Emory-Georgia Tech Predictive Health Initiative
DOM Clinical Biomarkers Laboratory

Universal Exposure Surveillance as a Component of Personalized Medicine

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EMORY
SCHOOL OF
MEDICINE

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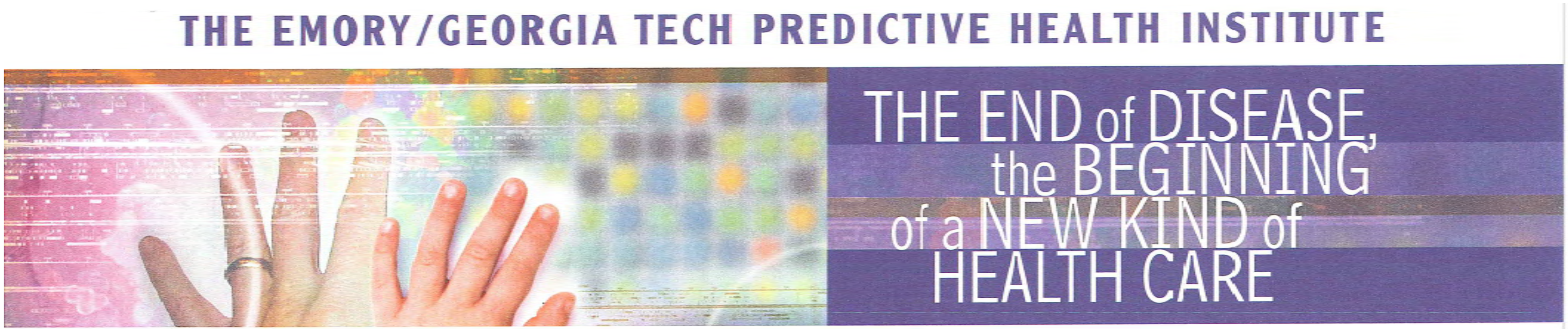

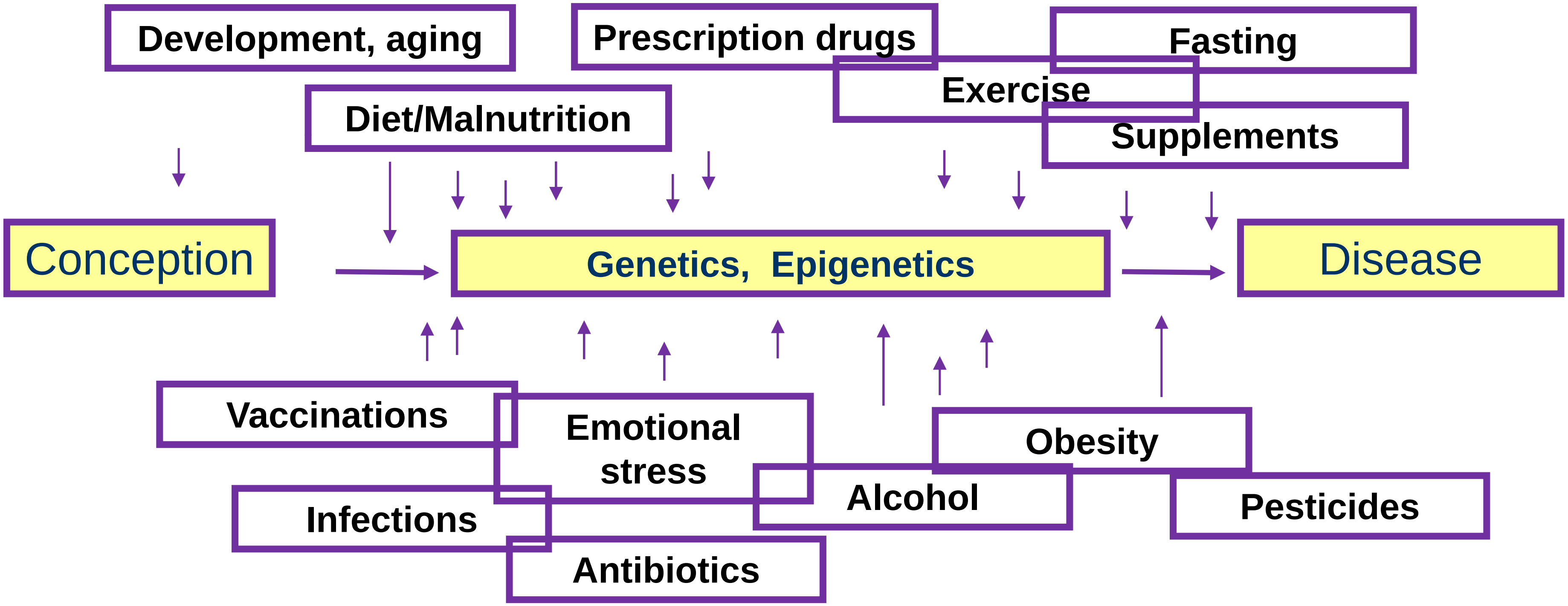

Development, aging

Prescription drugs

Fasting

Exercise

Supplements

Diet/Malnutrition

Conception

Genetics, Epigenetics

Disease

Vaccinations

Emotional stress

Obesity

Alcohol

Pesticides

Infections

Antibiotics

Exposome: the cumulative exposures of ones life

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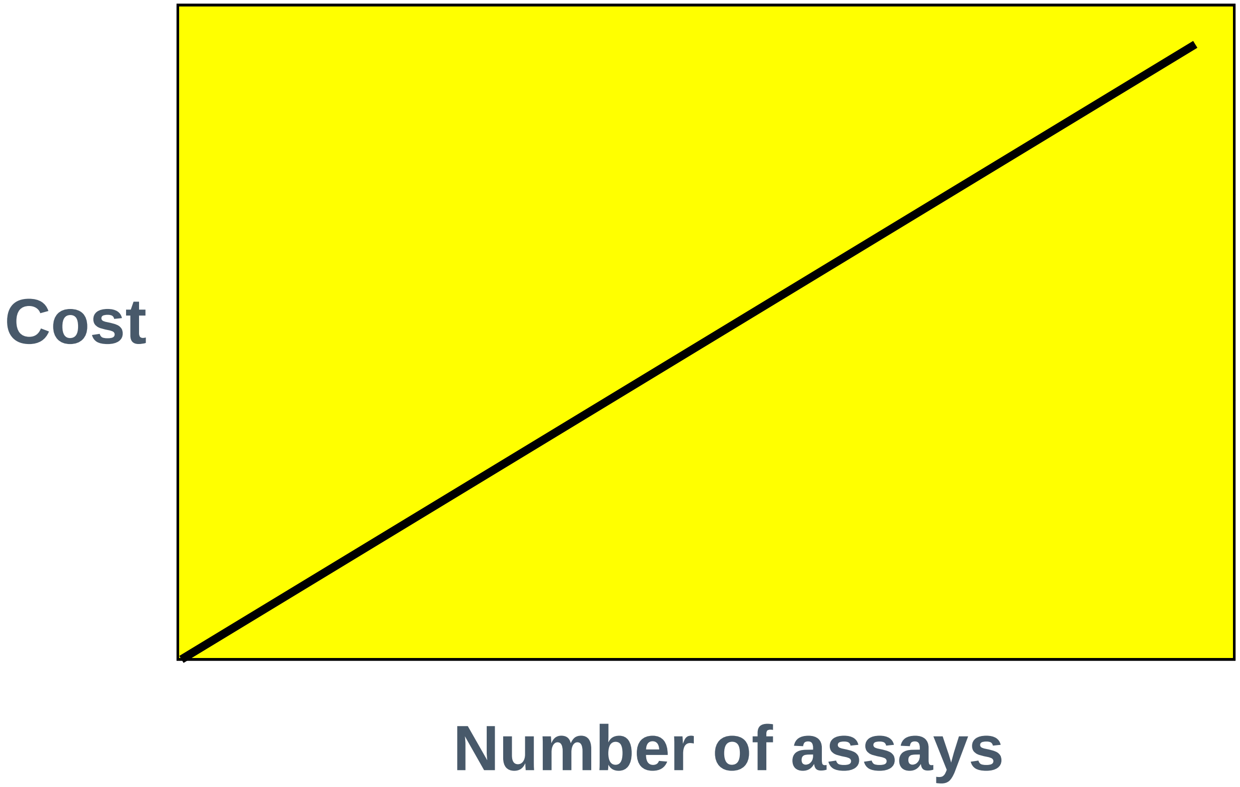
Personalized medicine will require thousands of measurements

Cost for metabolic profiling increases with number of measurements using traditional approaches, i.e., one at a time



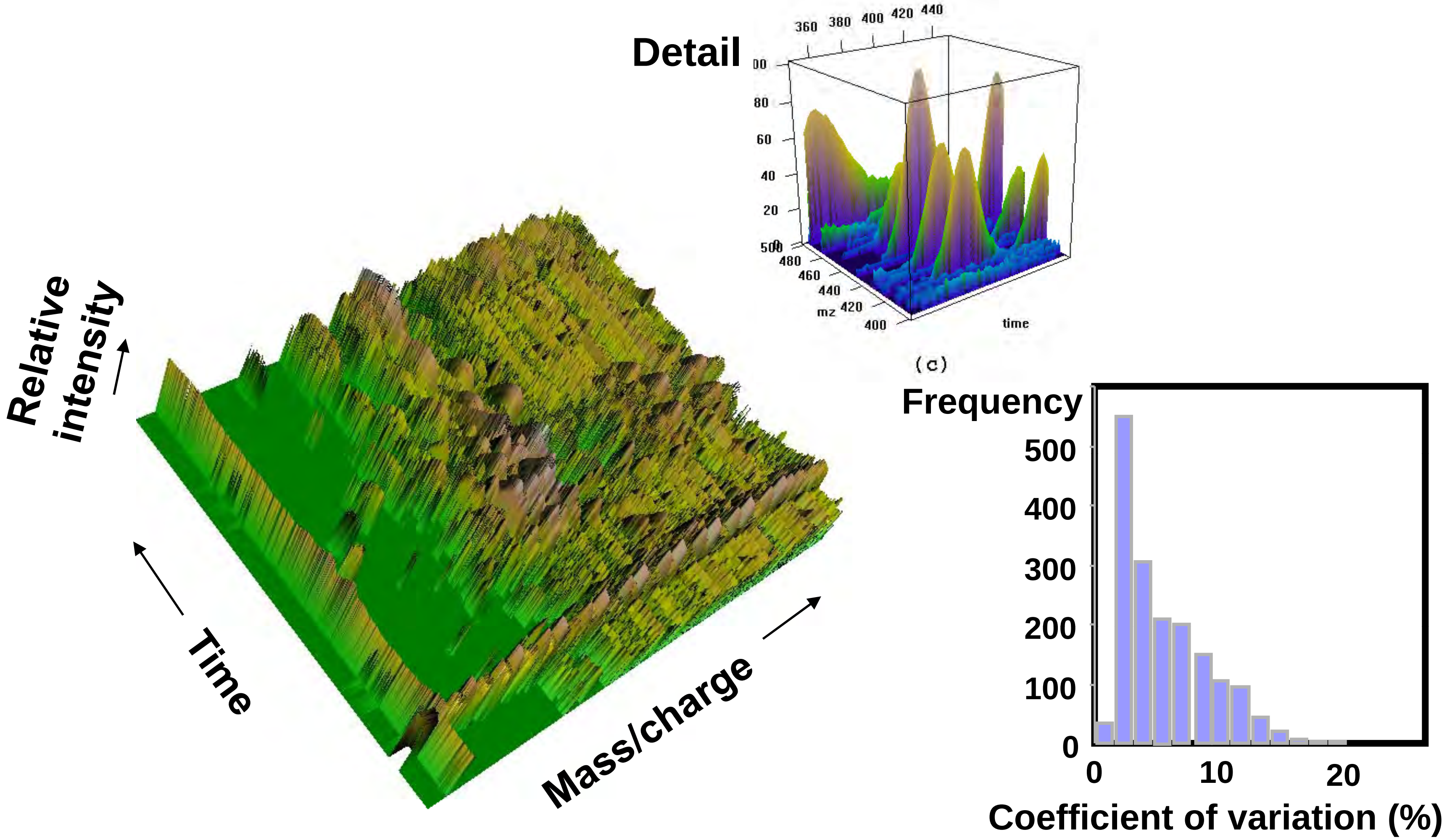
1000 @ $1 = $1,000

1000 @ $10 = $10,000

1000 @ $100 = $100,000

Multiplexed assays are the only viable approach

DP Jones, unpublished



Metabolic profiling with high-resolution mass spectrometry can measure up to 10,000 chemicals in 20 min analysis of a drop of plasma

JM Johnson, Analyst 2010; Q Soltow, Metabolomics 2011

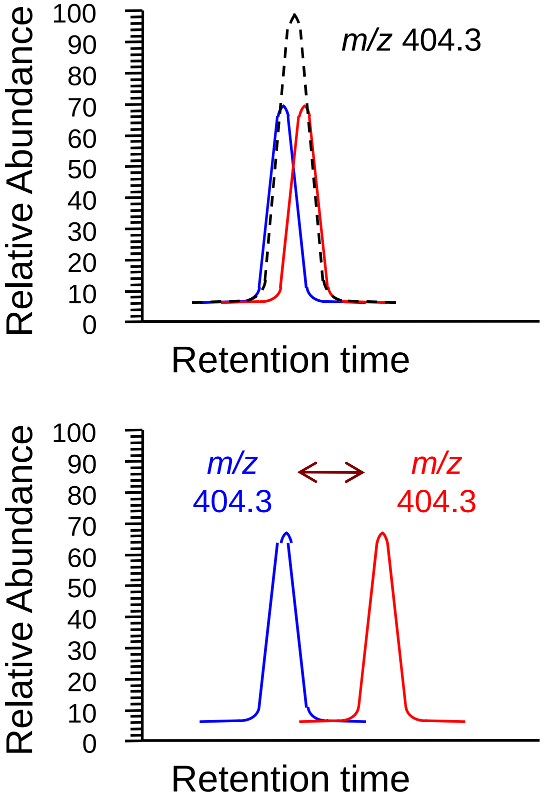
Comparison of MS profiling:

$C_{24}H_{36}O_5$
(m/z 404.256)

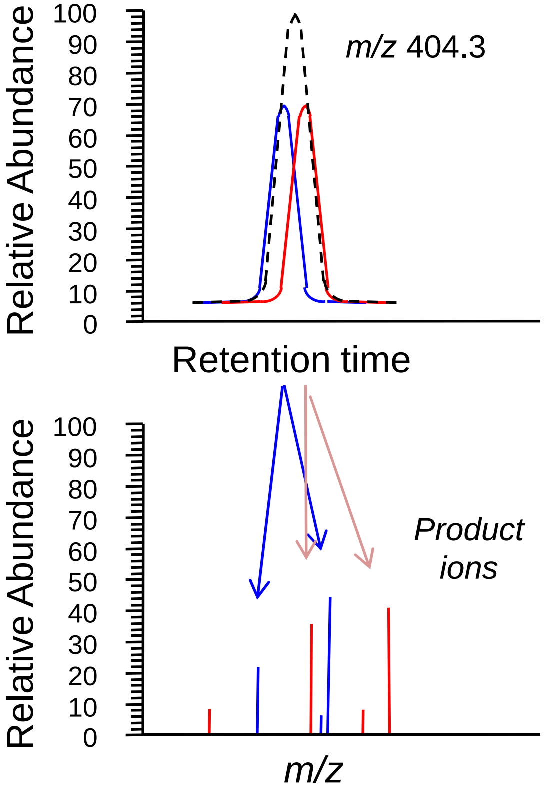
+

$C_{25}H_{40}O_4$
(m/z 404.293)

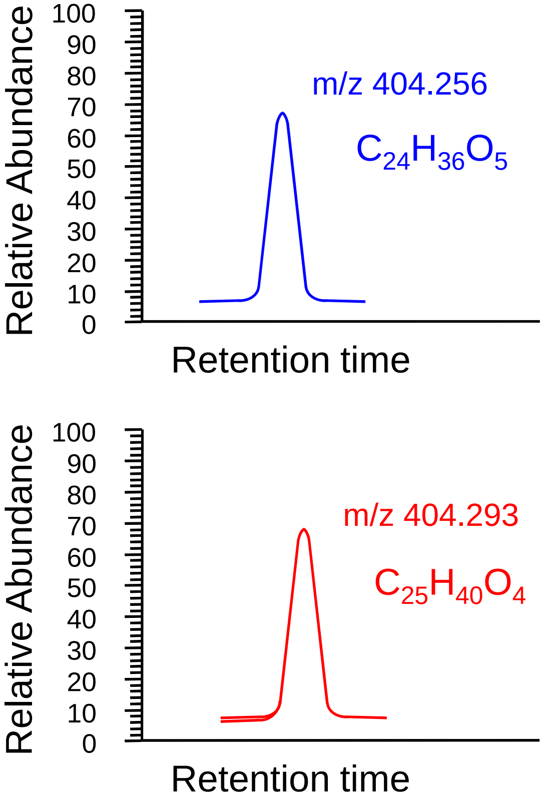
LC-MS or GC-MS
requires separation of same nominal mass prior to MS:



LC-MS/MS
measures based upon fragmentation pattern; less separation requirement



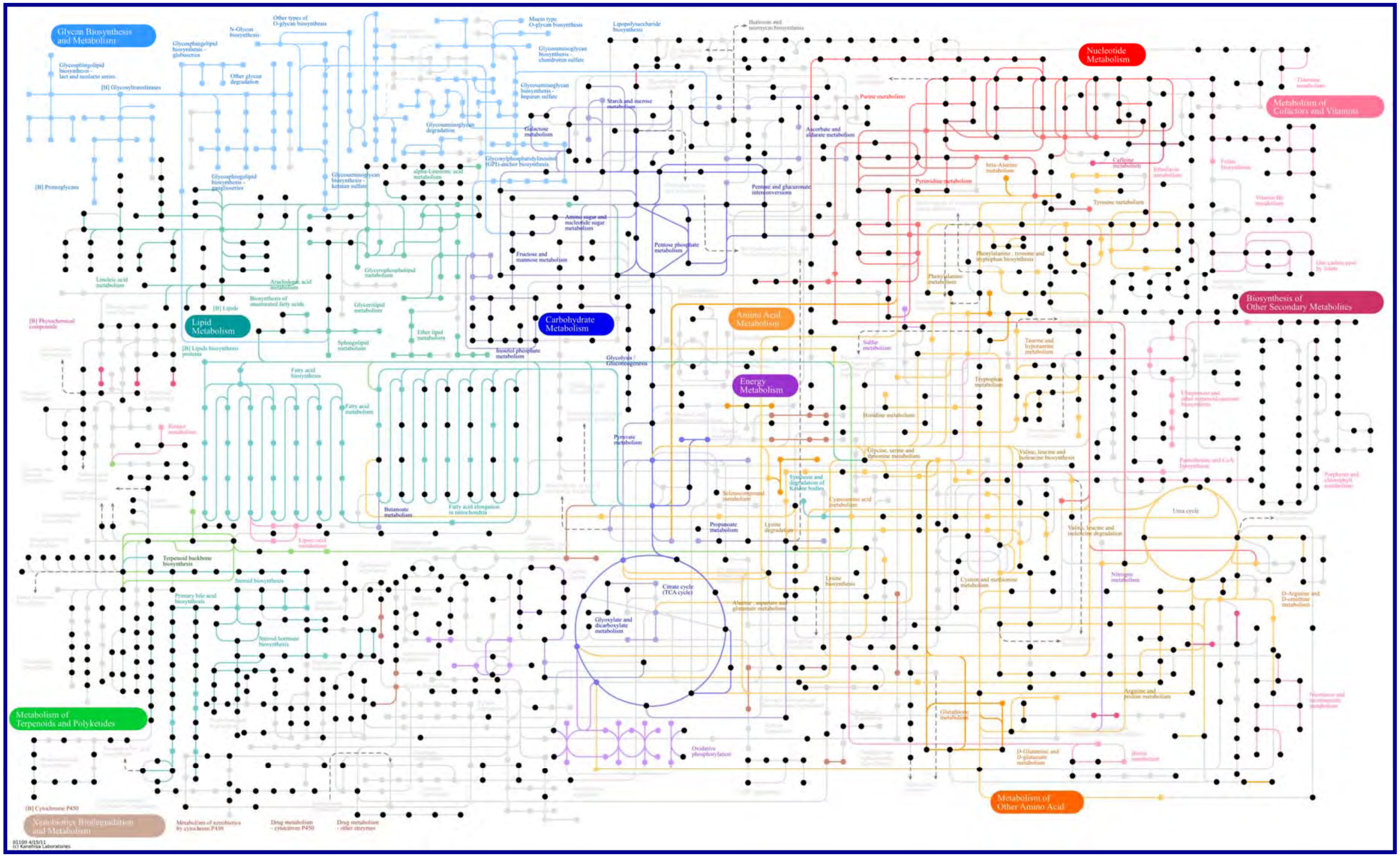
High-resolution MS
minimizes separation or fragmentation needs; often can predict elemental composition



DP Jones, unpublished

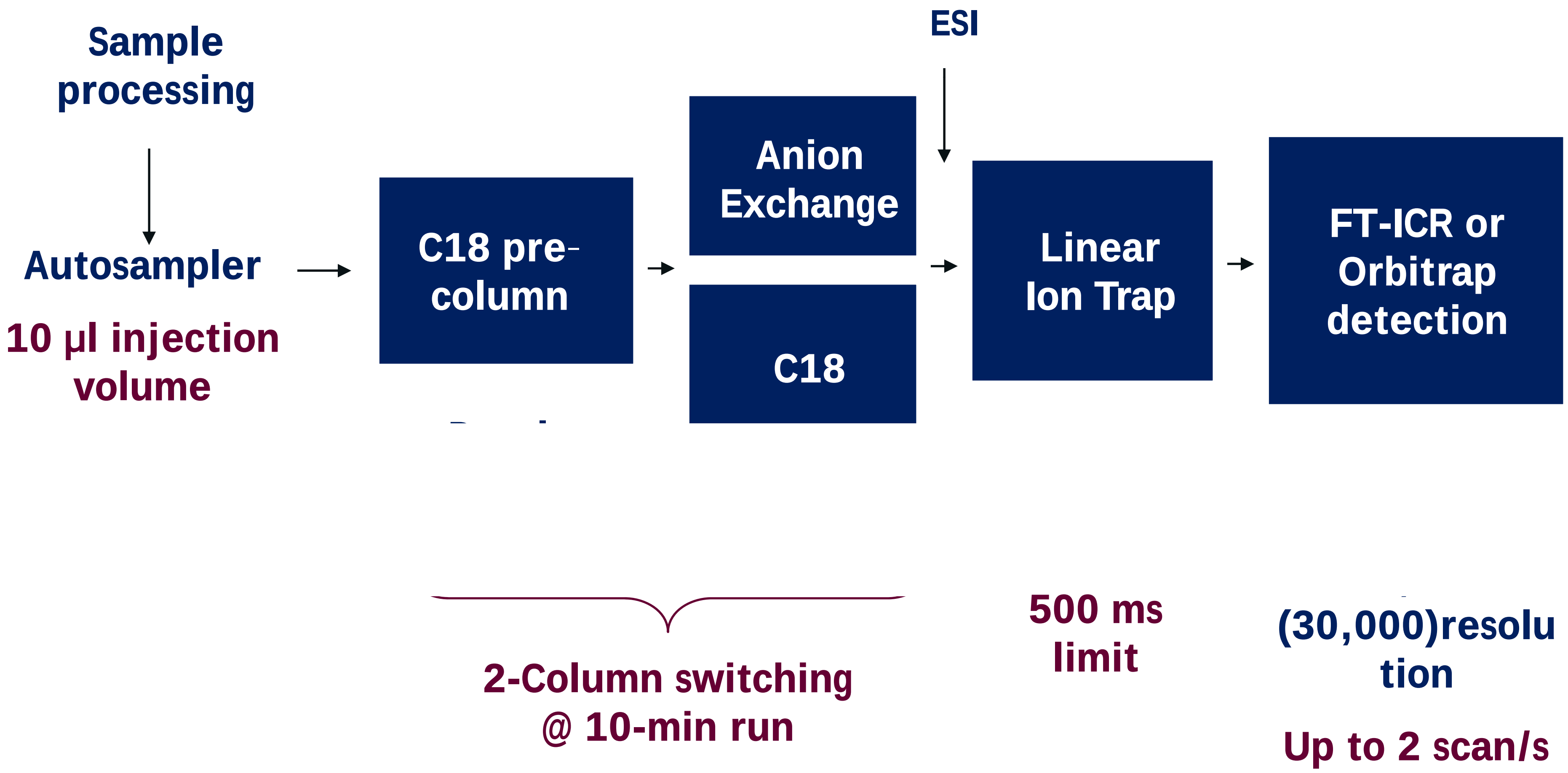
Metabolic profiling of human plasma: 20 min analysis
>90% of known human metabolites have unique elemental compositions

Black dots represent *m/z* of human plasma matched in KEGG pathways



Y Park, K Lee et al, unpublished

Metabolic profiling using DC-FTMS
(Dual chromatography-Fourier-transform mass spectrometry)



Sample processing

Autosampler

10 µl injection volume

C18 pre-column

Anion Exchange

C18

ESI

Linear Ion Trap

FT-ICR or Orbitrap detection

2-Column switching @ 10-min run

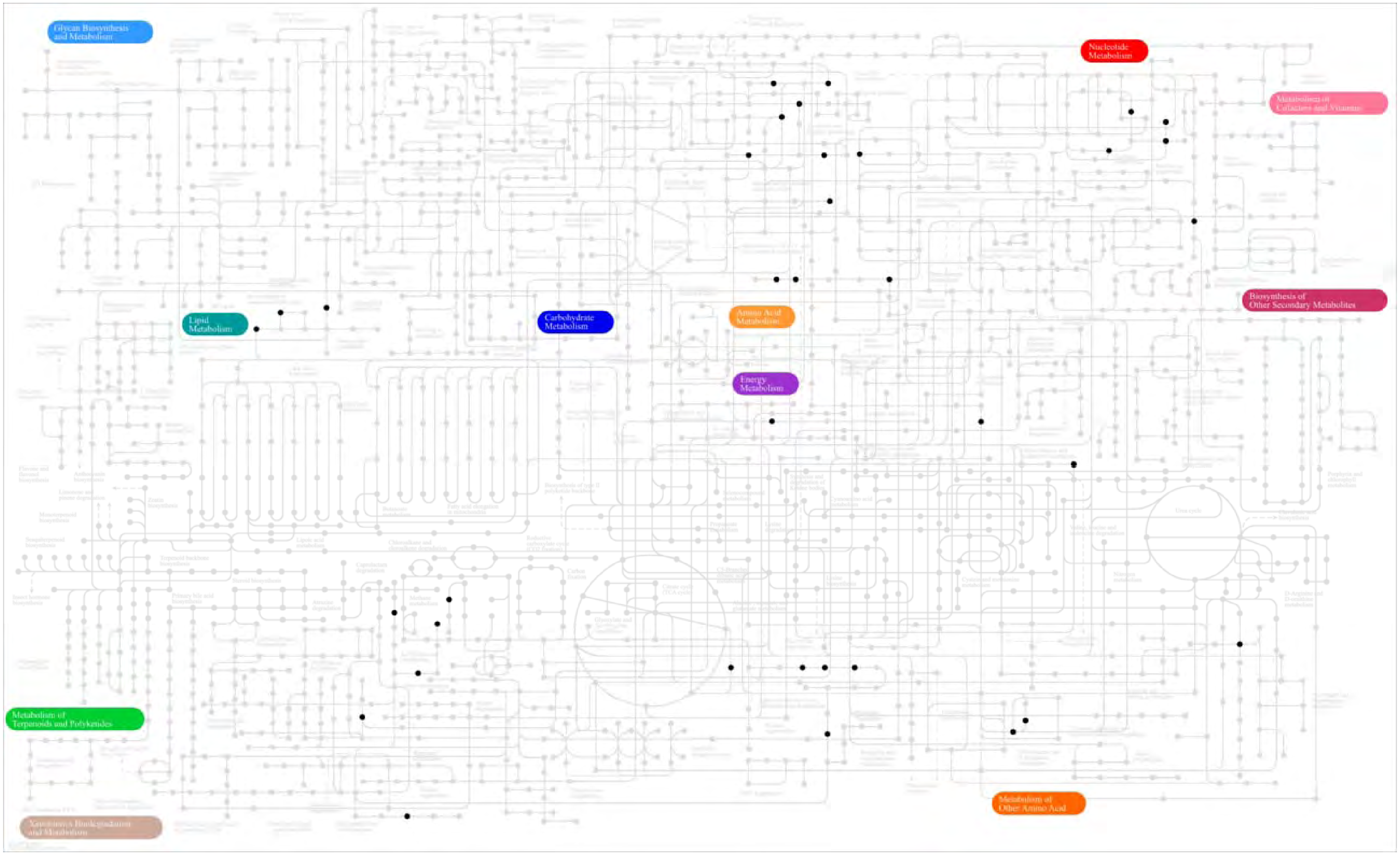
500 ms limit

(30,000)resolution

Up to 2 scan/s

Q Soltow et al, Metabolomics 2011

Personalized Medicine: Pathway Association with Disease Score



Q Soltow, N Kutner, A Quyyumi et al, unpublished

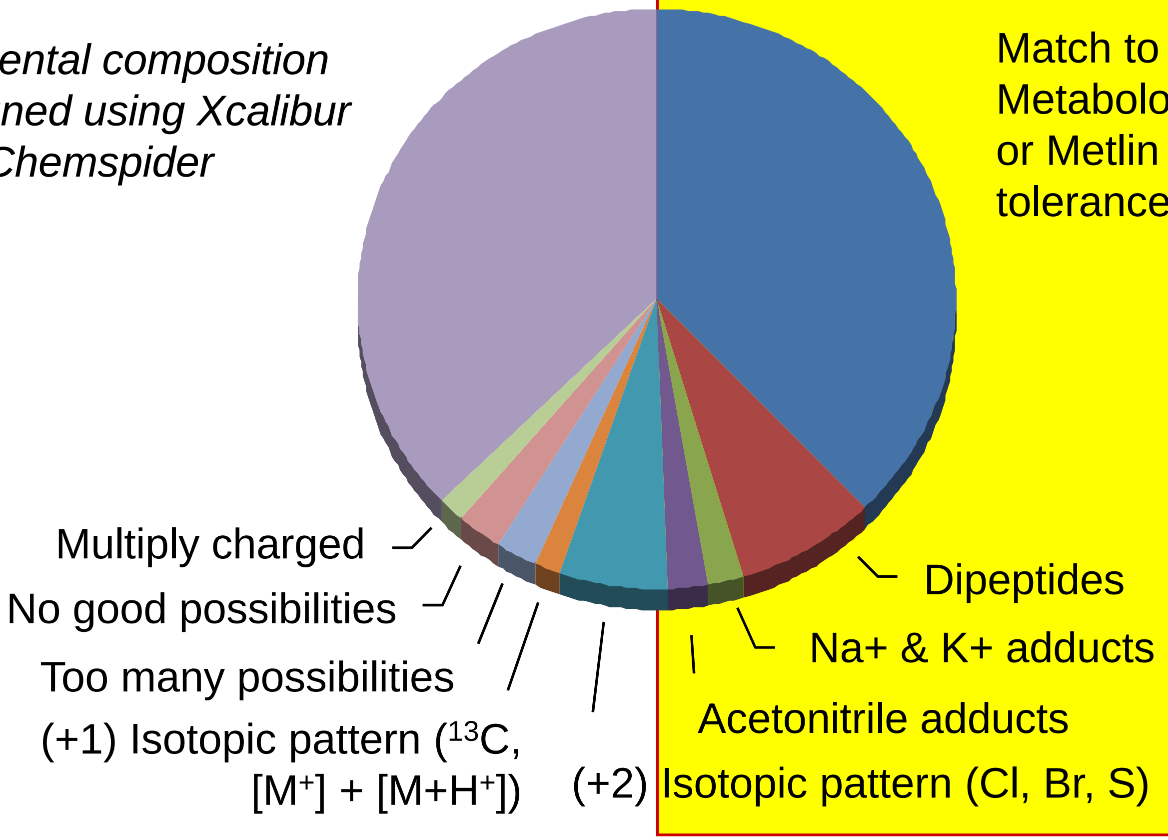
Only about half of the chemicals detected in plasma match chemicals in metabolomics databases

__Human plasma metabolites:__

Elemental composition assigned using Xcalibur and Chemspider

"KNOWN"

Match to Madison Metabolomics Consortium or Metlin Databases with tolerance of 5 or 10 ppm

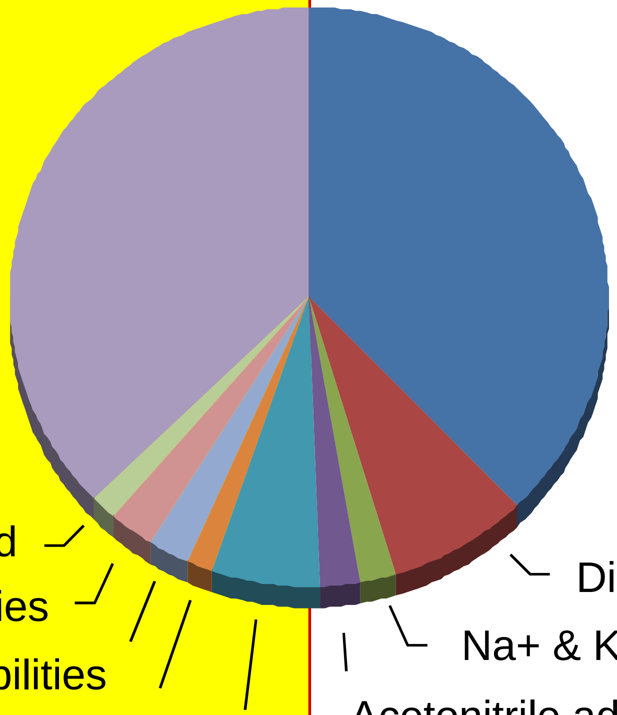


JM Johnson, DP Jones, unpublished

The other half of the chemicals detected in plasma are not present in human metabolite databases; these are an unidentified “exposome”

“UNKNOWN”

Elemental composition assigned using Xcalibur and Chemspider



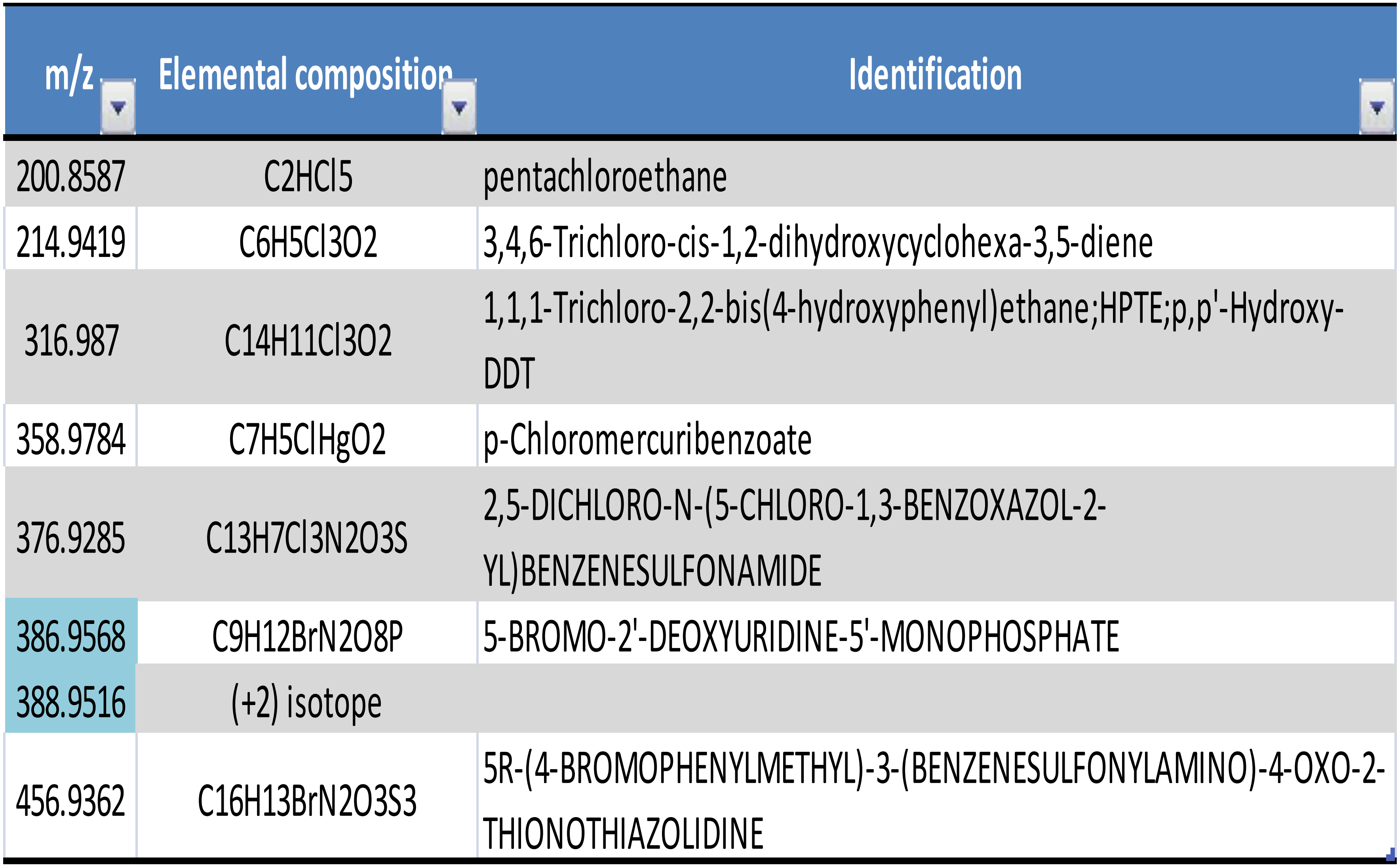
JM Johnson, DP Jones, unpublished

Do the “unknown” chemicals in human plasma provide information relevant to environmental exposures?

Data from 1960/70’s: DDT metabolites accumulate in depot fat and are released upon weight loss.

Is there evidence for environmental chemicals in LC-FTMS of human plasma under catabolic conditions?

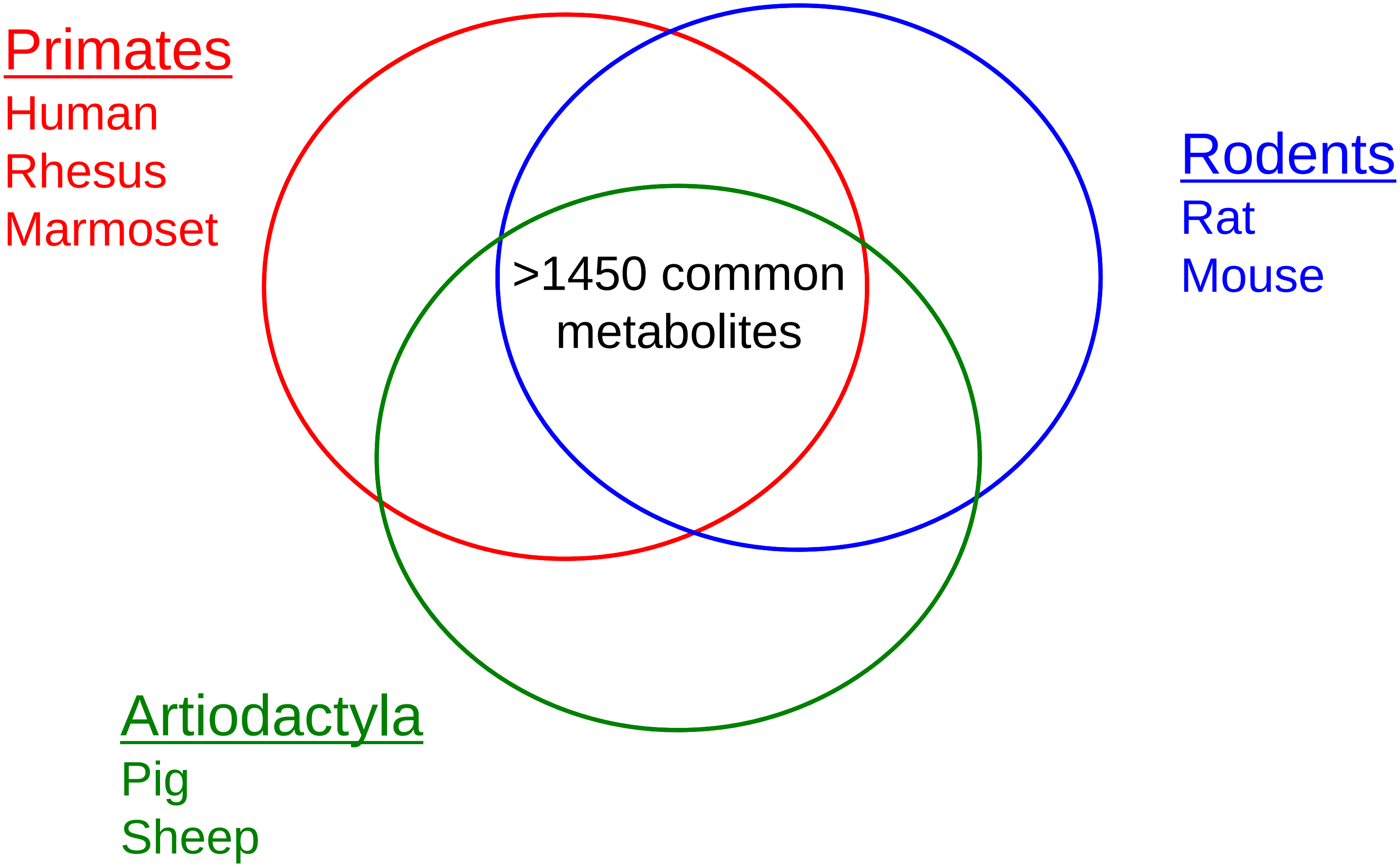
Catabolic ICU patients have increased halogenated hydrocarbons in plasma based upon high-resolution m/z match to databases



m/z	Elemental composition	Identification
200.8587	C2HCl5	pentachloroethane
214.9419	C6H5Cl3O2	3,4,6-Trichloro-cis-1,2-dihydroxycyclohexa-3,5-diene
316.987	C14H11Cl3O2	1,1,1-Trichloro-2,2-bis(4-hydroxyphenyl)ethane;HPTE;p,p'-Hydroxy-DDT
358.9784	C7H5ClHgO2	p-Chloromercuribenzoate
376.9285	C13H7Cl3N2O3S	2,5-DICHLORO-N-(5-CHLORO-1,3-BENZOXAZOL-2-YL)BENZENESULFONAMIDE
386.9568	C9H12BrN2O8P	5-BROMO-2'-DEOXYURIDINE-5'-MONOPHOSPHATE
388.9516	(+2) isotope	
456.9362	C16H13BrN2O3S3	5R-(4-BROMOPHENYLMETHYL)-3-(BENZENESULFONYLAMINO)-4-OXO-2-THIONOTHIAZOLIDINE

JM Johnson, DP Jones, unpublished

LC-FTMS analysis of plasma from 7 species of mammals: a subset of chemicals is common among species



Primates
Human
Rhesus
Marmoset

Rodents
Rat
Mouse

Artiodactyla
Pig
Sheep

>1450 common metabolites

Y Park, et al manuscript submitted

High-performance metabolic profiling matches to **environmental agents**

Pirimicarb	Insecticide
Rotenone	Insecticide
Dioctyl adipate	Plasticizer
Tris(butoxyethyl)phosphate	Plasticizer
Chlorsulfuron	Herbicide
Endosulfan	Insecticide
Di-n-heptyl phthalate	Plastics
p-Methylaminophenol sulfate	Photography developing agent
1,6-Dimethoxypyrene	Environmental metabolite of PAH
3-Hydroxycotinine glucuronide	Cigarette smoke
Benzyl sulfoxide	Metabolite of benzyl sulfide

Y Park, et al manuscript submitted

Co-elution and MS/MS studies verify identities of environmental chemicals in LC-FTMS analysis

Y Park, K Lee et al, submitted

Highlights: Universal Exposure Surveillance

PD and age-matched control samples contained *m/z* matching rotenone, a naturally occurring pesticide used in organic gardening, and related chemicals

395.1492355 m/z

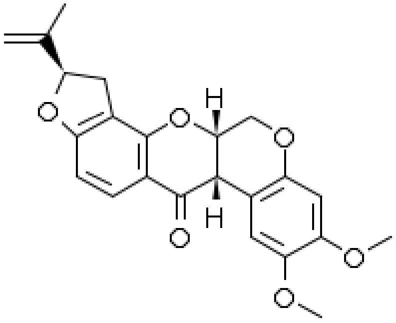
(394.138 - 394.1459 daltons): 14 Metabolites [M+H]$^+$

MetlinID	Mass	Δppm	Name	Formula	CAS	MS/MS
43615	394.1416	0	Dehydrodihydrorotenone	$C_{23}H_{22}O_{6}$	6659-45-6	NO
43639	394.1416	0	ISOROTENONE	$C_{23}H_{22}O_{6}$		
43722	394.1416	0	DEGUELIN(-)	$C_{23}H_{22}O_{6}$	522-17-8	
43834	394.1416	0	MACLUROXANTHONE	$C_{23}H_{22}O_{6}$		
43852	394.1416	0	Rotenone	$C_{23}H_{22}O_{6}$	83-79-4	
44511	394.1416	0	Robustic acid methyl ether	$C_{23}H_{22}O_{6}$		
47596	394.1416	0	Barbigerone	$C_{23}H_{22}O_{6}$		NO
48020	394.1416	0	Rotenone	$C_{23}H_{22}O_{6}$		NO
48030	394.1416	0	Myriconol	$C_{23}H_{22}O_{6}$		NO
48031	394.1416	0	(-)-cis-Deguelin	$C_{23}H_{22}O_{6}$		NO
49471	394.1416	0	Muxiangrin I	$C_{23}H_{22}O_{6}$		NO
51093	394.1416	0	8-C-Methylvellokaempferol 3,5-dimethyl ether	$C_{23}H_{22}O_{6}$		NO
52497	394.1416	0	Purpurin	$C_{23}H_{22}O_{6}$		NO
53054	394.1416	0	3',4'-Dihydroxy-7-methoxy-8-prenyl-5''-(2-hydroxyisopropyl)-[2'',3'':5,6]furanoflavanone	$C_{23}H_{22}O_{6}$		NO

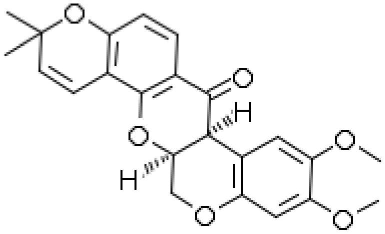
Y Park et al, unpublished

Highlights: Universal Exposure Surveillance

PD and age-matched control samples contain *m/z* matching rotenone, a naturally occurring pesticide used in organic gardening, and related chemicals



Rotenone



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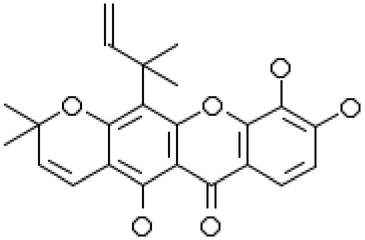


Macluraxanthone

Y Park et al, unpublished

Preliminary data suggest that >100 environmental chemicals are detected by LC-FTMS

Flame Retardants

Triphenyl phosphate	326.071
Dibromobisphenol A	

Plasticizers

Tetraethylene glycol	194.115
N-Butyl-benzenesulfonamide	213.082
Diethyl phthalate	222.089
Di-n-propylphthalate	250.121
Di-n-heptyl phthalate	362.246
Diethylhexylphthalate	391.288
Di(2-ethylhexyl) adipate	370.308
Diisononyl phthalate	418.308
Diisodecyl phthalate	446.340

Insecticides

Pirimicarb	238.143
Metofluthrin	360.135
Phosalone	366.987
Endosulfan	403.817
Benfuracarb	410.188
Rotenone	394.142

Herbicides

Desethylatrazine	187.630
Diaminochlorotriazine (DACT)	
Mefenacet	298.078
Chlorsulfuron	357.030
Sulfentrazone	385.982

Fungicides

Carbendazim	191.069
Benomyl	290.138
Tridemorph	297.303
Pencycuron	328.134
Famoxadone	374.127

Other

2,3-Benzofluorene	217.103

Q Soltow et al Metabolomics 2011; Park et al submitted

Universal Exposure Surveillance:
Opportunities and Challenges

100,000 agents are registered with EPA; recognized hazards/risks are monitored by targeted analysis

Millions of tons of chemicals are used in commerce; little ability to track the ultimate fates of many

Targeted analysis of everything is impractical, unaffordable and unwarranted

Universal Exposure Surveillance:
Opportunities and Challenges

High-resolution mass spectrometry is likely to be incorporated into personalized medicine

Opportunity for multiplexed biomonitoring of pesticides; more expanded coverage of environmental chemical space

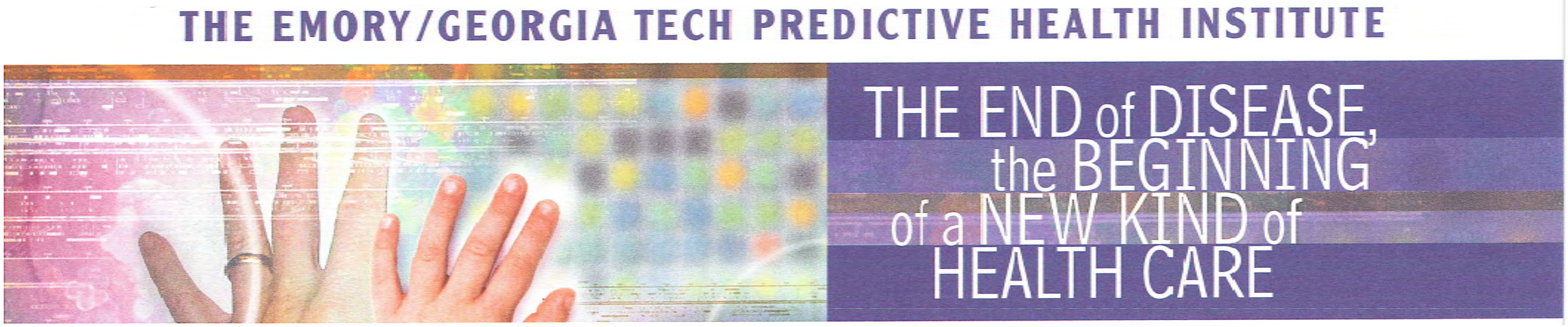
With appropriate development, common environmental agents could be measured as components of the metabolic phenotype

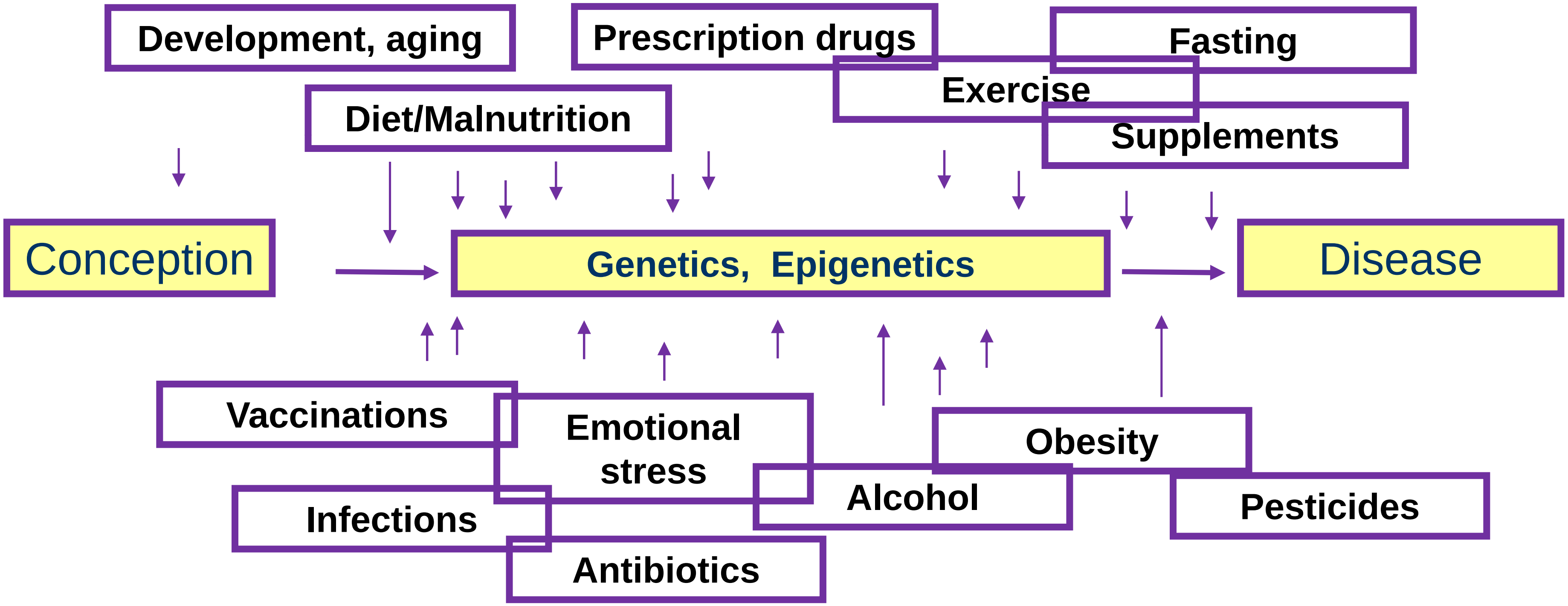
Universal Exposure Surveillance:
Opportunities and Challenges

De-identified sampling:

- Military recruits
- Emergency room patients
- Newborn screening (bloodspots)
- ICU patients (catabolic state)

Component of annual physical examinations





Development, aging

Prescription drugs

Fasting

Exercise

Diet/Malnutrition

Supplements

Conception → Genetics, Epigenetics → Disease

Vaccinations

Emotional stress

Obesity

Alcohol

Infections

Pesticides

Antibiotics

Universal surveillance strategy could support improved G x E research

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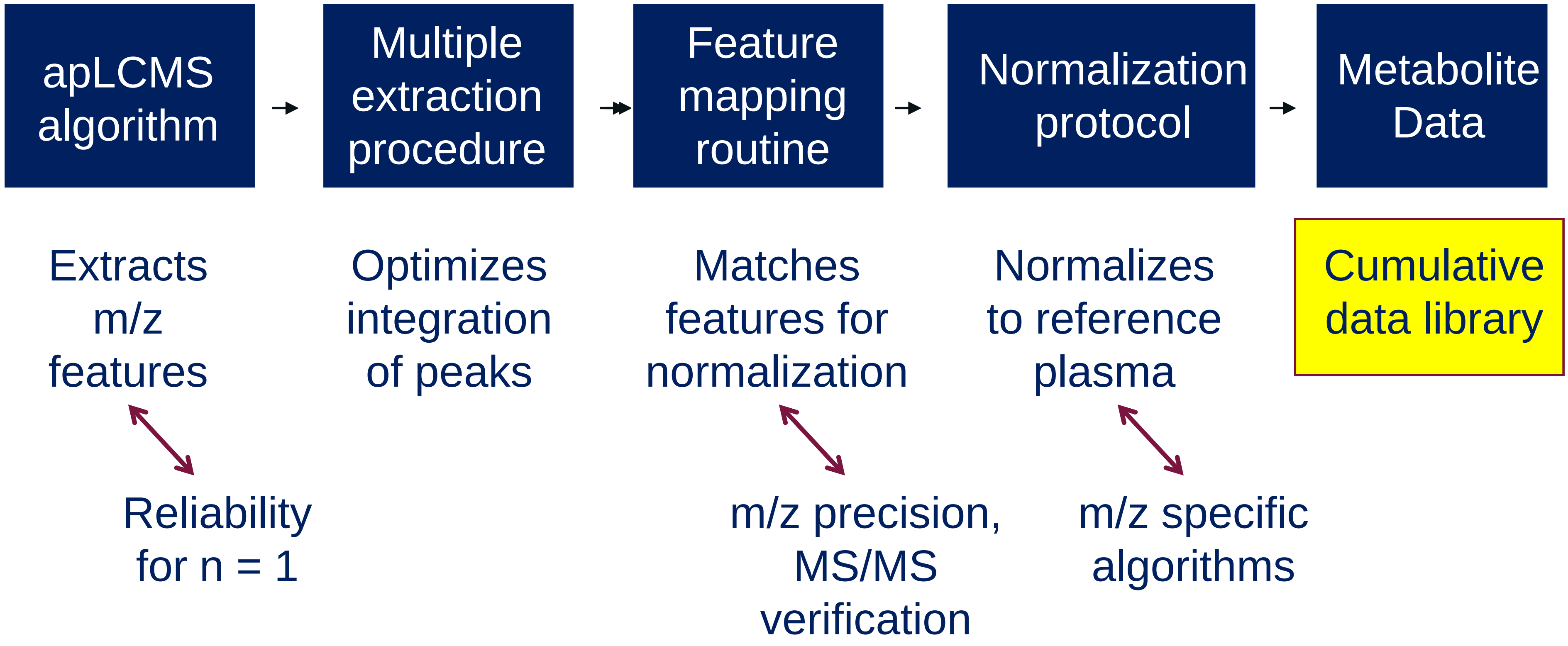
Clinical Biomarkers Laboratory Metabolic Profiling

MS data processing to cumulative data library

apLCMS algorithm →	Multiple extraction procedure →	Feature mapping routine →	Normalization protocol →	Metabolite Data
Extracts m/z features	Optimizes integration of peaks	Matches features for normalization	Normalizes to reference plasma	Prototype cumulative data library
T Yu, 2009	K Uppal, submitted	S Le, unpublished	Q Soltow, unpublished	

High-performance Metabolic Profiling (LC-FTMS)

Needs: MS data processing to cumulative data library



apLCMS algorithm

Extracts m/z features

Reliability for n = 1

Multiple extraction procedure

Optimizes integration of peaks

Feature mapping routine

Matches features for normalization

m/z precision, MS/MS verification

Normalization protocol

Normalizes to reference plasma

m/z specific algorithms

Metabolite Data

Cumulative data library

Summary: Universal Exposure Surveillance and Personalized Medicine

1. High-performance metabolic profiling can be cost-effective for personalized medicine

2. “Routine” human metabolic profiles include pesticides

3. High-performance metabolic profiling provides opportunity to multiplex pesticide surveillance

4. With appropriate development, biomonitoring could become a component of personalized medicine

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