1

9 am after breakfast

↓ ↓

Metabolome 2

4 pm sports

↓ ↓

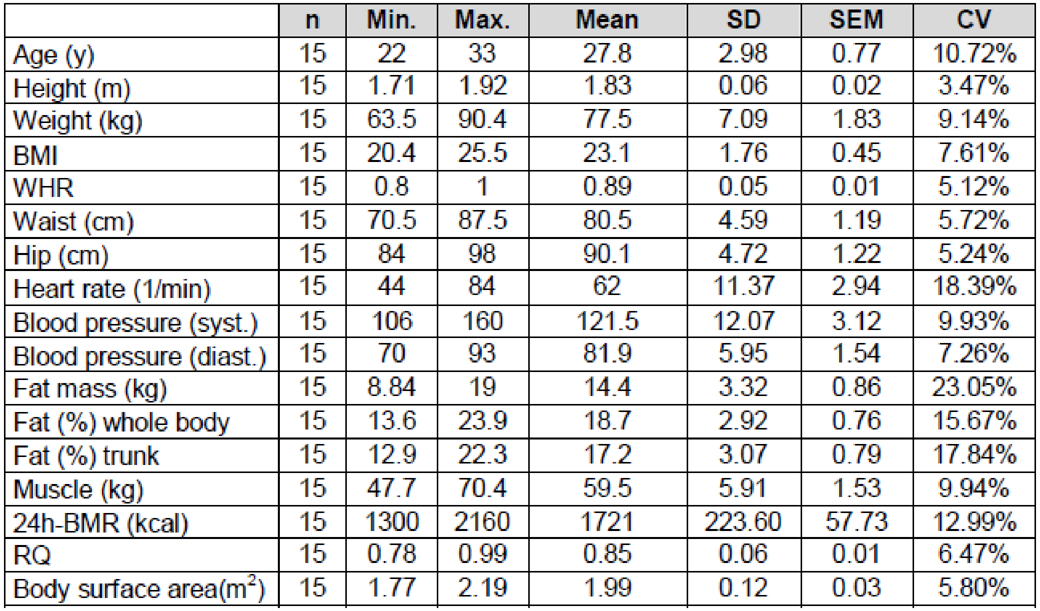
Metabolome 3

HuMet: Studying the “normal” human metabolome

- 15 young healthy men:

age: 22-33

BMI: 20-25 kg/m^2



	n	Min.	Max.	Mean	SD	SEM	CV
Age (y)	15	22	33	27.8	2.98	0.77	10.72%
Height (m)	15	1.71	1.92	1.83	0.06	0.02	3.47%
Weight (kg)	15	63.5	90.4	77.5	7.09	1.83	9.14%
BMI	15	20.4	25.5	23.1	1.76	0.45	7.61%
WHR	15	0.8	1	0.89	0.05	0.01	5.12%
Waist (cm)	15	70.5	87.5	80.5	4.59	1.19	5.72%
Hip (cm)	15	84	98	90.1	4.72	1.22	5.24%
Heart rate (1/min)	15	44	84	62	11.37	2.94	18.39%
Blood pressure (syst.)	15	106	160	121.5	12.07	3.12	9.93%
Blood pressure (diast.)	15	70	93	81.9	5.95	1.54	7.26%
Fat mass (kg)	15	8.84	19	14.4	3.32	0.86	23.05%
Fat (%) whole body	15	13.6	23.9	18.7	2.92	0.76	15.67%
Fat (%) trunk	15	12.9	22.3	17.2	3.07	0.79	17.84%
Muscle (kg)	15	47.7	70.4	59.5	5.91	1.53	9.94%
24h-BMR (kcal)	15	1300	2160	1721	223.60	57.73	12.99%
RQ	15	0.78	0.99	0.85	0.06	0.01	6.47%
Body surface area(m^2)	15	1.77	2.19	1.99	0.12	0.03	5.80%

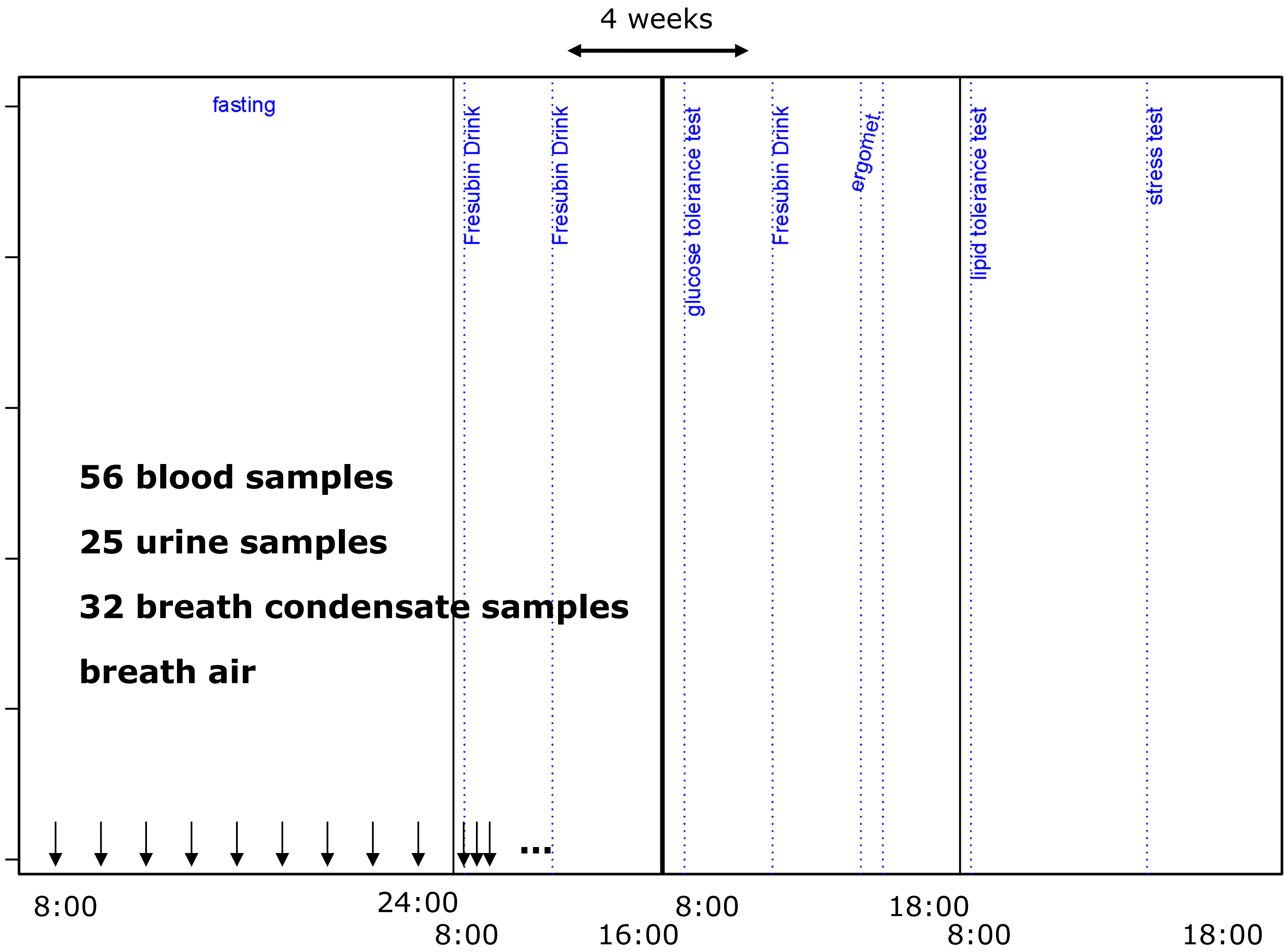
- Controlled trial over the time course of 4 days

4 nutritional interventions: (fasting, standard meal, OGTT, OLTT)

physical exercise

stress test

HuMet: Study design



56 blood samples

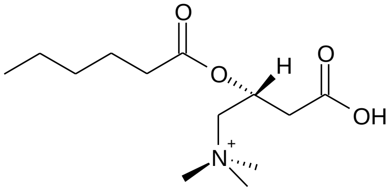
25 urine samples

32 breath condensate samples

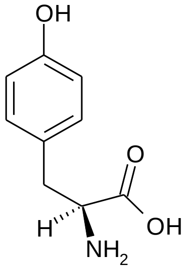
breath air

HuMet plasma samples @ *metaP*

15 individuals x 56 plasma samples



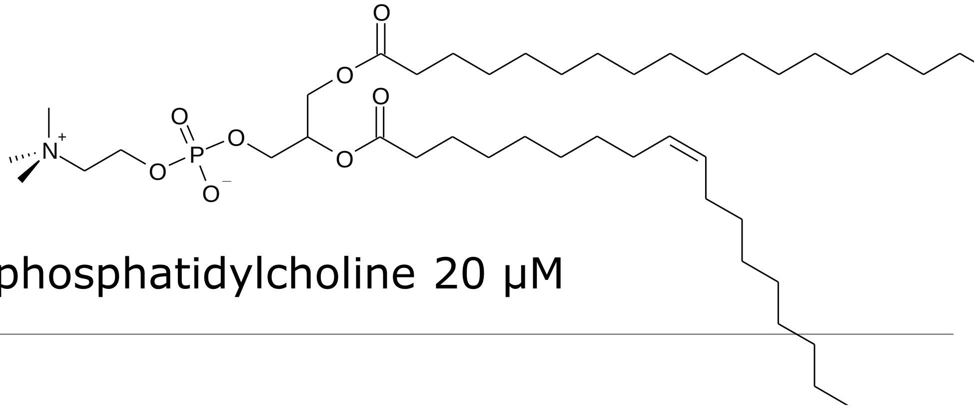
hexanoylcarnitine 0.05 µM



tyrosine 100 µM

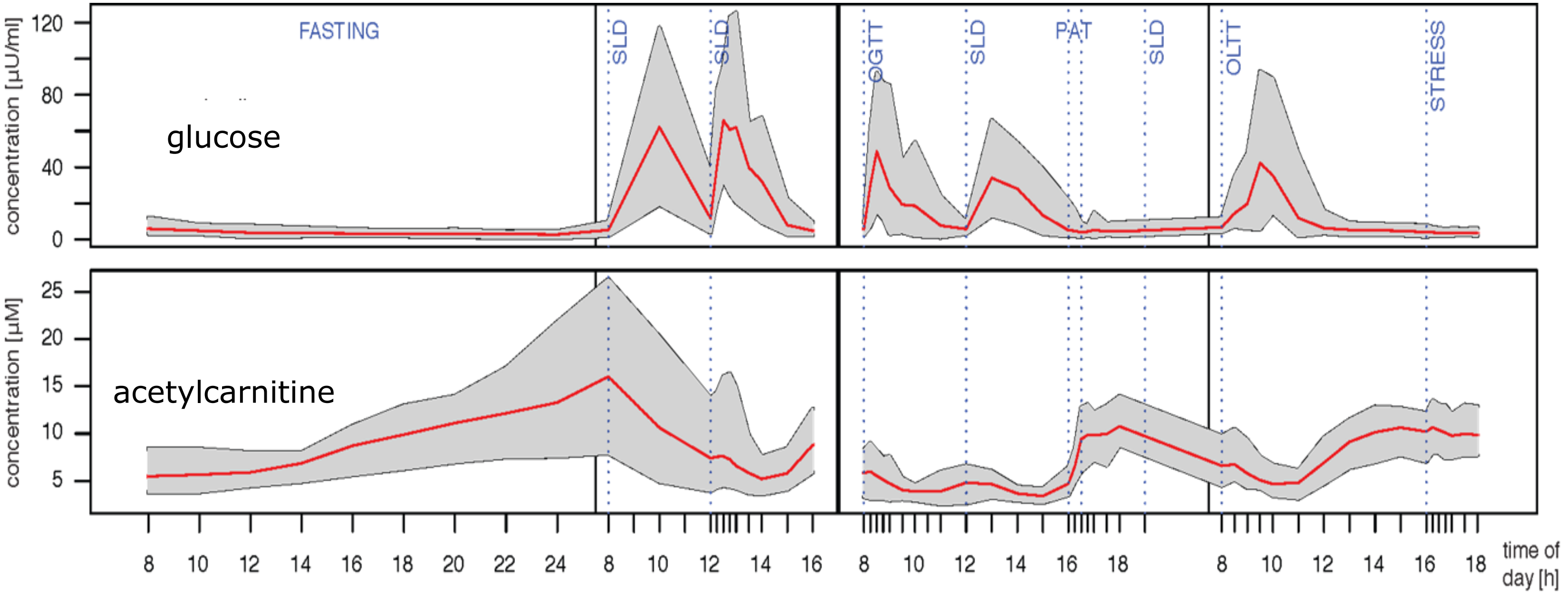
Targeted metabolomics

163 metabolites per sample



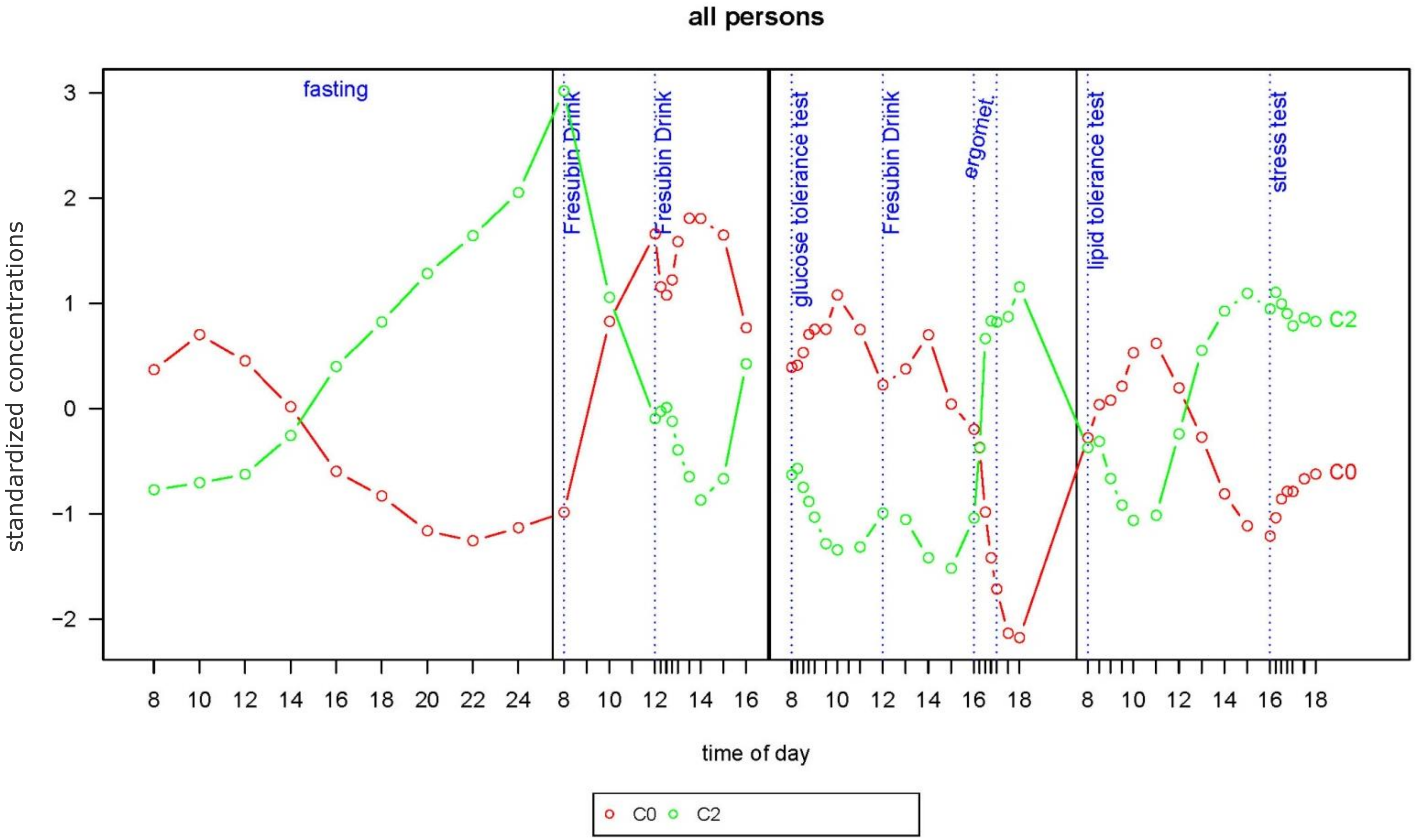
phosphatidylcholine 20 µM

Metabolites levels largely vary during the day and on response to challenges

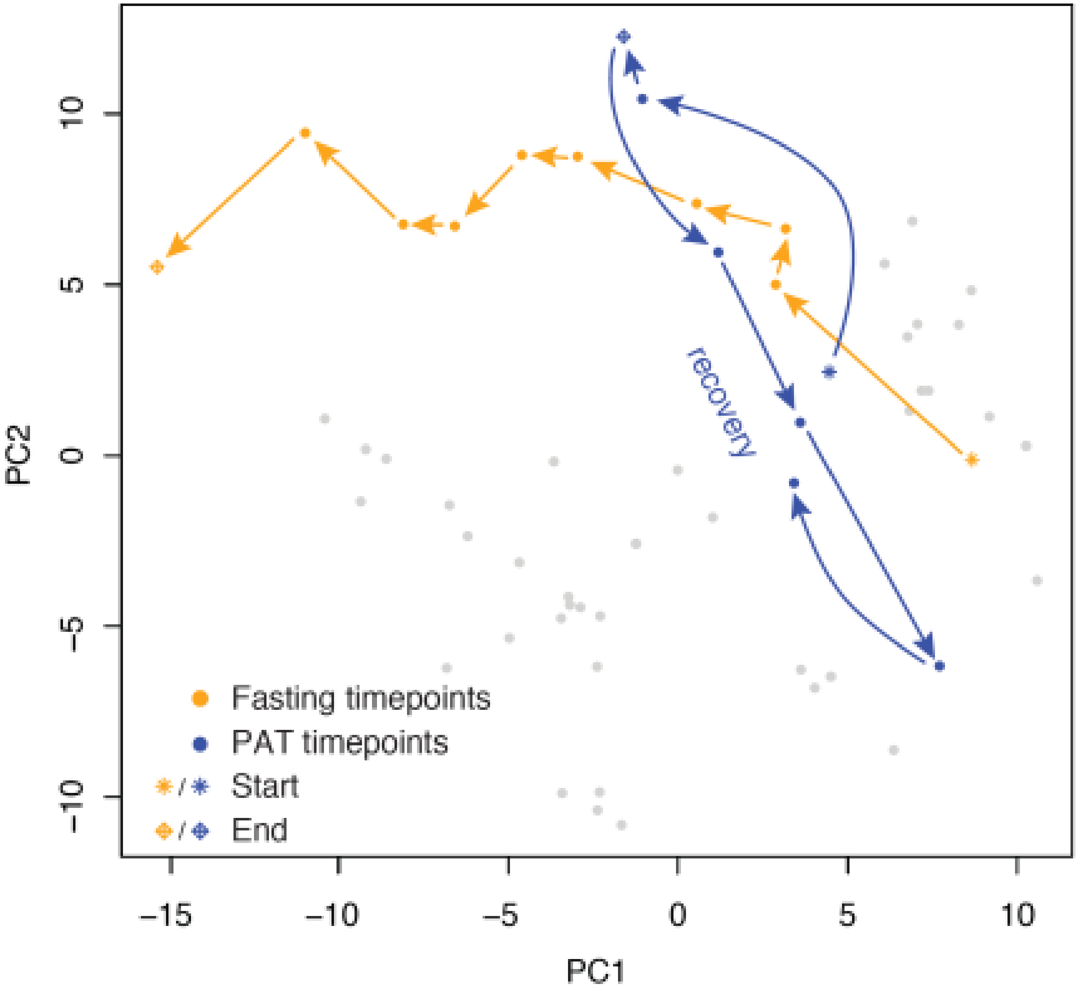


Krug et al., FASEB, 2012

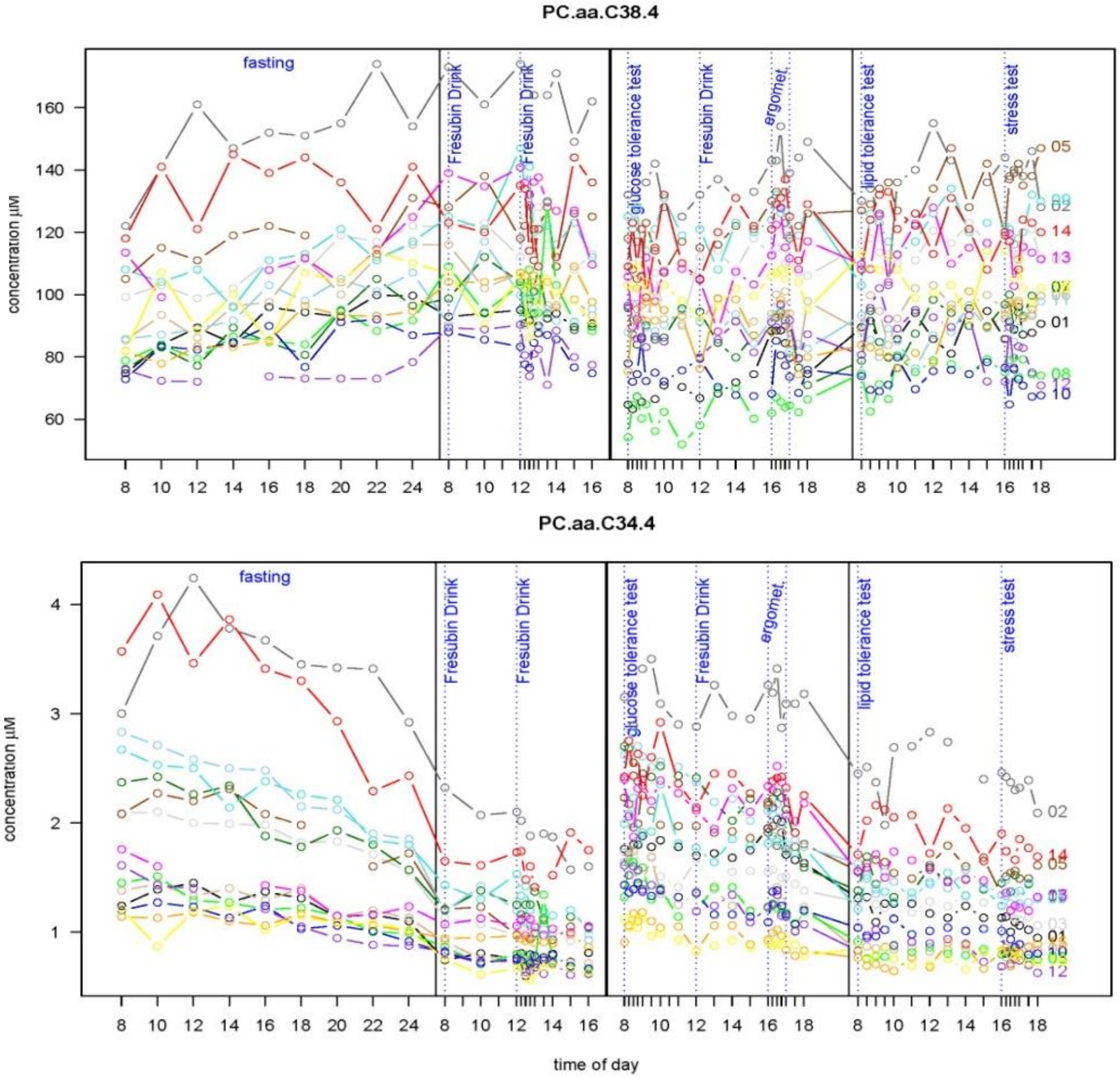
Switching from anabolism to catabolism and back …



… pushing volunteers through the metabolic space



Metabolite levels differ between individuals

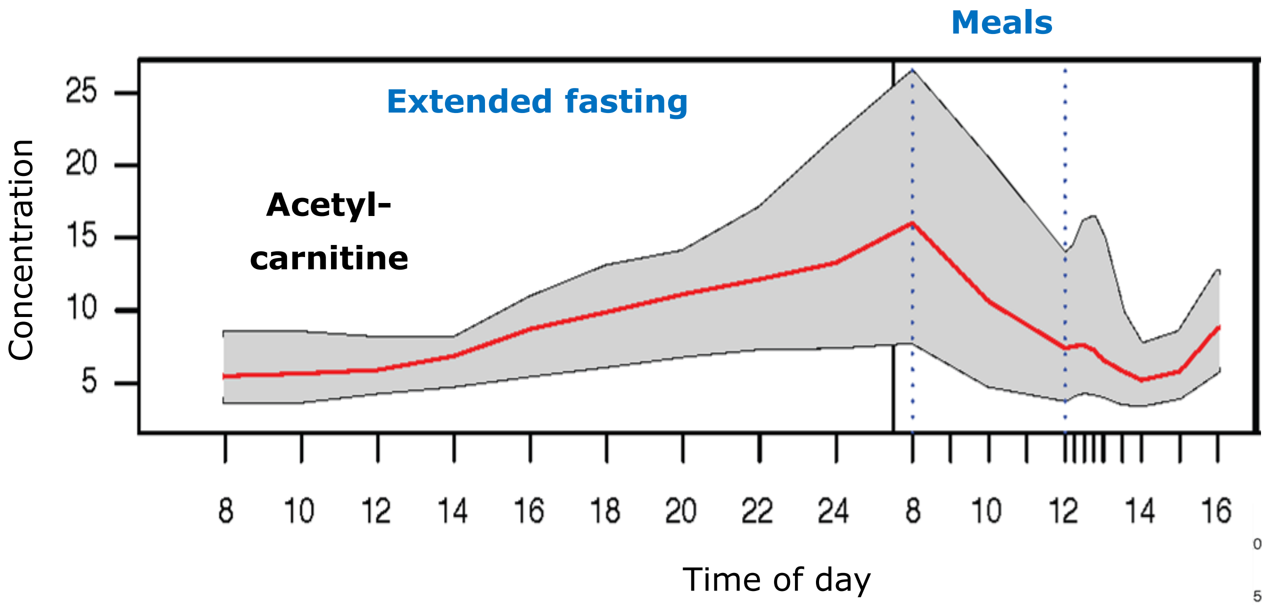


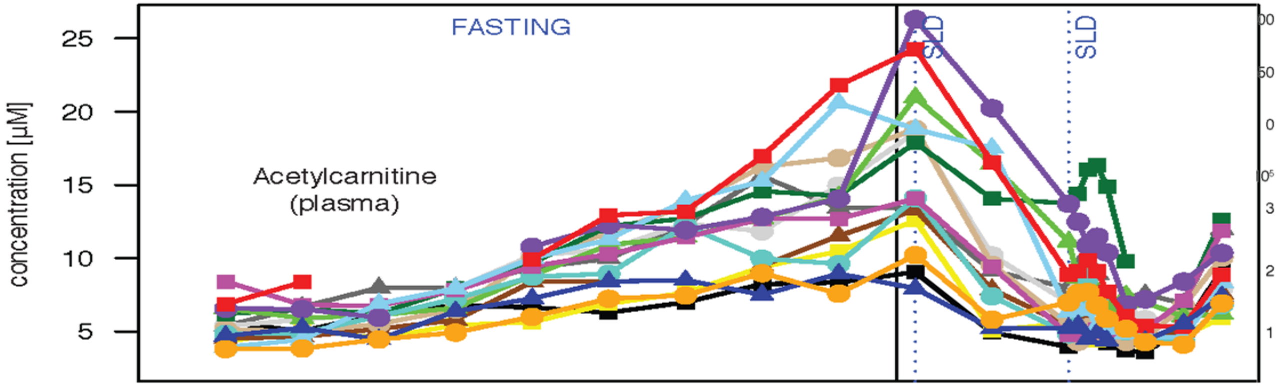
color for persons

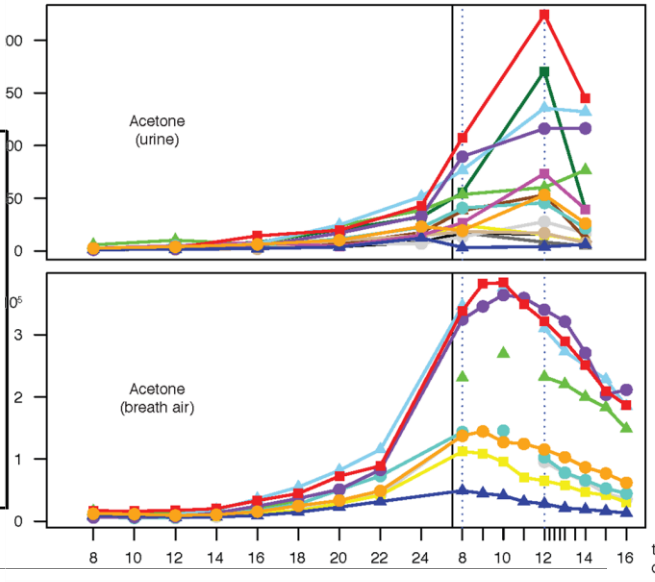
● 01
● 02
● 03
● 05
● 06
● 07
● 08
● 09
● 10
● 11
● 12
● 13
● 14
● 15
● 16

Normal ranges!

Metabolic response differs between individuals

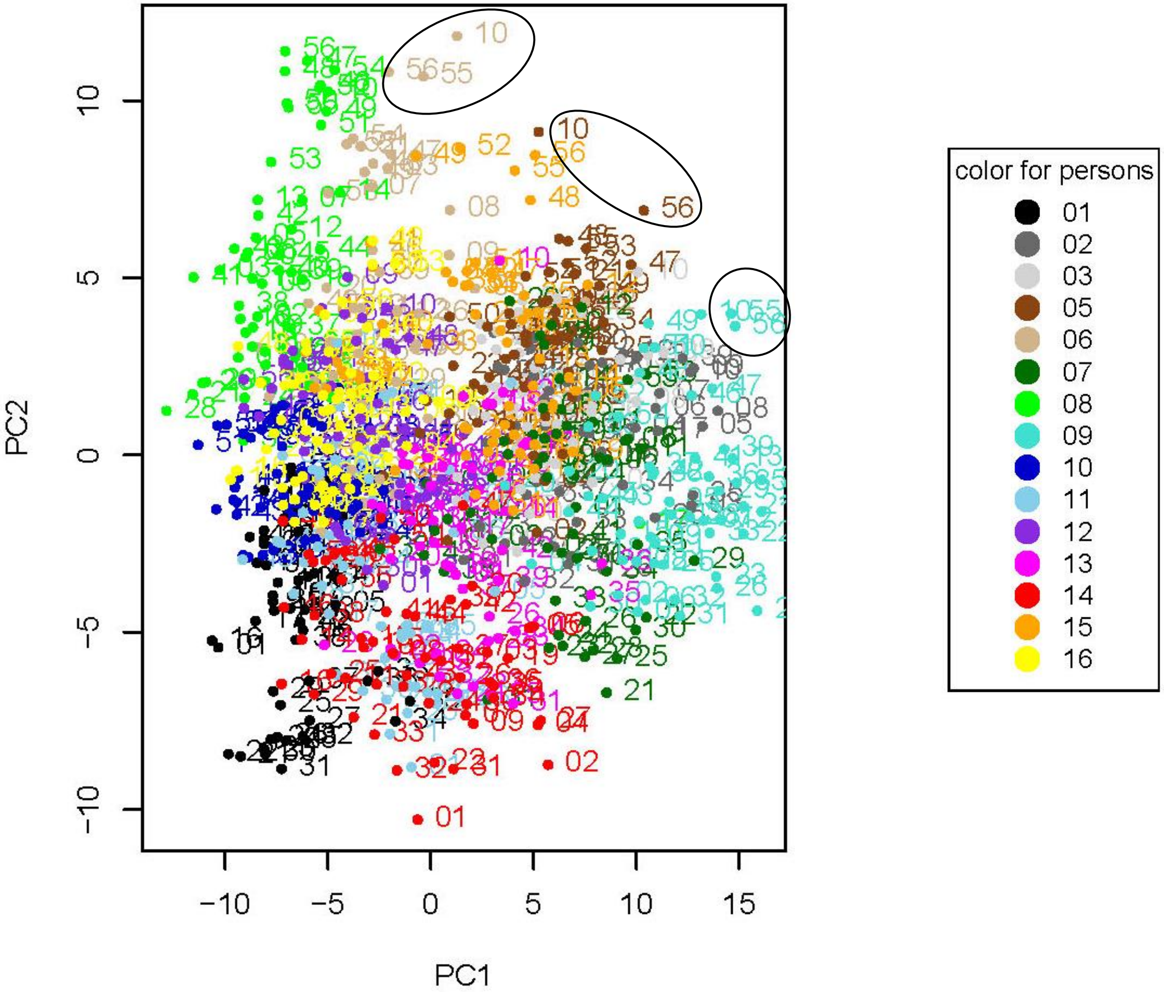






Krug et al., FASEB, 2012

Metabolite profiles are individual

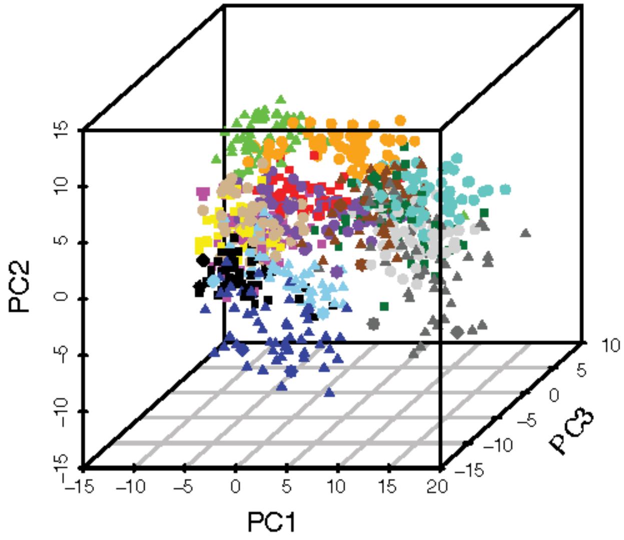


Personal metabolomes are stable

- **Short-term** (days), challenges

  plasma, 15 young men, MS-based

  Krug et al., FASEB, 2012

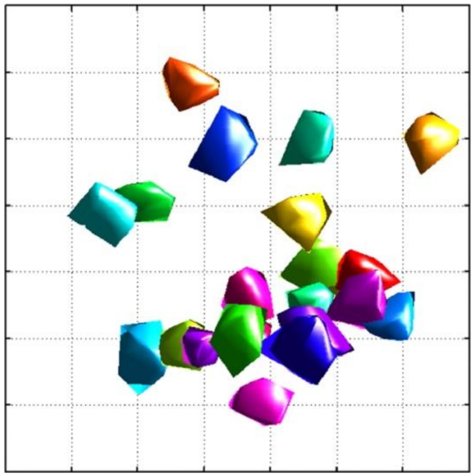


Metabolic individuality

- **Mid-term** (months)

  urine, 22 subjects, NMR-based

  Assfalg et al., PNAS, 2008



Assfalg et al., PNAS, 2008

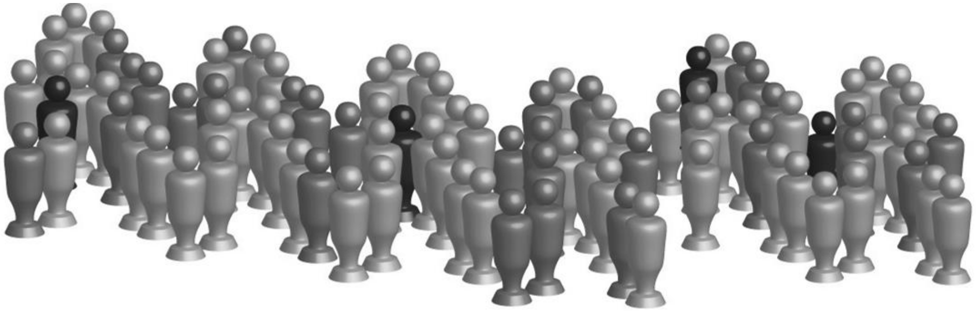
Yousri et al., Metabolomics, 2014

Krug et al., FASEB, 2012

Chua et al., PNAS, 2013

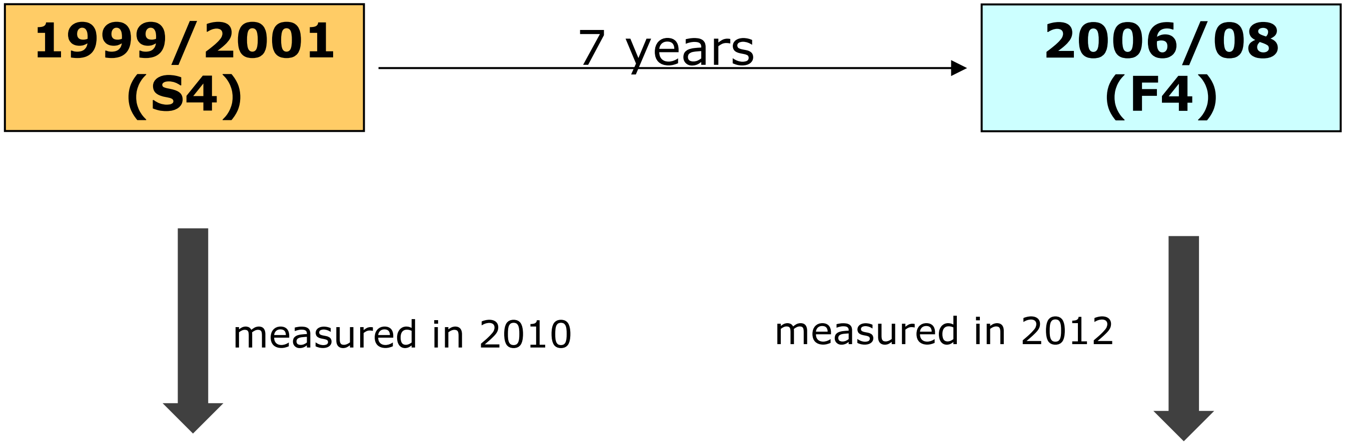
Long-term stability (years)?

Long-term stability of metabolite profiles?



KOOPERATIVE GESUNDHEITSFORSCHUNG IN DER REGION AUGSBURG KORA

n=818



1999/2001 (S4)

7 years

2006/08 (F4)

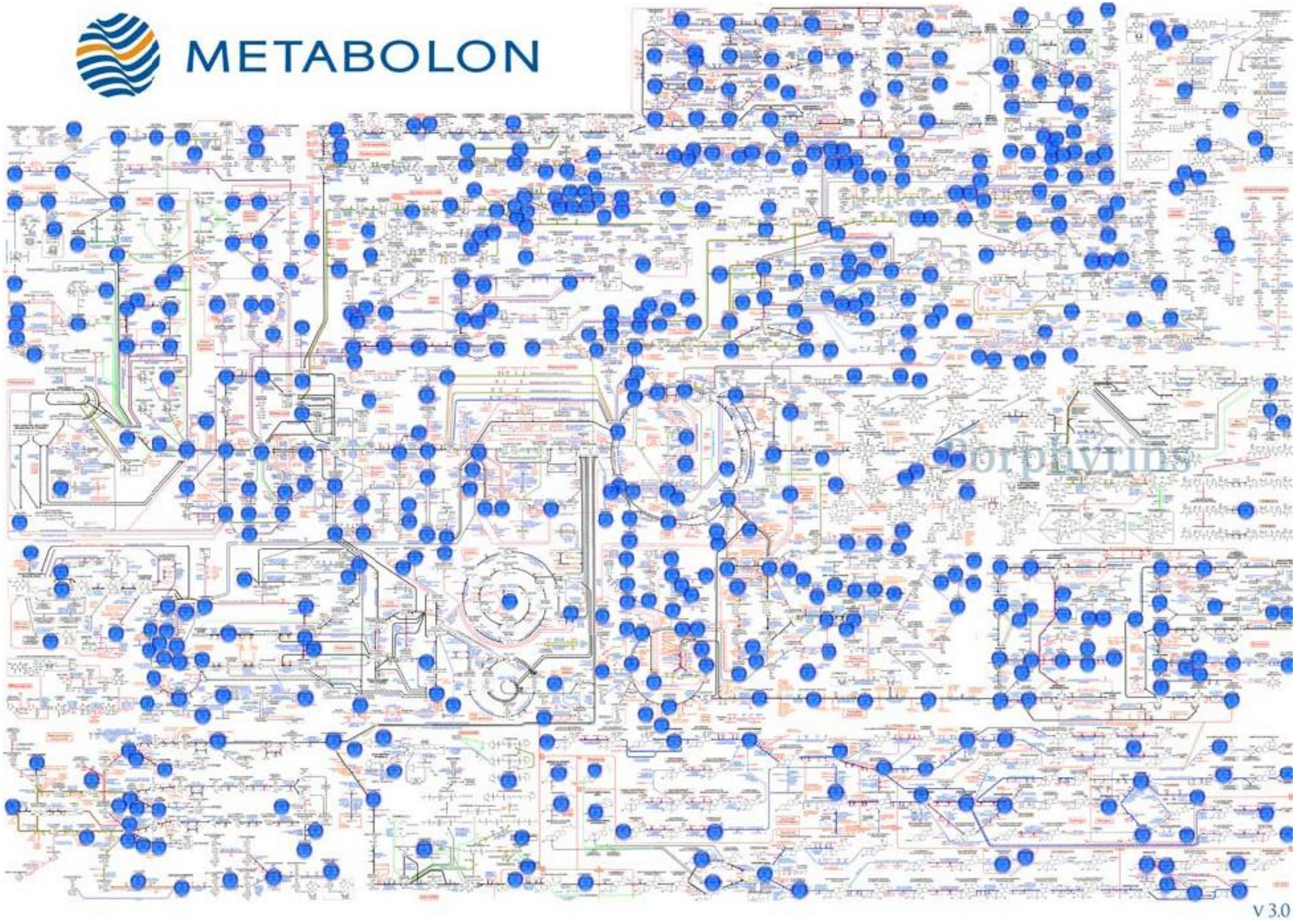
measured in 2010

measured in 2012

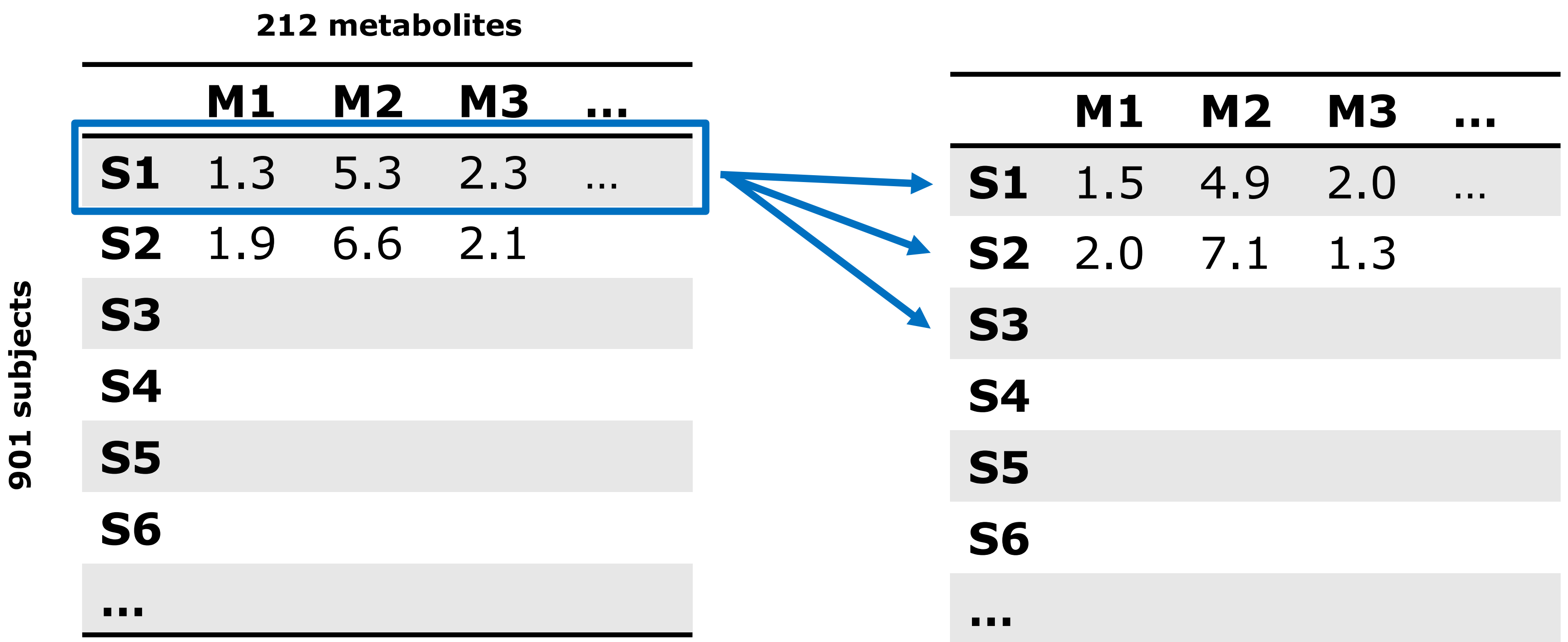
Non-targeted metabolomics: METABOLON

212 metabolites

METABOLON



Assessing ranks of “self-correlation”



Baseline

212 metabolites

901 subjects

	M1	**M2**	**M3**	**…**
S1	1.3	5.3	2.3	…
S2	1.9	6.6	2.1	
S3				
S4				
S5				
S6				
…				

7-year follow-up

	M1	**M2**	**M3**	**…**
S1	1.5	4.9	2.0	…
S2	2.0	7.1	1.3	
S3				
S4				
S5				
S6				
…				

Yousri et al., Metabolomics, 2014

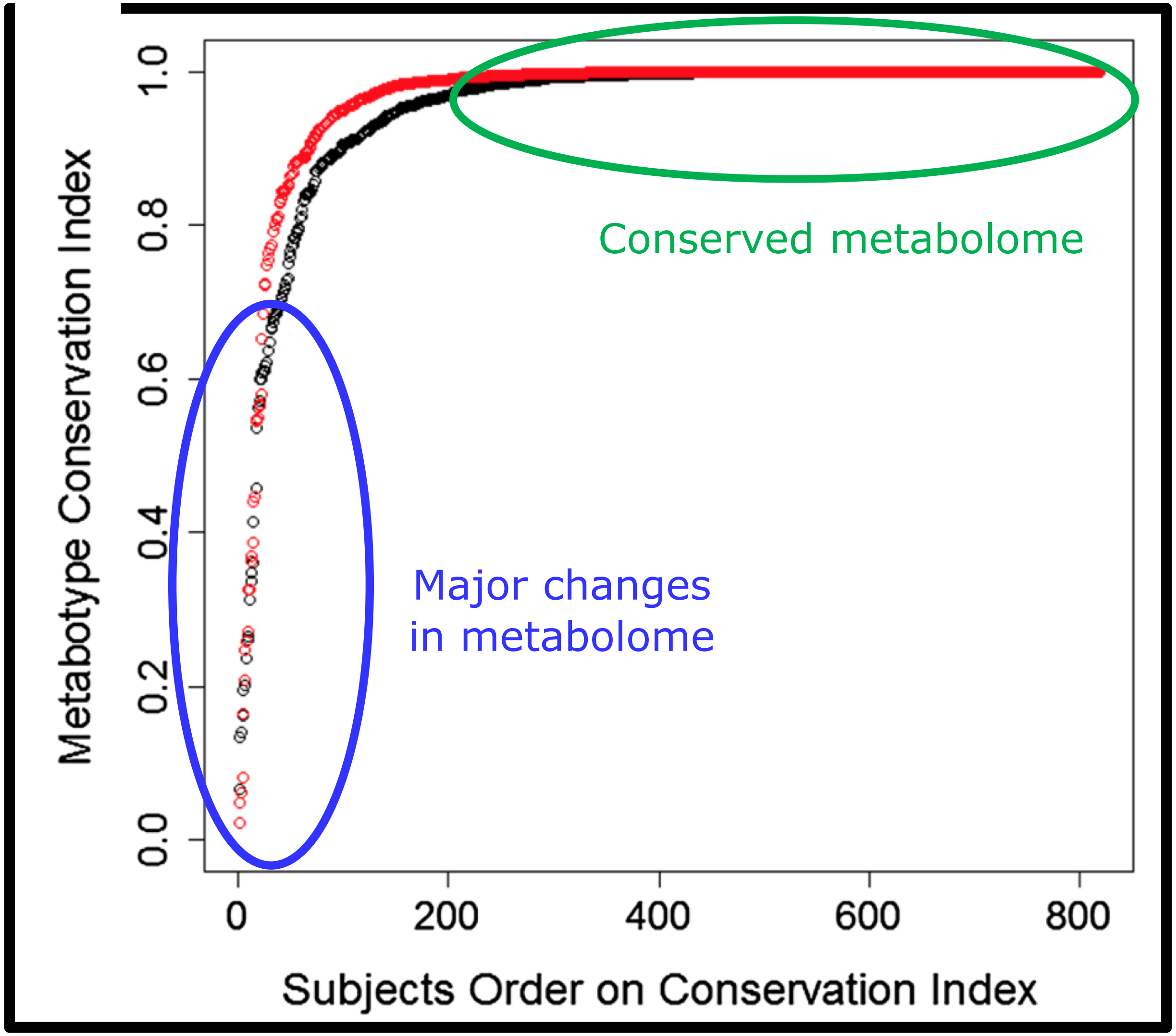
High long-term stability of metabolite profiles

40% of subjects:

strongest correlation with own profiles in 7y follow-up

95% of subjects:

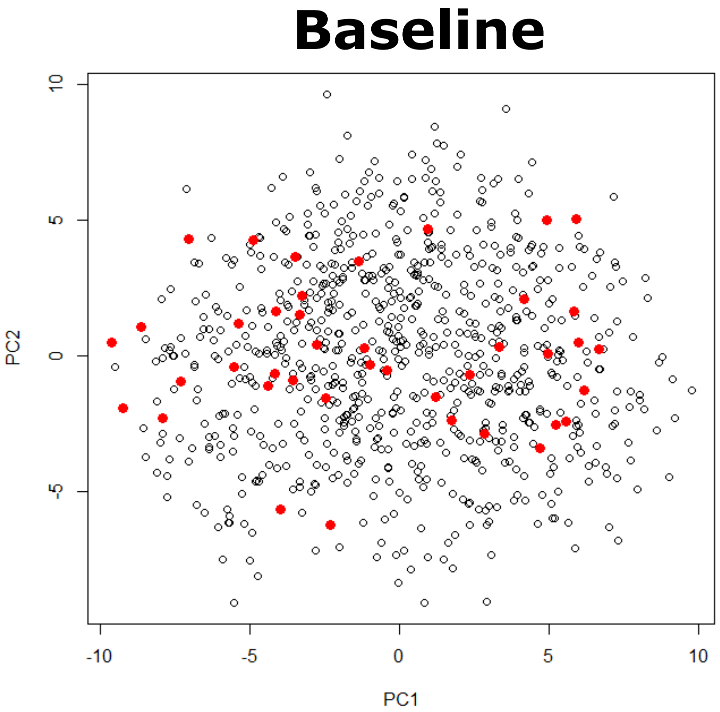
“self” correlation ranked among the 30% strongest

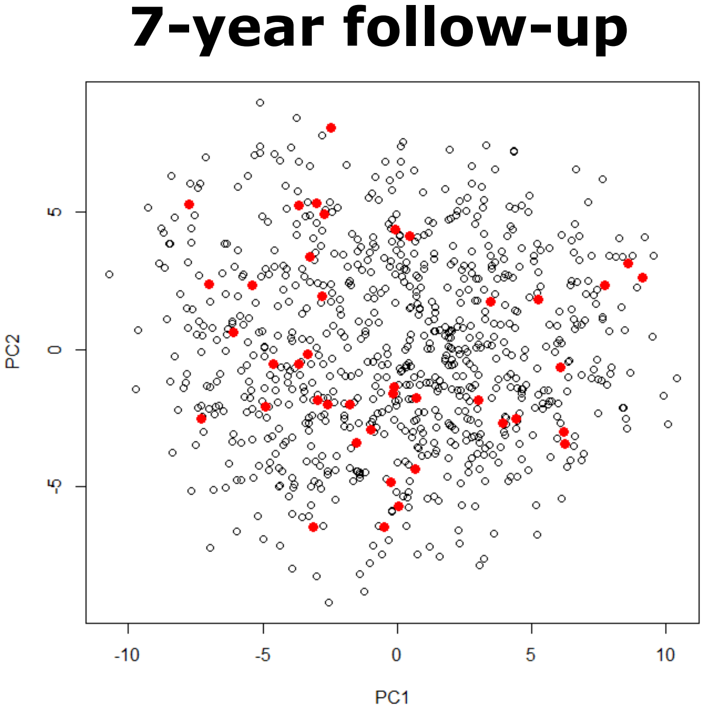


Yousri et al., Metabolomics, 2014

Are metabolomes with major changes special?

Individuals with
major changes in their metabolome



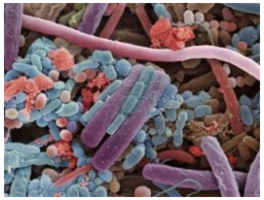


HelmholtzZentrum münchen
Deutsches Forschungszentrum für Gesundheit und Umwelt

Yousri et al., Metabolomics, 2014

Reasons for high conservation?

Genetic variation



Steve Gschmeissner/SPL

Microbiome

Lifestyle

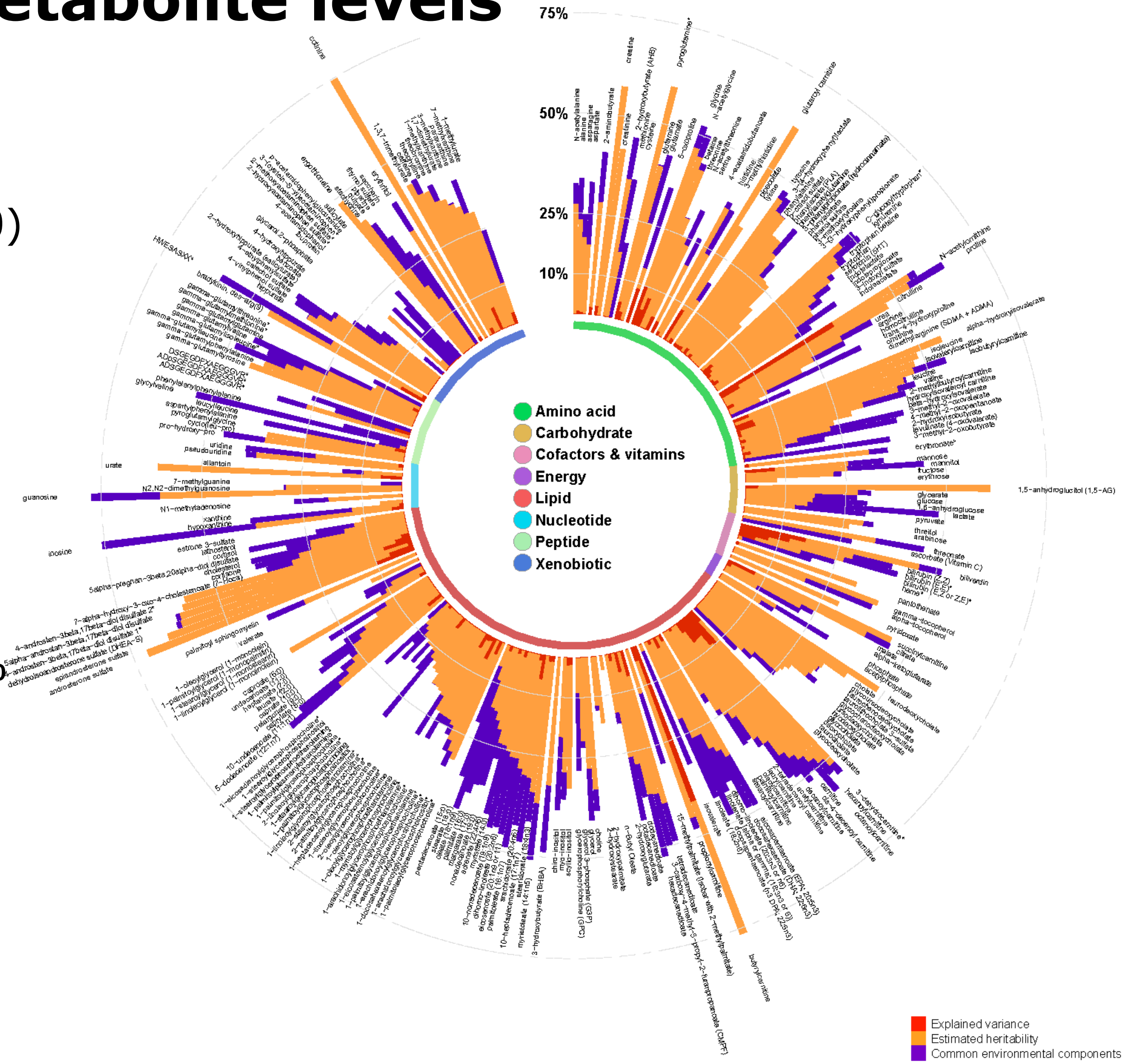
Heritability of metabolite levels

Estimation of heritability
based on twins (n=6000)
(ACE model)

Which fraction of variance
inherited, which due to
common environment?

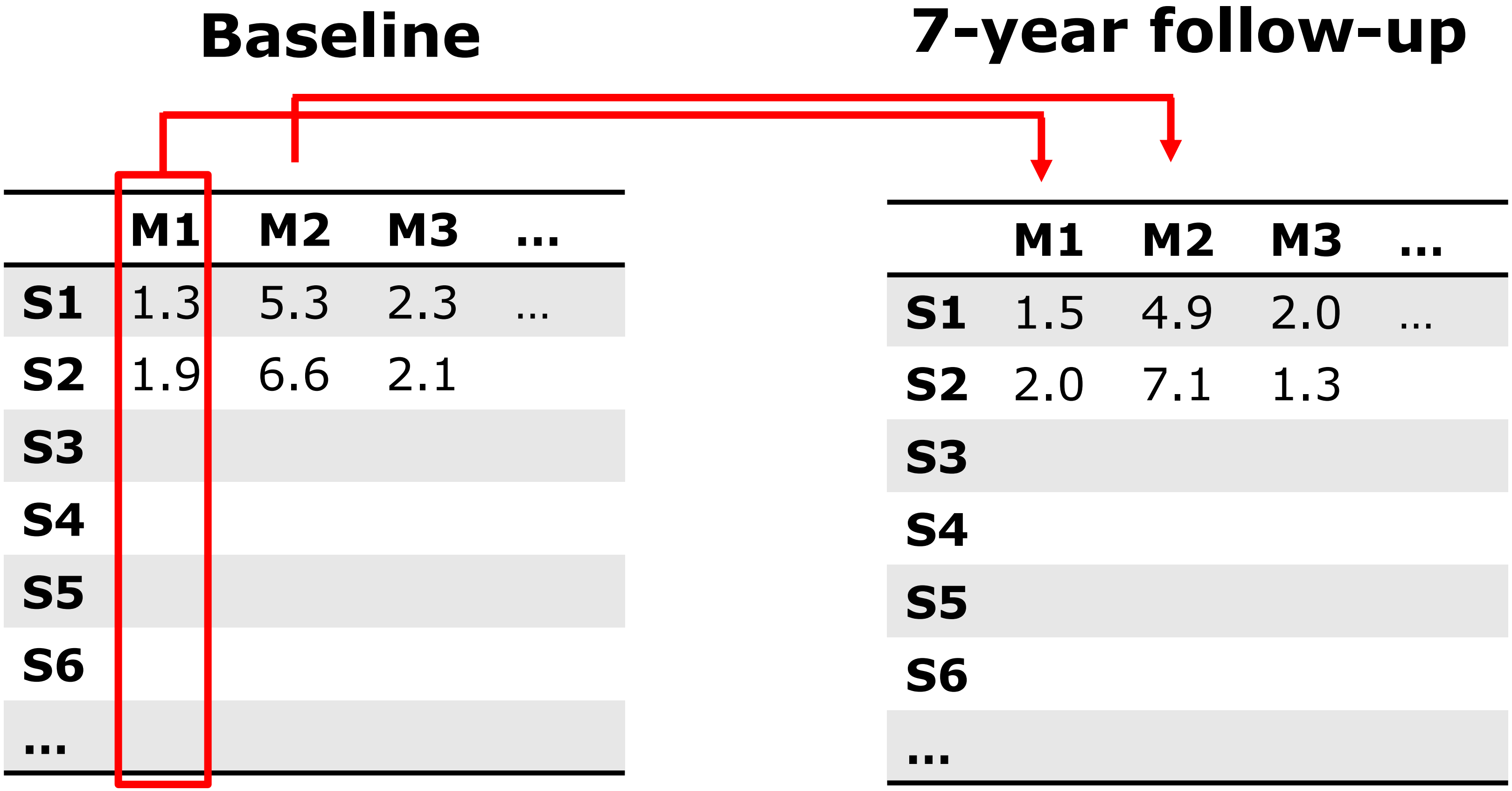
⇒ Median heritability: 25%

⇒ Max heritability: 76%
(butyrylcarnitine)



Shin et al., Nature Genet, 2014

Conservation of metabolites



Baseline

	M1	M2	M3	…
S1	1.3	5.3	2.3	…
S2	1.9	6.6	2.1	
S3				
S4				
S5				
S6				
…				

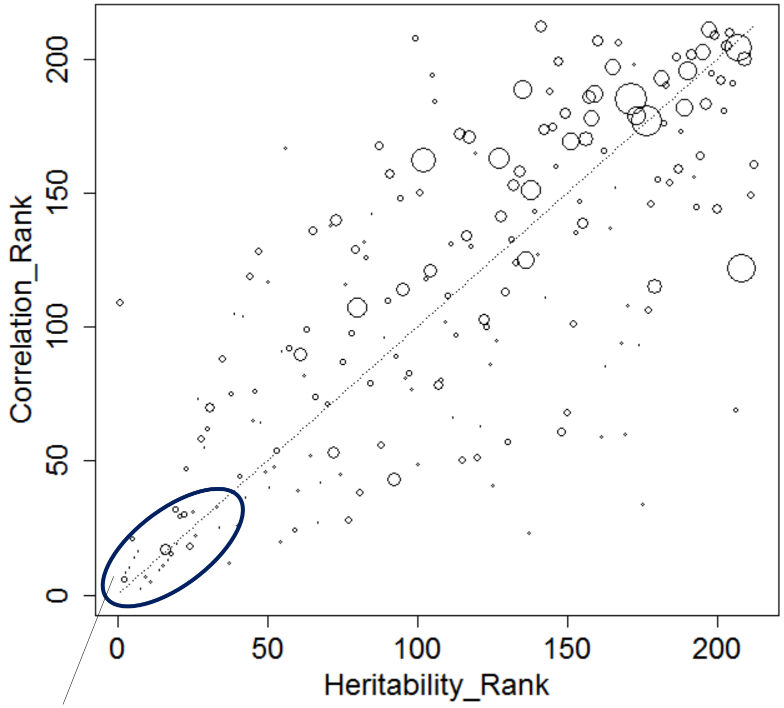
7-year follow-up

	M1	M2	M3	…
S1	1.5	4.9	2.0	…
S2	2.0	7.1	1.3	
S3				
S4				
S5				
S6				
…				

pairwise correlations -> rank correlations -> most stable metabolites?

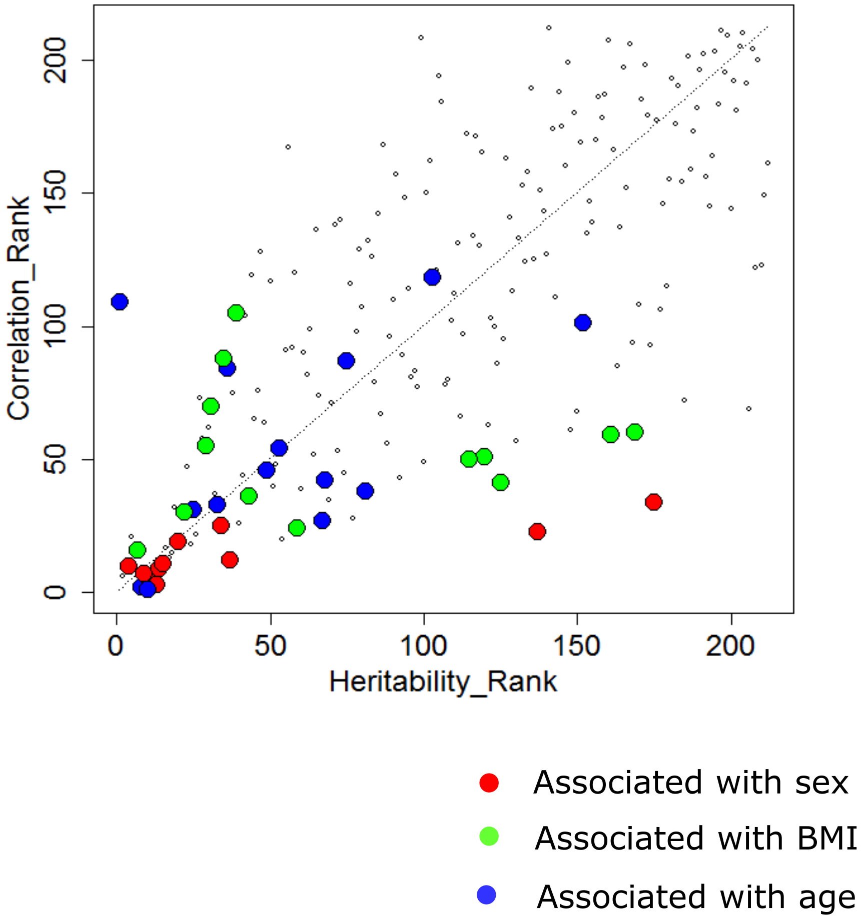
Yousri et al., Metabolomics, in press

Conservation vs heritability



C4 carnitine

hormones



Can heritability be explained by common genetic variants?

Thank you for your attention !

© K. Suhre