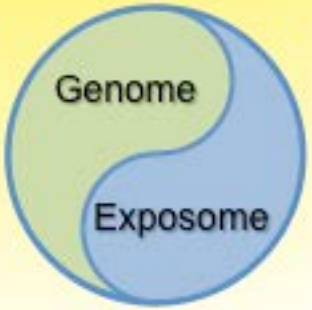

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EPA's CompTox Communities of Practice

February 28, 2013 Webinar

High-Resolution Metabolomics for Environmental Chemical Surveillance and Bioeffect Monitoring

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Department of Medicine/Division of Pulmonary, Allergy and Critical Care Medicine
Emory University, Atlanta

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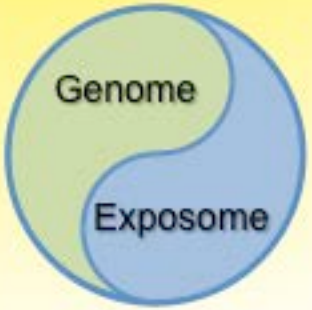
No financial COI to disclose

Funding acknowledgements for system development: **NIEHS**, NIA, NCI, NHLBI, NIDDK, NIAAA, NIAID, Woodruff Foundation, Emory-Georgia Tech Predictive Health Initiative; Georgia Research Alliance, Emory Department of Medicine

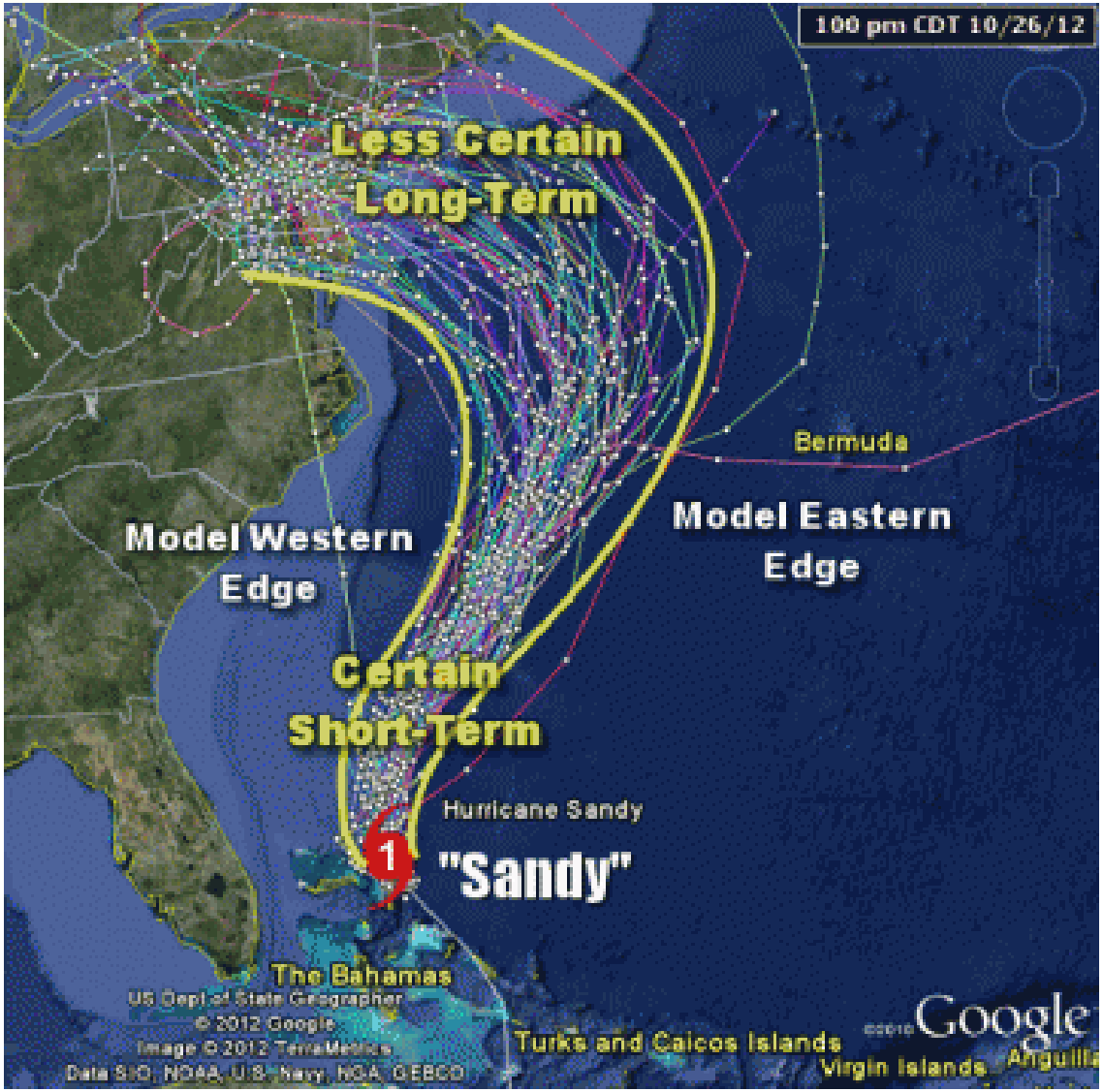


Toward a National Health Surveillance & Forecasting System

A central concept of personalized medicine and predictive health is that the ability to predict

risk of disease,
timing of disease onset and
intensity of impact

would make the healthcare system more efficient and empower individuals in health management



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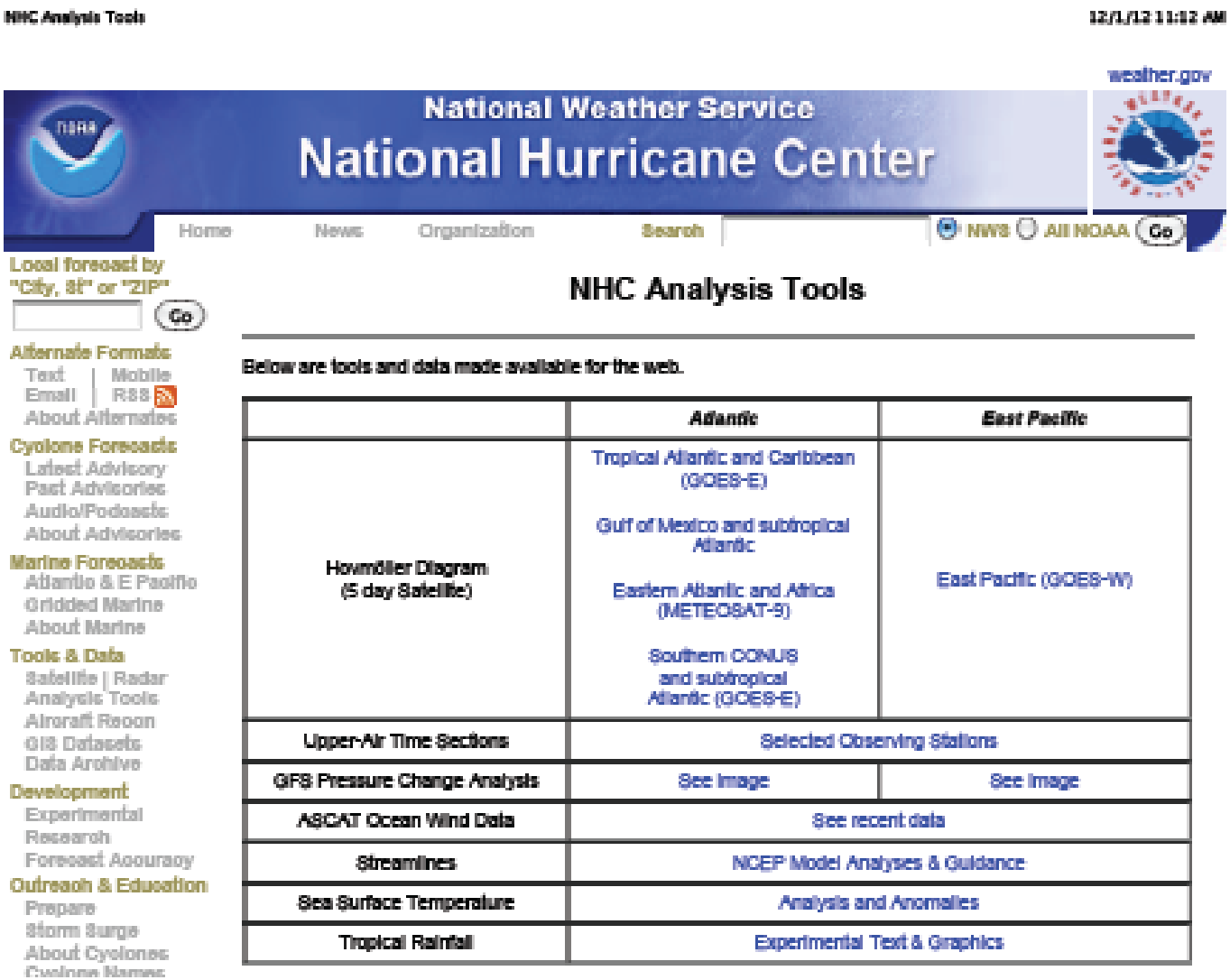
We can learn from The National Weather Service:

National Hurricane Center uses data input from multiple sources and multiple analysis tools



NHC Analysis Tools 12/1/12 11:12 AM

weather.gov

National Weather Service
National Hurricane Center

Home | News | Organization | Search | NWS | All NOAA | Go

Local forecast by "City, St" or "ZIP" Go

Alternate Formats
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Cyclone Forecasts
Latest Advisory
Past Advisories
Audio/Podcasts
About Advisories

Marine Forecasts
Atlantic & E Pacific
Gridded Marine
About Marine

Tools & Data
Satellite | Radar
Analysis Tools
Aircraft Recon
GIS Datasets
Data Archive

Development
Experimental
Research
Forecast Accuracy

Outreach & Education
Prepare
Storm Surge
About Cyclones
Cyclone Names

NHC Analysis Tools

Below are tools and data made available for the web.

	Atlantic	*East Pacific*
Hovmöller Diagram (5 day Satellite)	Tropical Atlantic and Caribbean (GOES-E) Gulf of Mexico and subtropical Atlantic Eastern Atlantic and Africa (METEOSAT-9) Southern CONUS and subtropical Atlantic (GOES-E)	East Pacific (GOES-W)
Upper-Air Time Sections	Selected Observing Stations	
GFS Pressure Change Analysis	See Image	See Image
ASCAT Ocean Wind Data	See recent data	
Streamlines	NCEP Model Analyses & Guidance	
Sea Surface Temperature	Analysis and Anomalies	
Tropical Rainfall	Experimental Text & Graphics	

Lessons from NOAA

Don’t rely upon a small number of isolated measurements; i.e., “biomarkers”

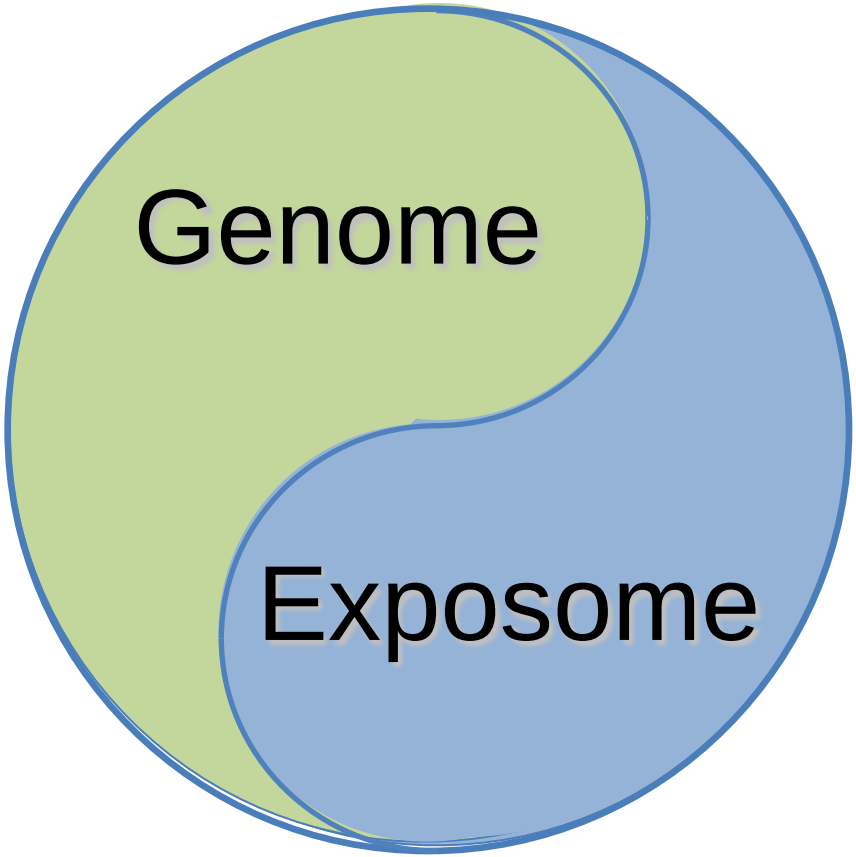
Rely upon cumulative data and multiple models; no model is infallible

Expect reliability to be gained with

1) time,
2) understanding of failures and
3) introduction of improved models

Scientific underpinning for National Health Surveillance & Forecasting System: Disease risk is determined by genetics in combination with lifelong exposures, i.e., the exposome

10-20% of disease risk

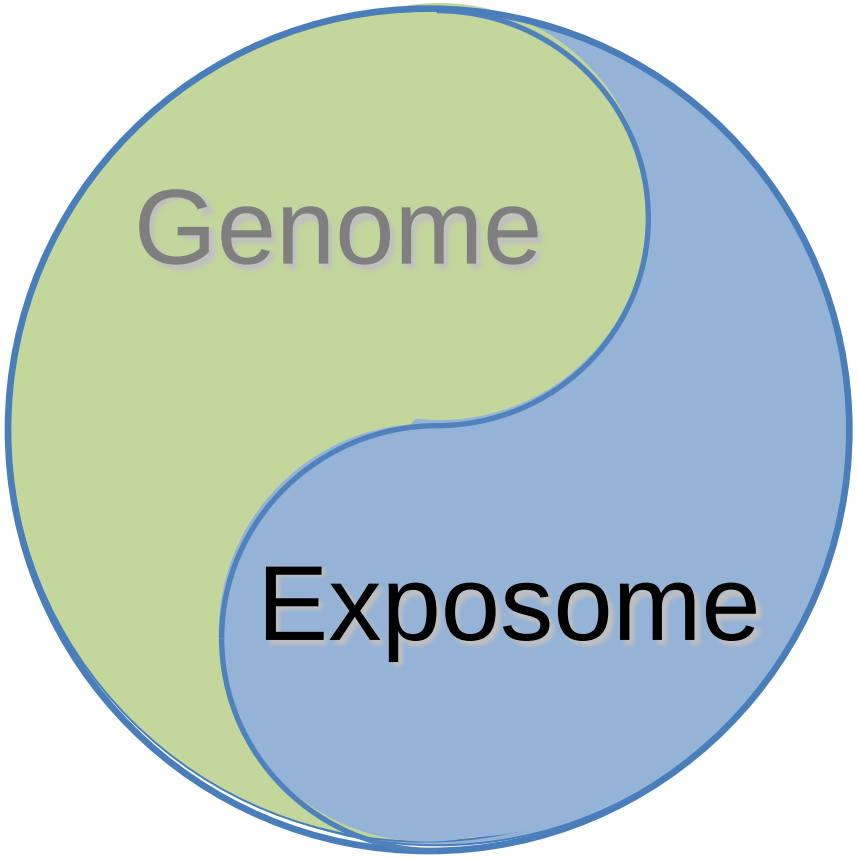


80-90% of disease risk

Wild (2005) Cancer Epidemiol Biomark Prevent 14:1847

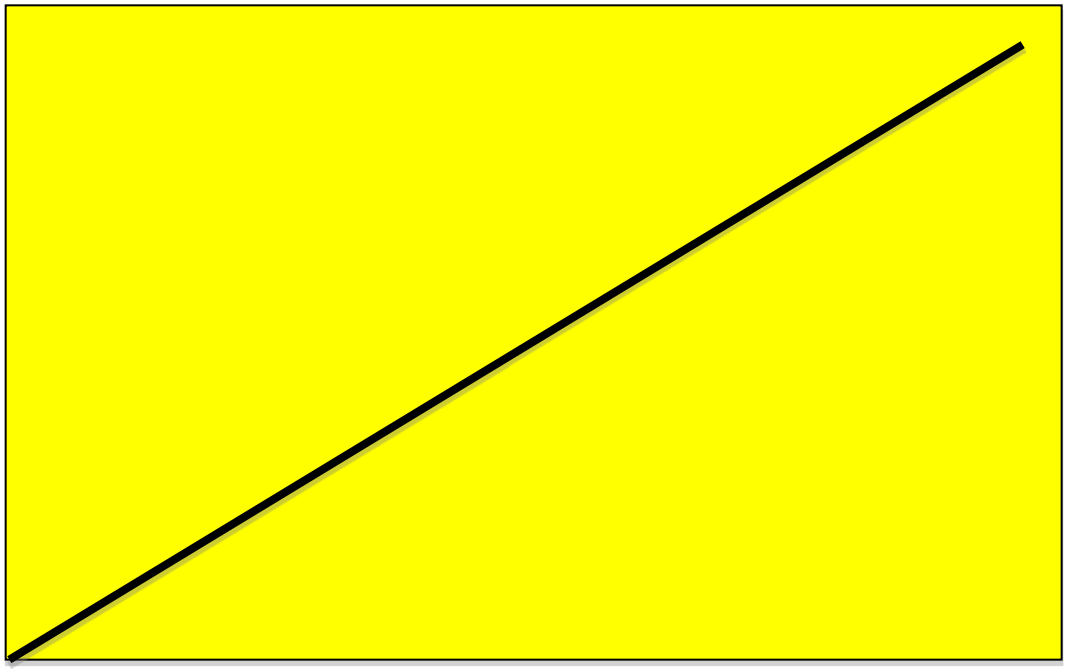
Genotyping and gene sequencing capabilities are available

How can we address the exposome?



Problem: Thousands of targeted measurements requires thousands of measurements

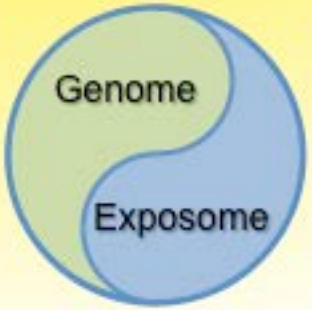
Cost for chemical profiling increases with number of measurements using traditional approaches



1000 @ $1 = $1,000

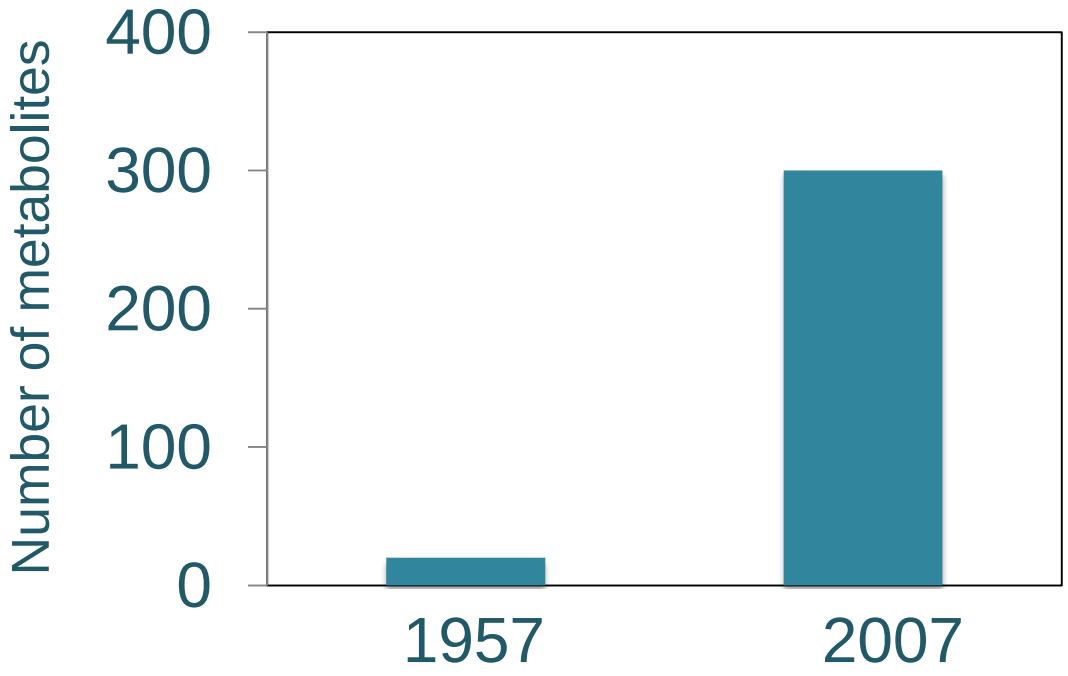
1000 @ $10 = $10,000

1000 @ $100 = $100,000



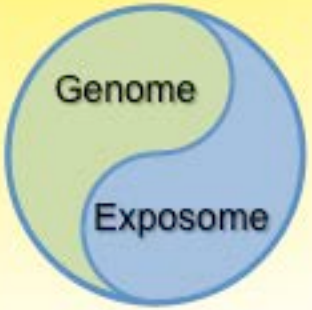
Progress in chemical profiling has been slow relative to progress in gene sequencing

Number of metabolites measurable by routine analysis



Moore et al, automated Amino acid analysis

Combined GC/MS & LC/MS



In 2003, we proposed to use high resolution mass spectrometry for metabolic profiling

NIH Peer Review: "Waste of a good instrument"

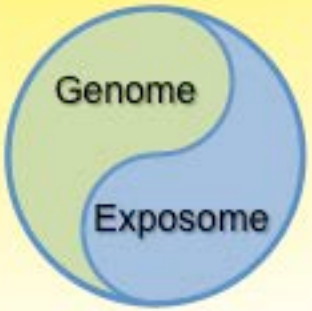
High-resolution Ion Cyclotron Resonance mass spectrometers:

Orbit ions in electromagnetic field: allows detection with improved mass resolution and mass accuracy

Fourier-transform mass spectrometry (FTMS): Can obtain accuracy sufficient to predict elemental composition for small molecules

Ion detection as perturbation of magnetic field allows more sensitive detection than ion counting

Marshall AG, Hendrickson CL. 2008. High-resolution mass spectrometers. *Annu. Rev. Anal. Chem.* 1:579–99



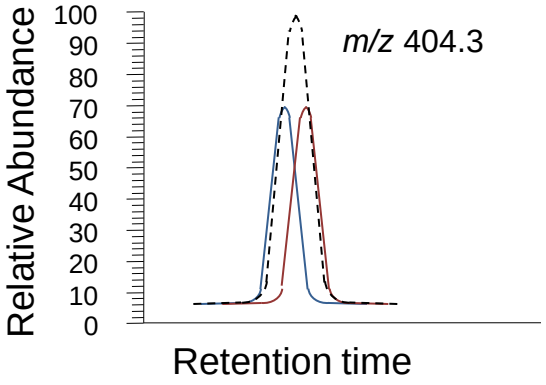
Mass spectral measurement of chemicals with same nominal mass

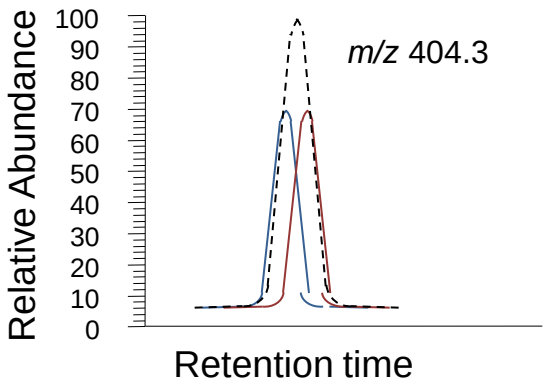
$C_{24}H_{36}O_5$ (m/z 404.256) + $C_{25}H_{40}O_4$ (m/z 404.293)

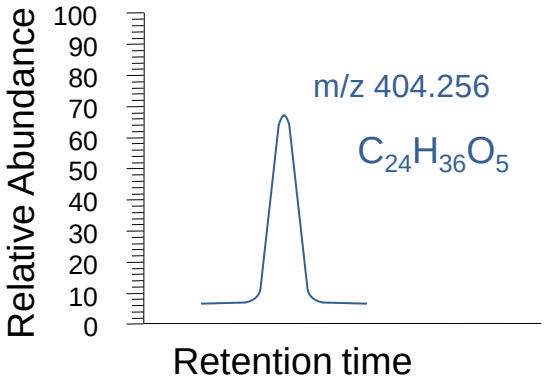
LC-MS or GC-MS
requires separation of same nominal mass prior to MS:

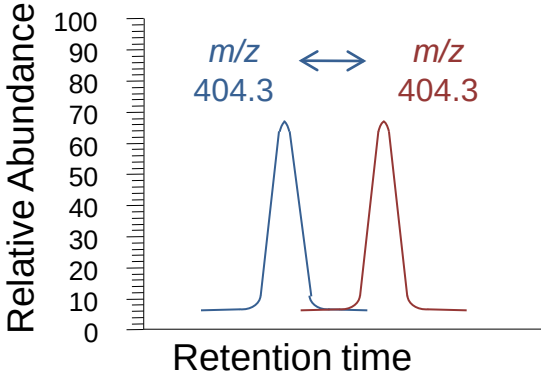
LC-MS/MS
measures based upon fragmentation pattern; less separation requirement

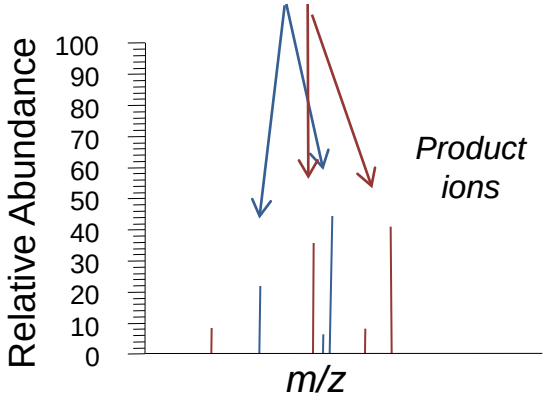
High-resolution MS
minimizes separation or fragmentation needs; often can predict elemental composition

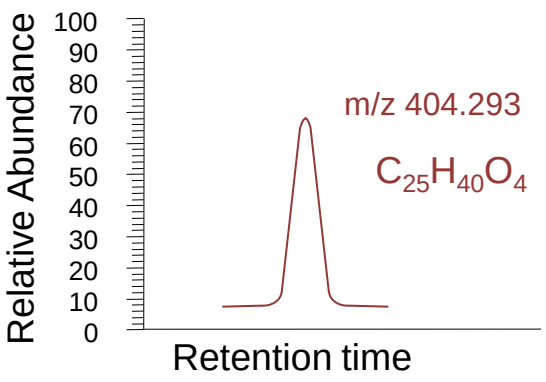




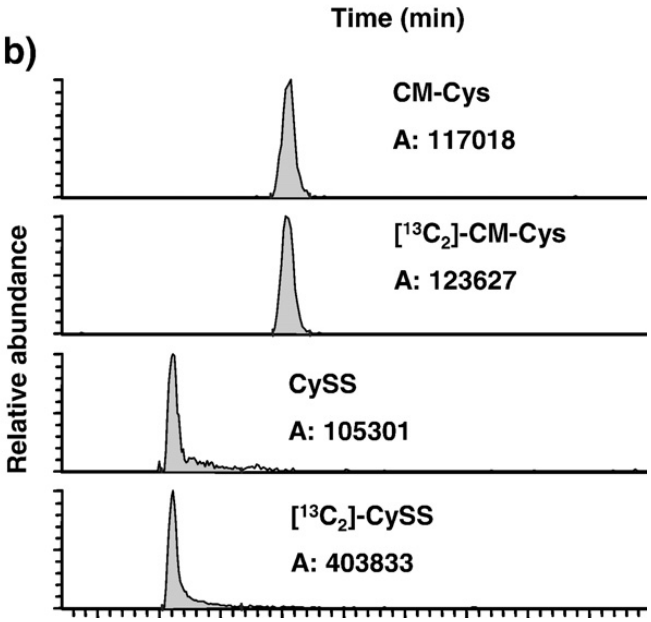








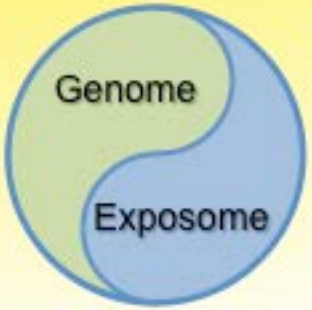
High-resolution metabolomics: Initially showed that LC-FTMS was reliable and quantitatively accurate for targeted analysis of metabolites in biological samples



Common HPLC methods used to measure and quantify Cys and CySS in human plasma

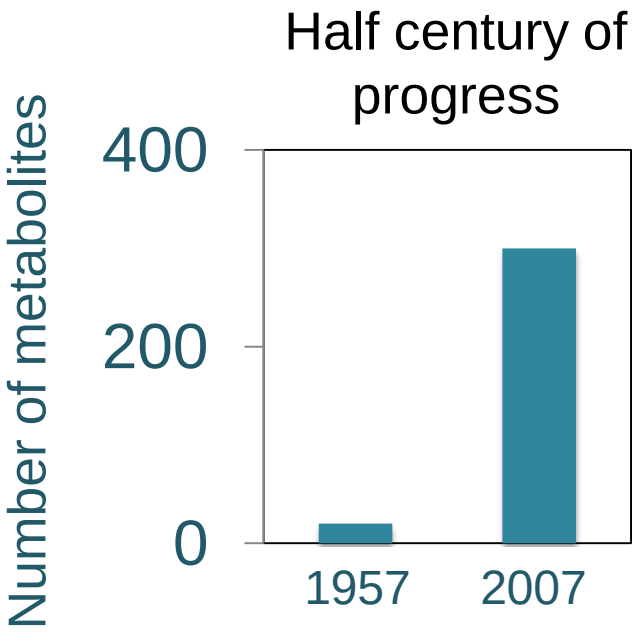
Method	Prep time[a]	HPLC run time
Fluorescent: mBrB [23,26]	1 h	35 min per fraction[b]
Ultraviolet: CMQT [27]	40 min	10 min per fraction[b]
Colorimetric: DTMB [28]	4 h	2 h per fraction[b]
Amino acid analyzer: ninhydrin [9,29]	none	80 min per fraction[b]
Electrochemical: dual electrode [30]	30 min	15 min
Colorimetric: DNFB [31]	5+ h	40 min
Fluorescent: Dansyl [11,12]	16+ h	1 h
MS/MS: [15,32]	2 min	15 min
Current method: FTMS	2 min	9 min

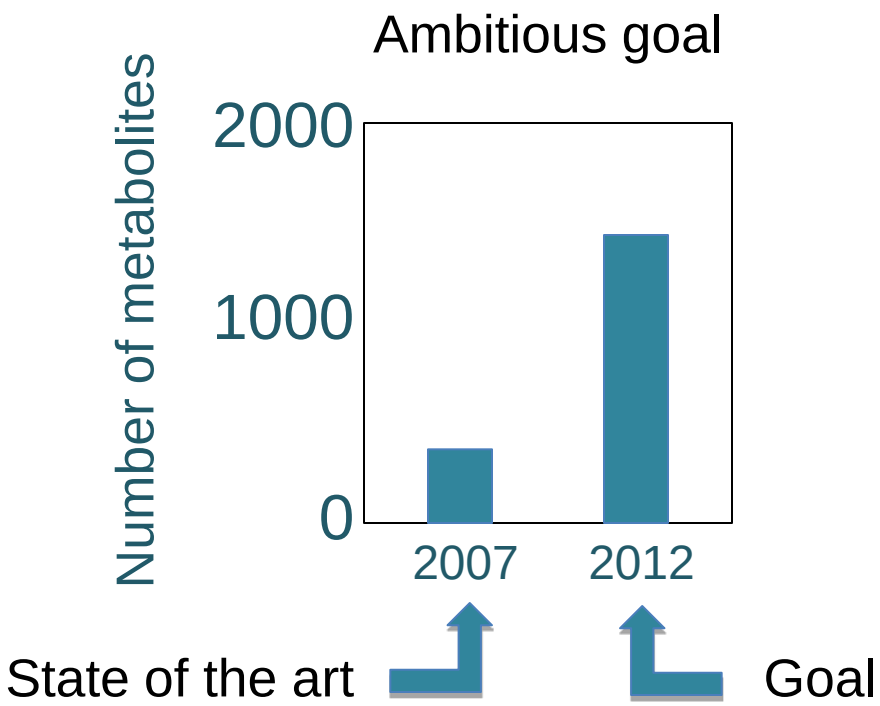
JM Johnson, 2008 Clin Chim Acta 396:43-48

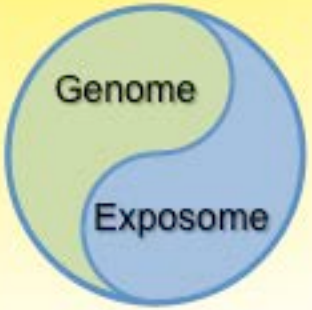


Goal in 2007: use high-resolution mass spectrometry to improve detection from 300 metabolites to 1500 metabolites

NIEHS funding: Environmental Parkinson's Disease (PD-CERC)

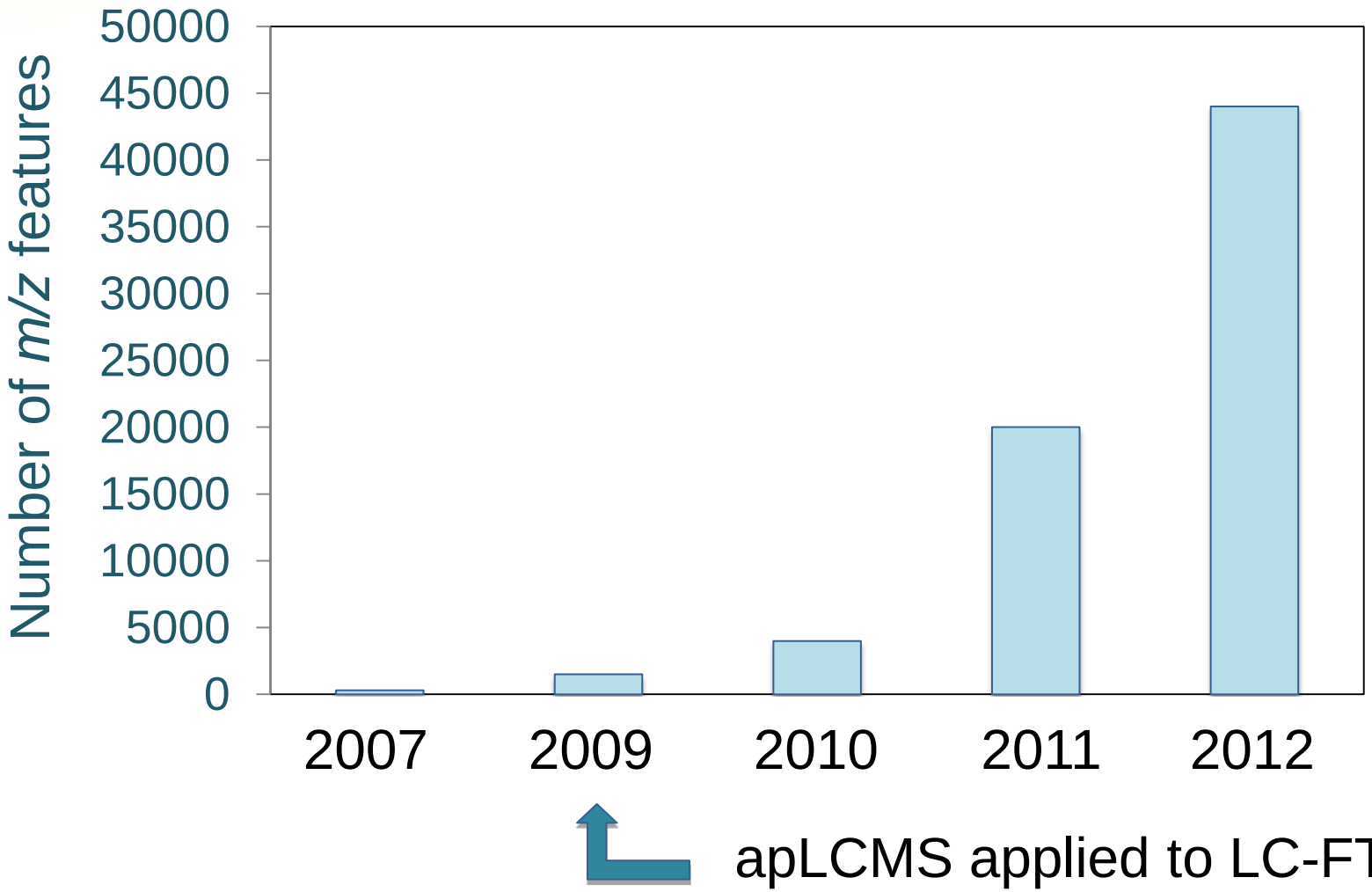


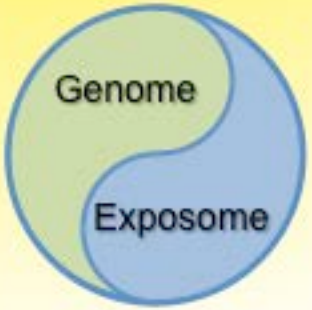




With apLCMS data extraction algorithms, achieved 5-fold improvement in one year

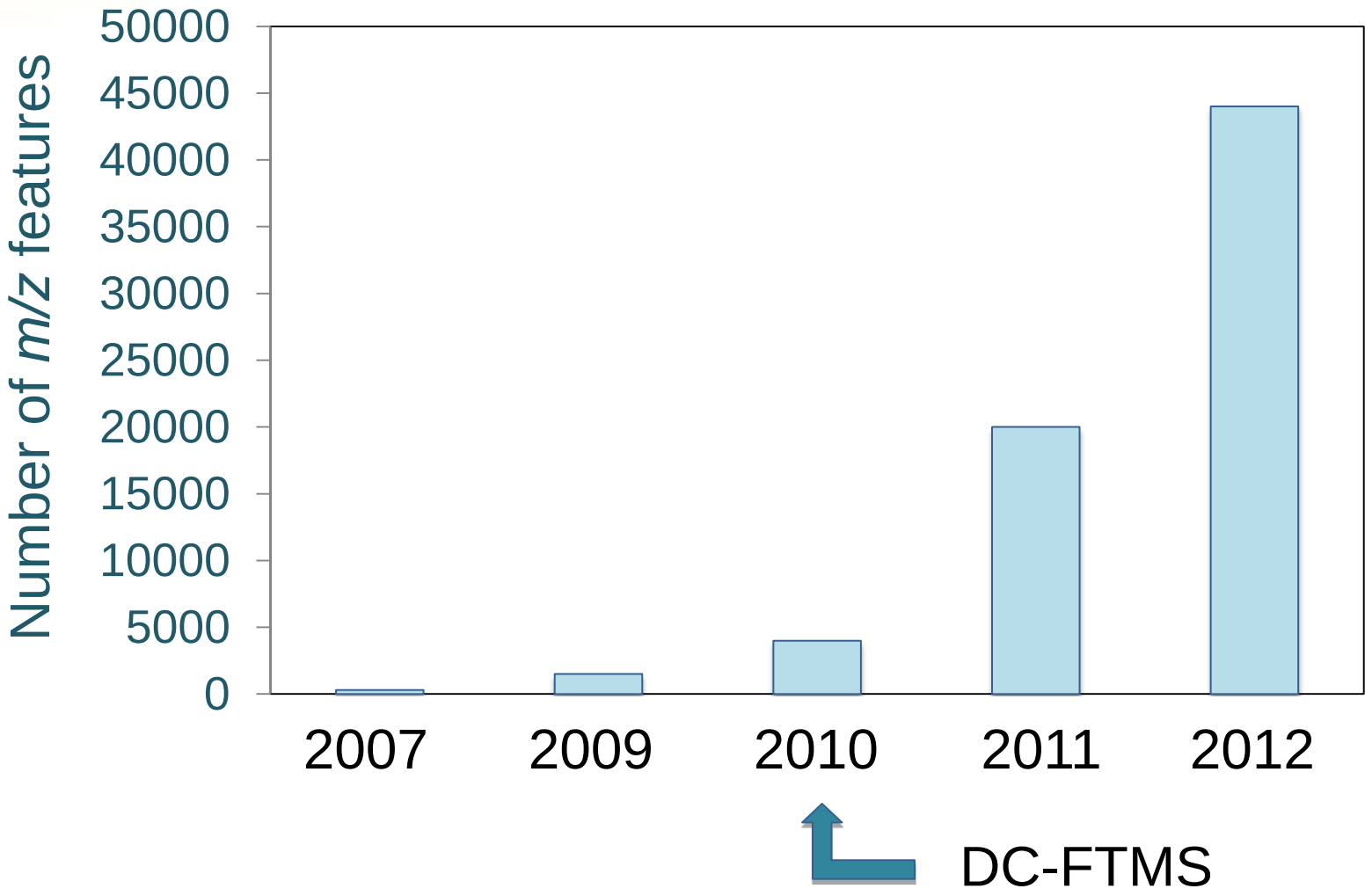
(Yu et al *Bioinformatics* 2009; Johnson et al *Analyst* 2010)

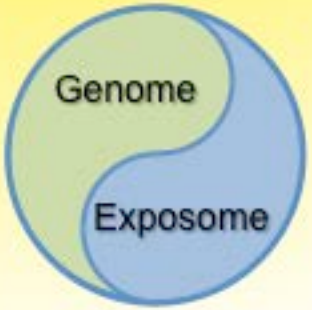




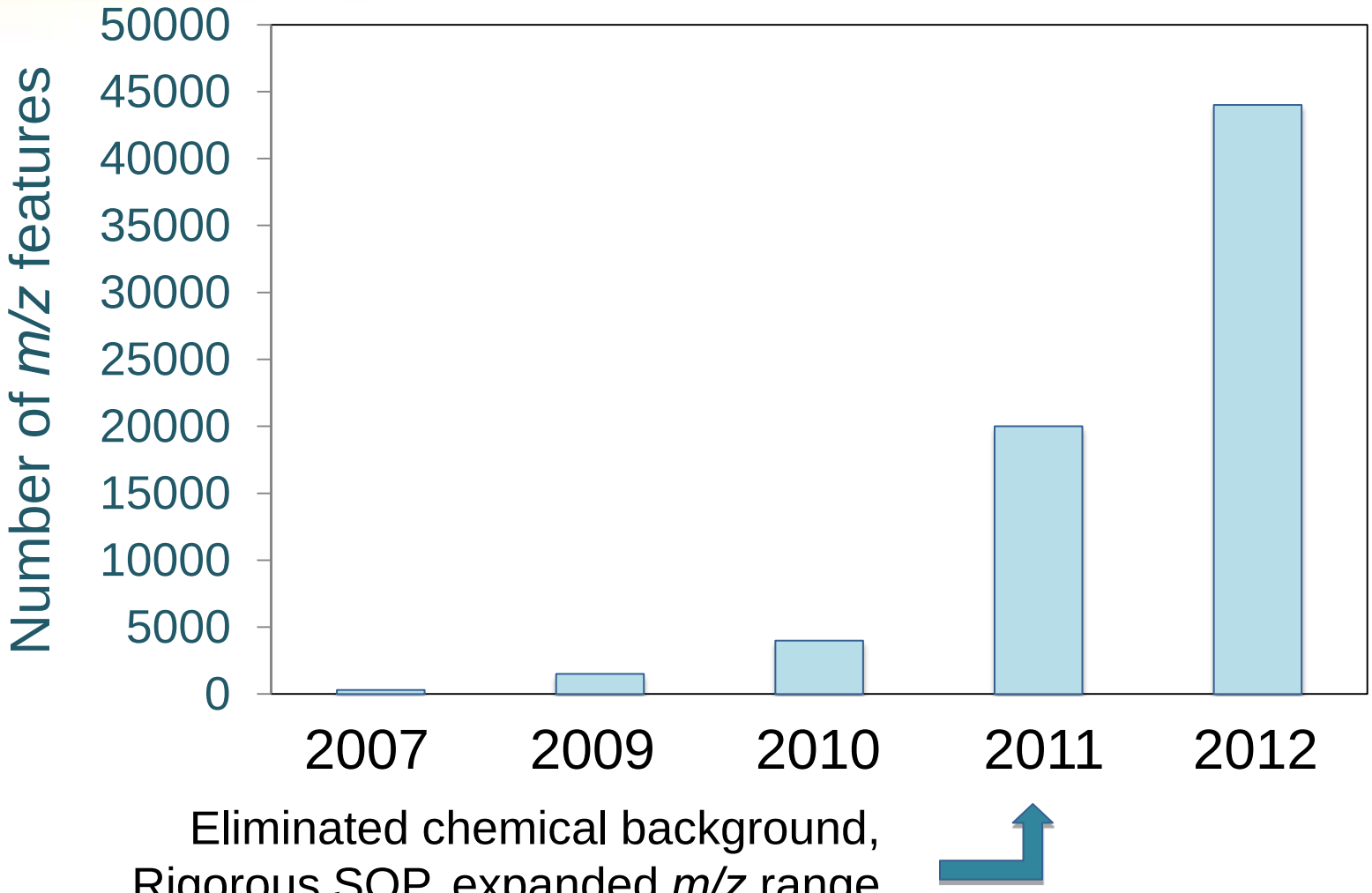
Capability was more than doubled by introduction of dual chromatography strategy

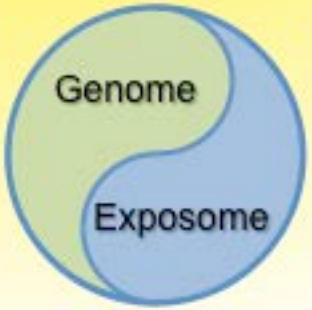
Soltow et al *Metabolomics* 2011





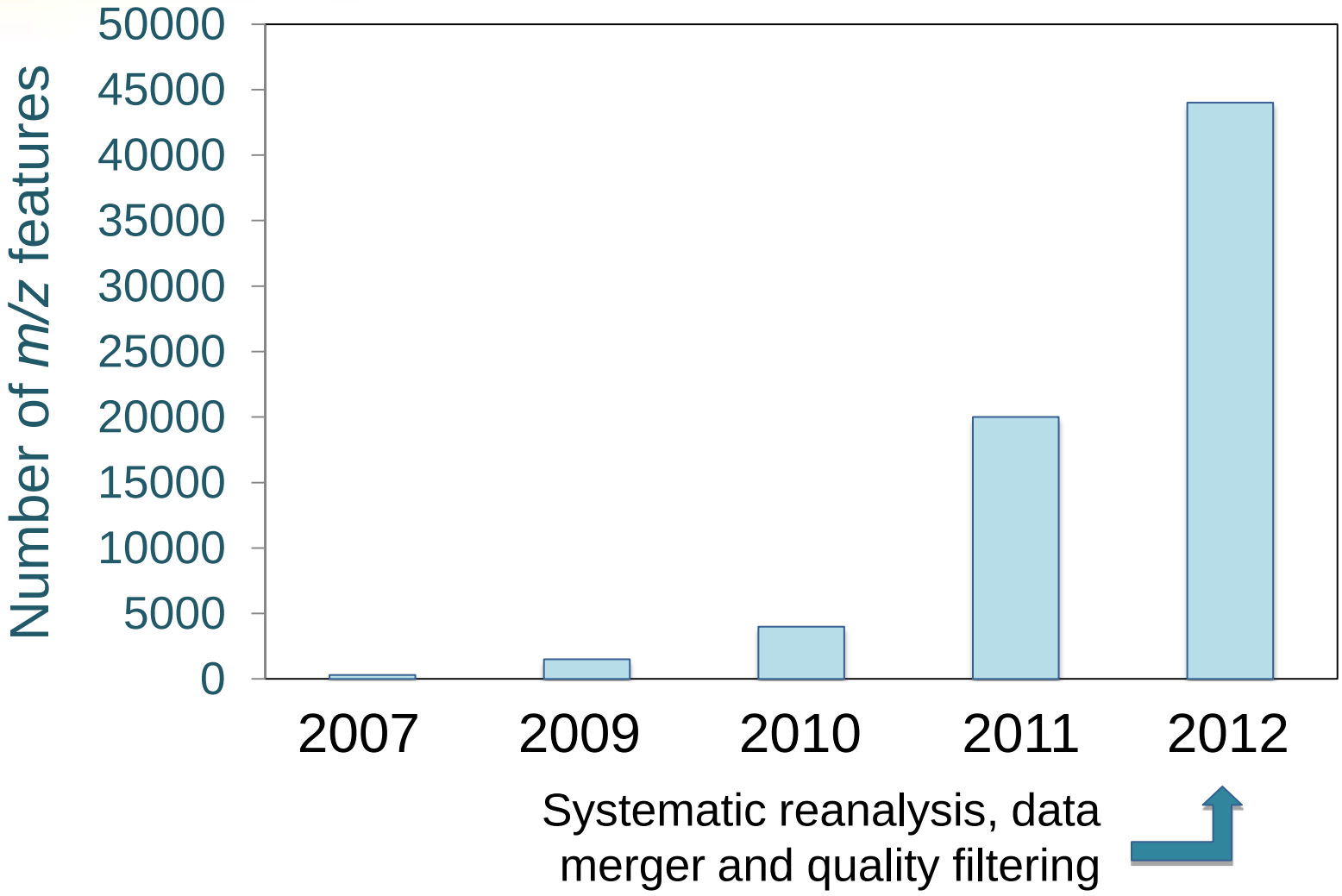
Capability was increased 4- to 5-fold by acquisition of an LTQ-Velos Orbitrap dedicated 24/7 to high-resolution metabolomics

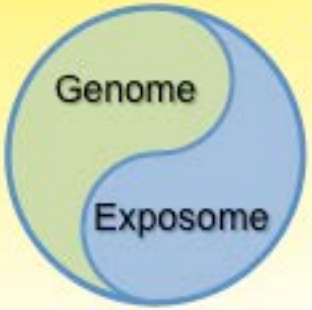




Development of xMSanalyzer has now doubled extraction of useful metabolic data

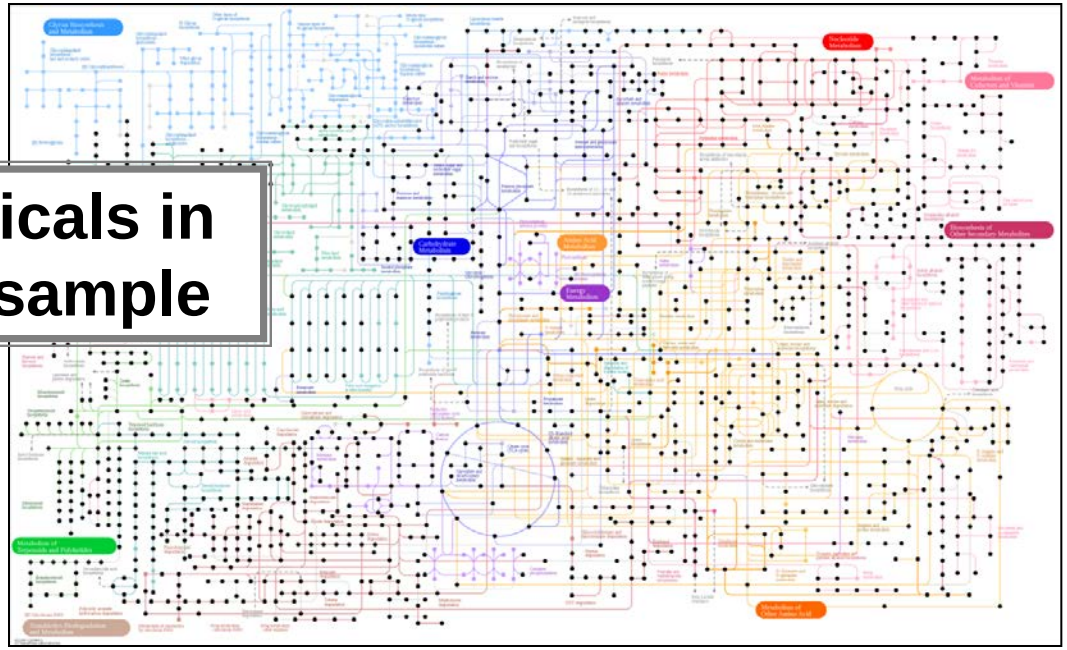
(K Uppal et al, BMC Bioinformatics 2013)

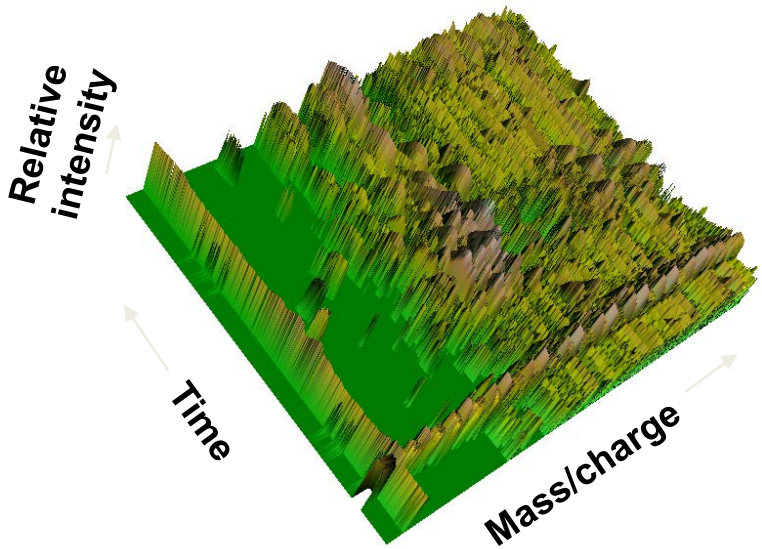




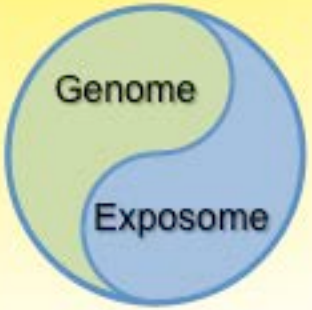
High-resolution metabolomics developed at Emory measures individual biochemistry with resolution approaching that for genomics

Measure 20,000 chemicals in an individual plasma sample





Measure 200,000 ions among individuals in population studies



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Practical aspects of high-resolution metabolomics:

Instrument cost is double that for common LC-MSMS; total operation cost is only 10-20% more because other expenses are comparable

Sensitivity is driven by

1) Analysis under rigorously defined, routine conditions
2) Dedicated continuous use (24/7); 10 µl; 10 min/analysis
3) Analysis in triplicate; dual chromatography
4) Advanced computational methods for data extraction

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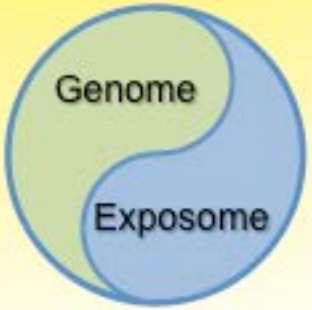
T Yu et al 2009 Bioinformatics 25:1930-6
JM Johnson et al 2010 Analyst 135: 2864-2870
Q Soltow et al 2011 Metabolomics DOI: 10.1007/s11306-011-0332-1
K Uppal et al 2013 BMC Bioinformatics in press



Nutritional and Environmental Metabolomics

Current Metabolomic capabilities: >20,000 “metabolites” in plasma or urine

Core Nutritional Metabolome

Non-nutritive Chemicals in Diet

Food metabolome

40 Essential nutrients and about 2000 metabolites formed by enzymes encoded by the genome

Plant metabolome >200,000 chemicals

Microbiome-related Chemicals

Largely uncharacterized (may be 10-40% of plasma metabolome)

Supplements and Pharmaceuticals

>1000 drugs in use

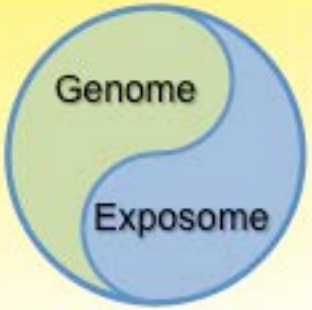
Environmental metabolome

Commercial Products

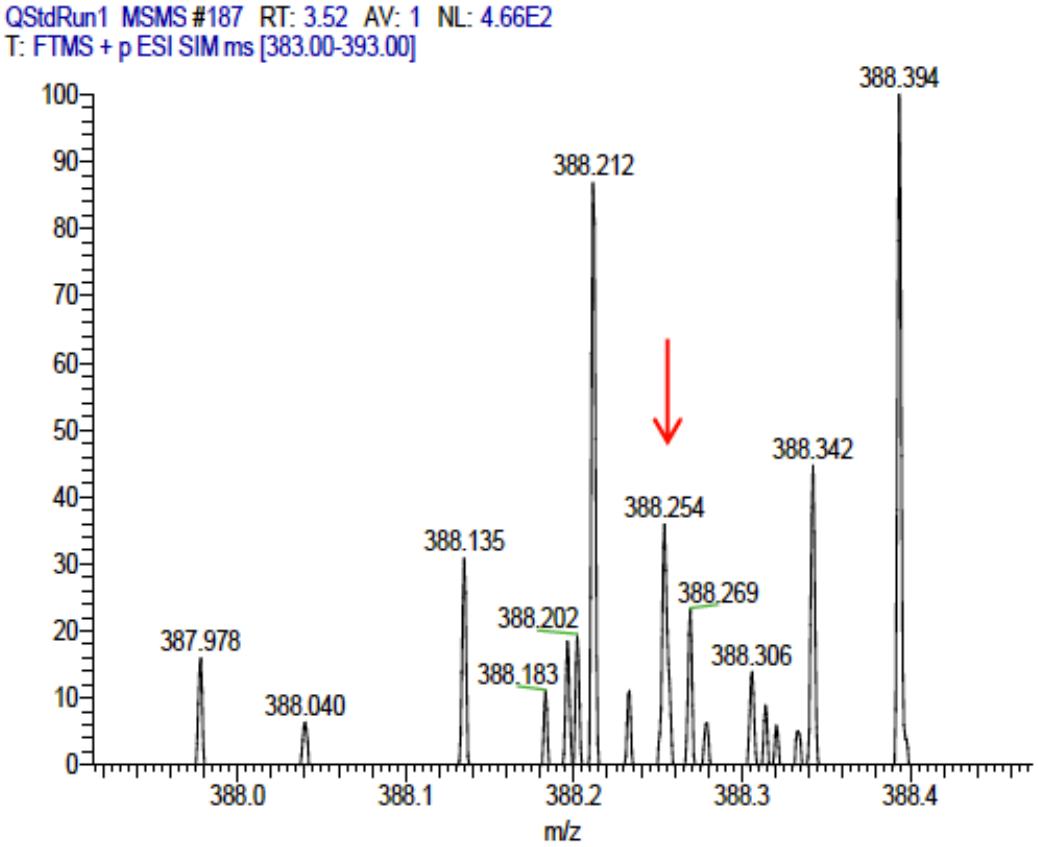
Environmental Chemicals

>10,000 agents used
>80,000 registered with EPA

Jones et al 2012 Annu Rev Nutr 32:183-202



Mass resolution and high sensitivity allow improved detection of low-abundance ions



Upppal et al 2013 BMC Bioinformatics

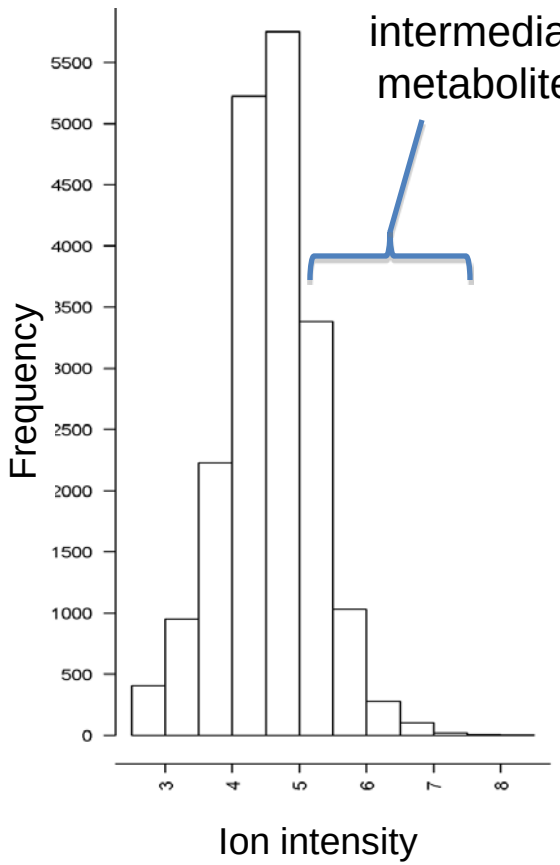
High-resolution metabolomics data for 174 subclinical CVD subjects

Improved data extraction over most approaches: 34,768 ions, triplicate analyses

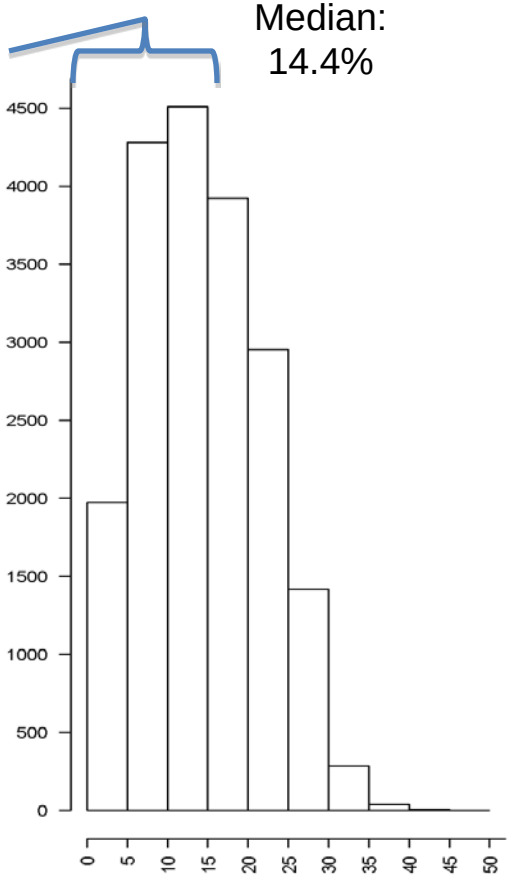
Summary for C18: 19,383 ions Range of detection over 5 orders of magnitude of intensity

With triplicate analyses, CV is obtained for each metabolite in each sample:
6,247 had median CV < 10% Mean intensity of ions with CV <10%: 3.0 x 10^5

Intensity
Mean: 1.2 x 10^5
Median: 2.0 x 10^4

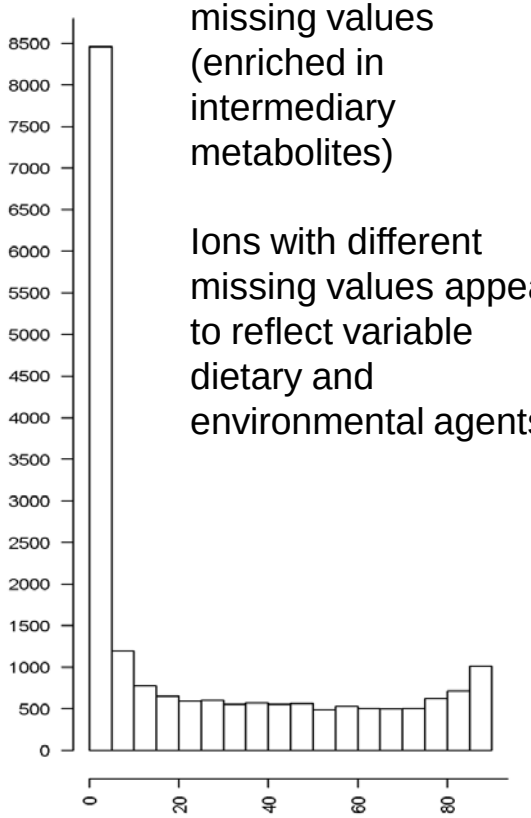


CV
Median: 14.4%



Missing values
>8000 had <5% missing values (enriched in intermediary metabolites)

Ions with different missing values appear to reflect variable dietary and environmental agents



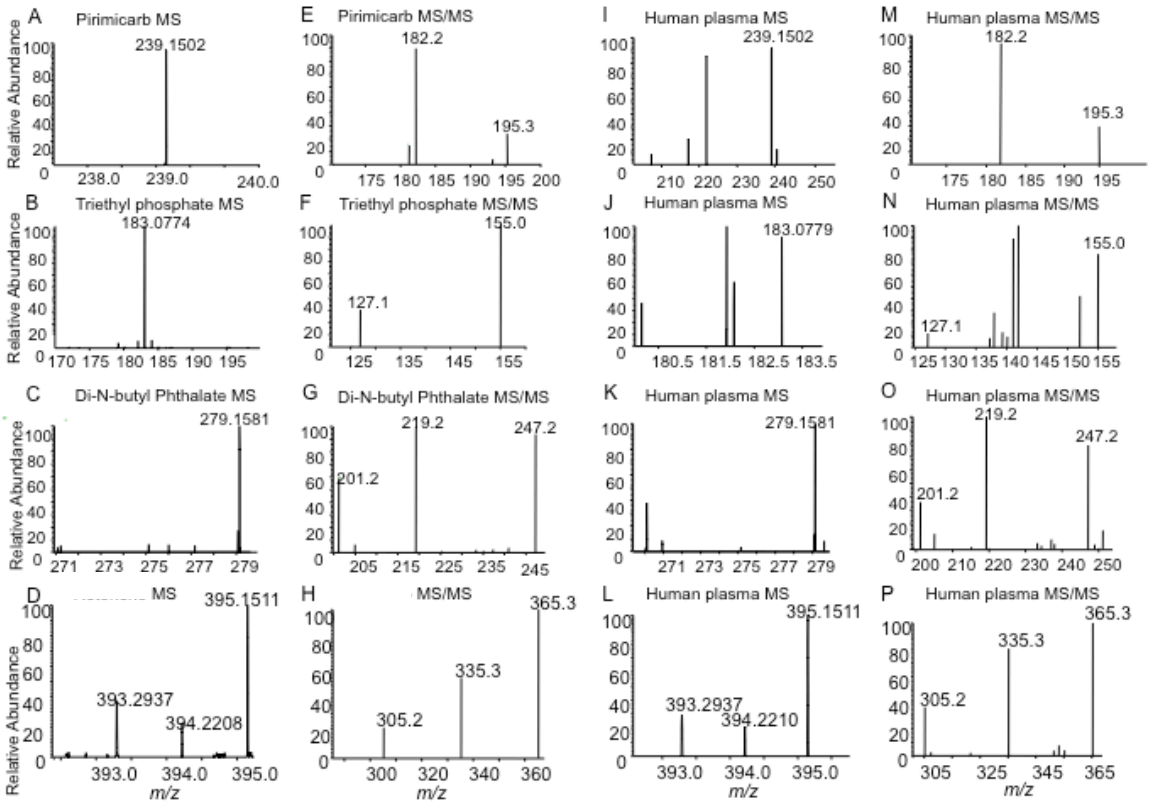
Highlights: Universal Exposure Surveillance

Many environmental chemicals are measured by high-resolution metabolomics

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

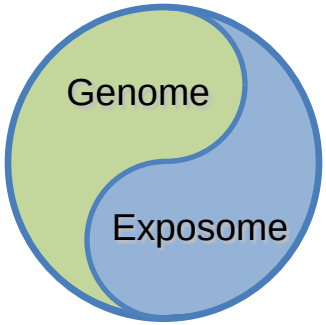
Q Soltow et al, Metabolomics 2011; Y Park et al, Toxicology 2012

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



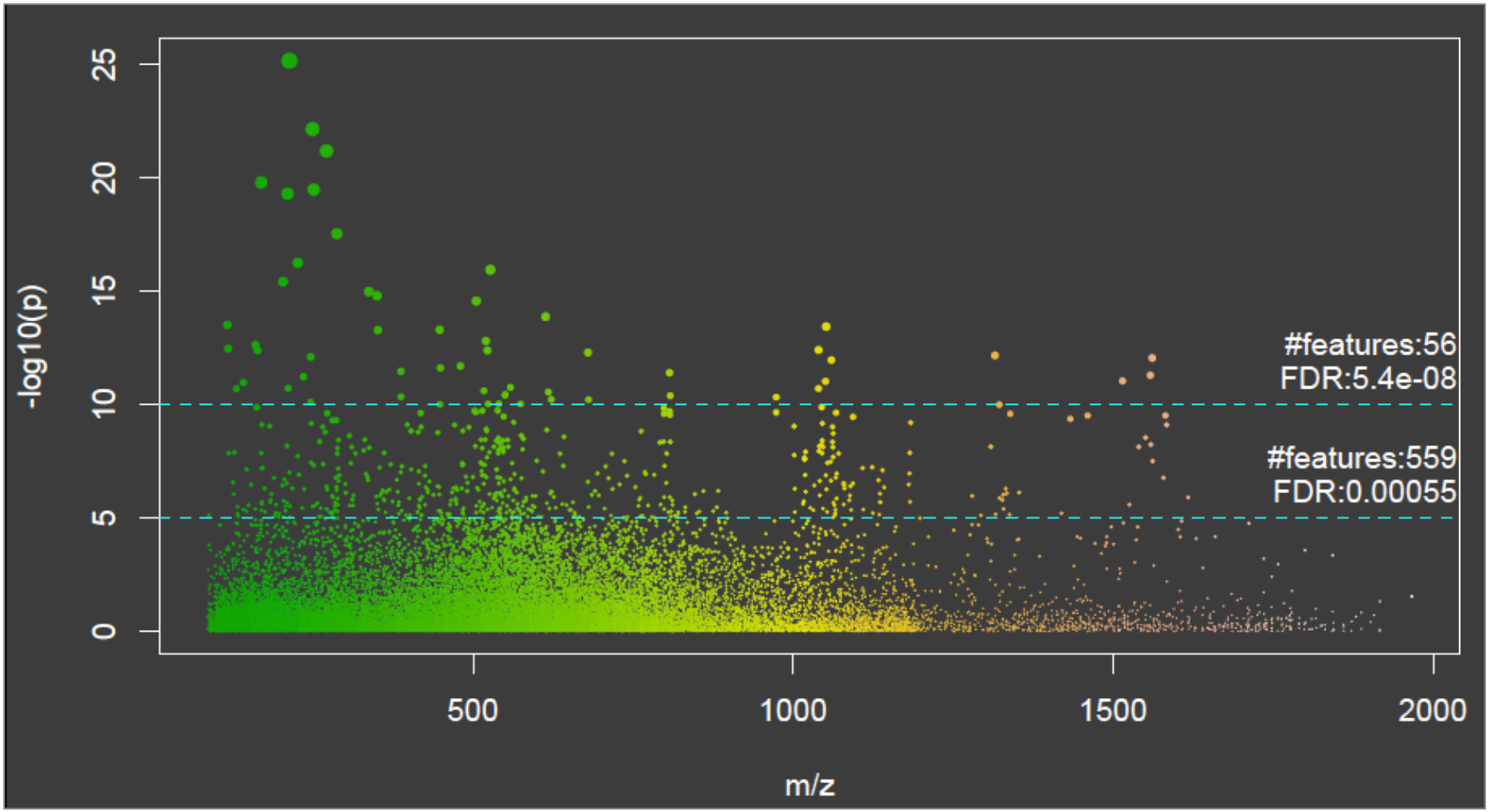
Minor component of commercial rotenone

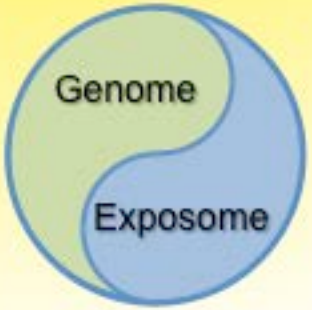
Y Park, K Lee et al, 2012 Toxicology 295:47-55



Metabolome-wide association study (MWAS) of BMI

Controlled for age, sex and race/ethnicity



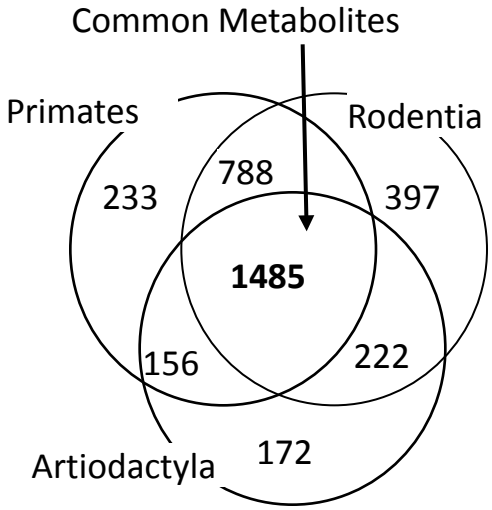


Comparative study of 7 mammalian species: less than half of chemicals detected are common

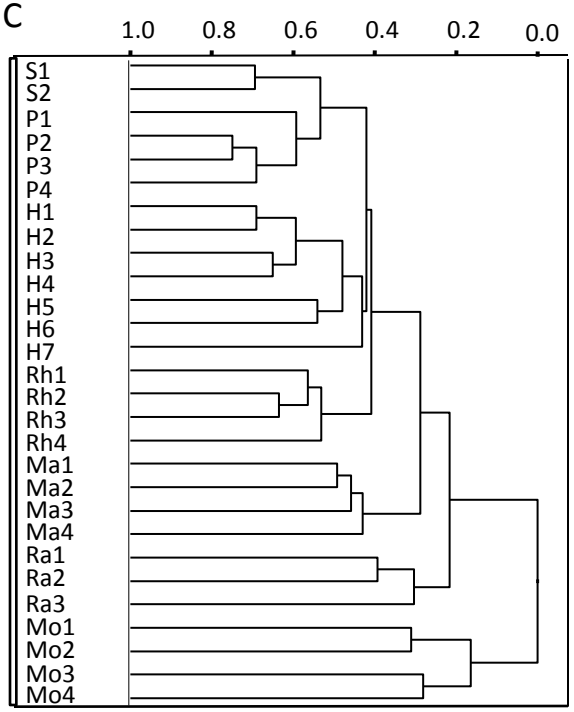
A

Order	Family		Metabolites in species
Class Mammalia	Primates 3723	Hominidae	
		human	**3221**
		Cercopithecidae	
		rhesus	**3379**
		Callithrix	
		marmoset	**3292**
	Artiodactyla 3382	Suidae	
		pig	**2537**
		Bovidae	
		sheep	**2877**
	Rodentia 3704	Muridae	
		rat	**3220**
		mouse	**3373**

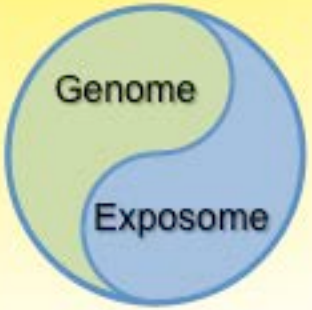
B



C



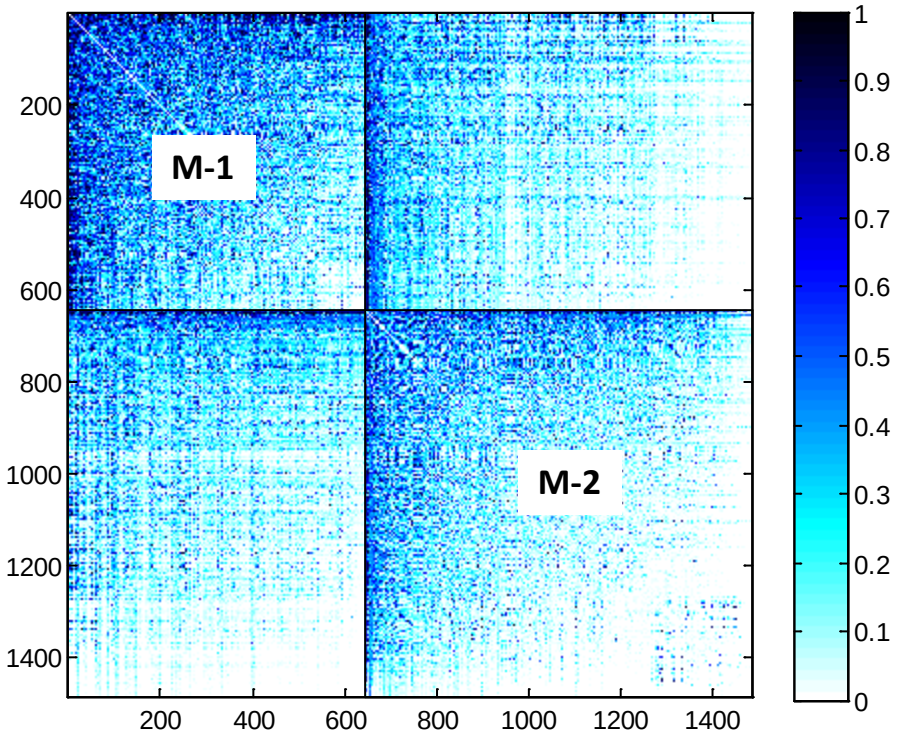
Park et al 2012 Toxicology



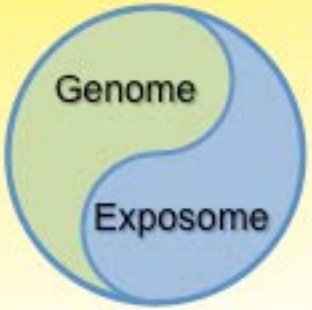
Probability-based clustering of metabolomics in 7 mammalian species discriminates environmental chemicals and metabolites useful for bioeffect monitoring

Module-1: Useful for bioeffect monitoring
Intermediary metabolites, e.g,
Ile, Citrulline, Cystine

Module-2: Environmental chemicals and detoxification systems:
Pirimicarb
Triethyl phosphate
Di-N-butyl phthalate
GSH



Park et al 2012 Toxicology



Nutritional and Environmental Metabolomics

Current Metabolomic capabilities: >20,000 “metabolites” in plasma or urine

Core Nutritional Metabolome

Non-nutritive Chemicals in Diet

Food metabolome

40 Essential nutrients and about 2000 metabolites formed by enzymes encoded by the genome

Plant metabolome >200,000 chemicals

Microbiome-related Chemicals

Largely uncharacterized (may be 10-40% of plasma metabolome)

Supplements and Pharmaceuticals

>1000 drugs in use

Environmental metabolome

Commercial Products

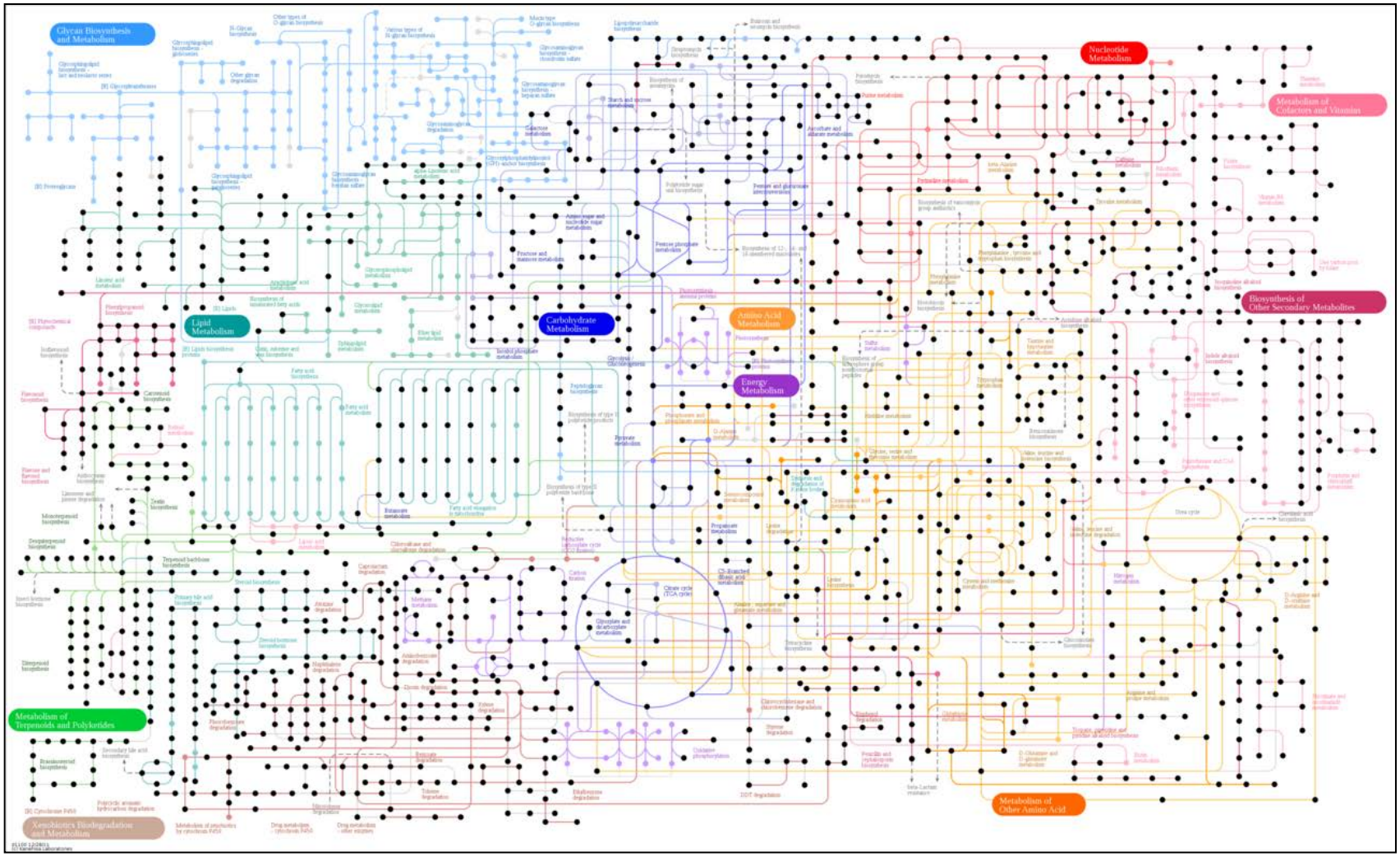
Environmental Chemicals

>10,000 agents used
>80,000 registered with EPA

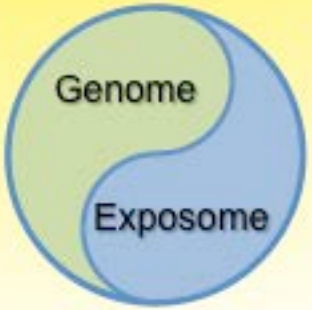
Jones et al Annu Rev Nutr 2012

The high-resolution metabolomics platform provides precise metabolic phenotyping to support personalized medicine

Detected *m/z* features matching half of known human intermediary metabolites (KEGG) are shown in black; most human metabolic pathways are represented

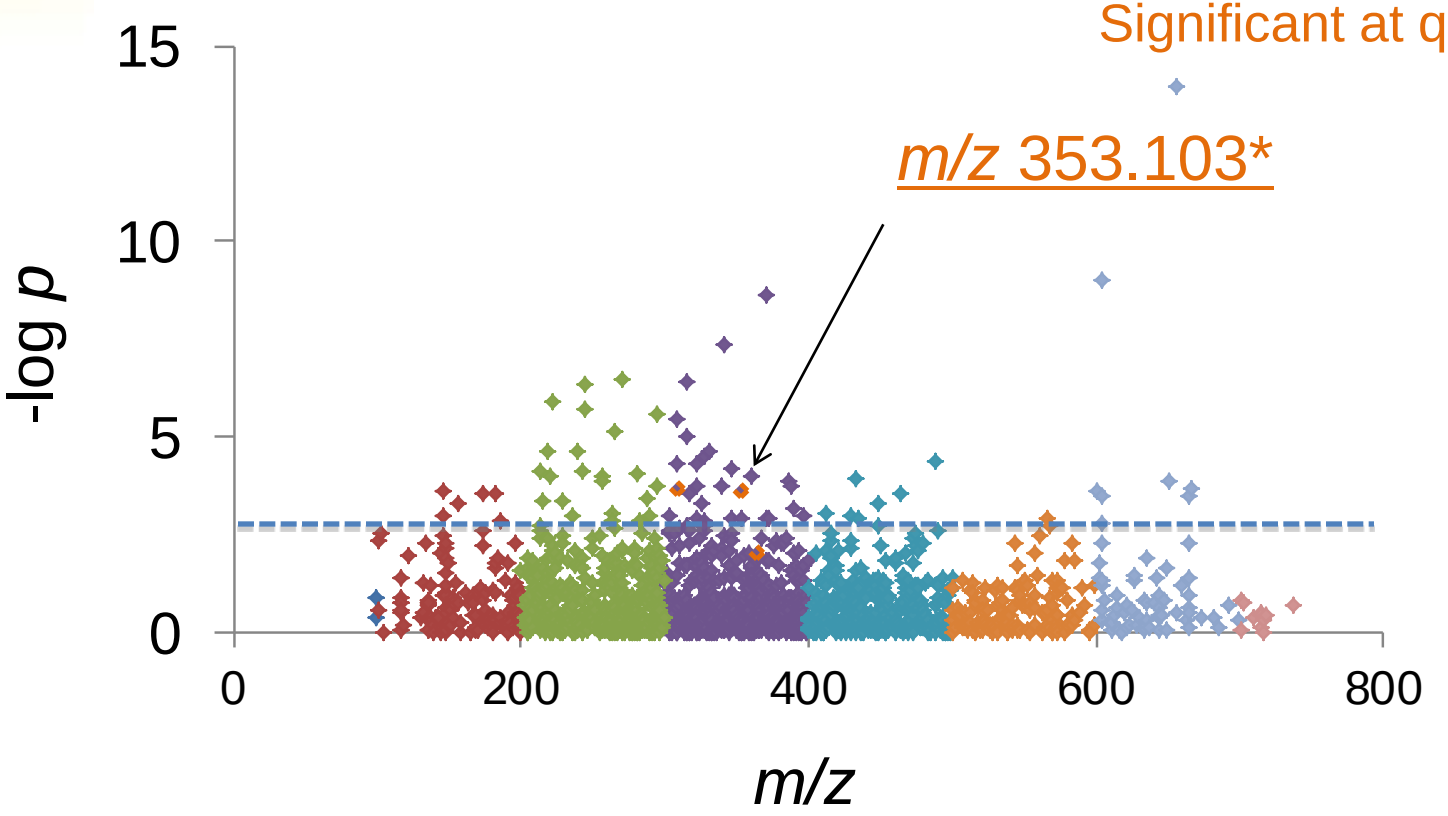


Soltow et al *Metabolomics* 2011; Yu et al *Bioinformatics 2009;* Uppal et al BMC Bioinformatics 2013

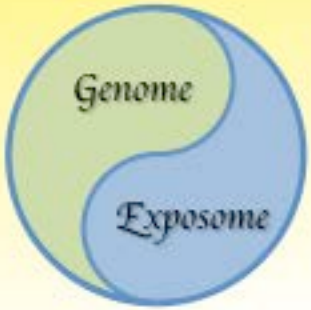


MWAS of age-related macular degeneration

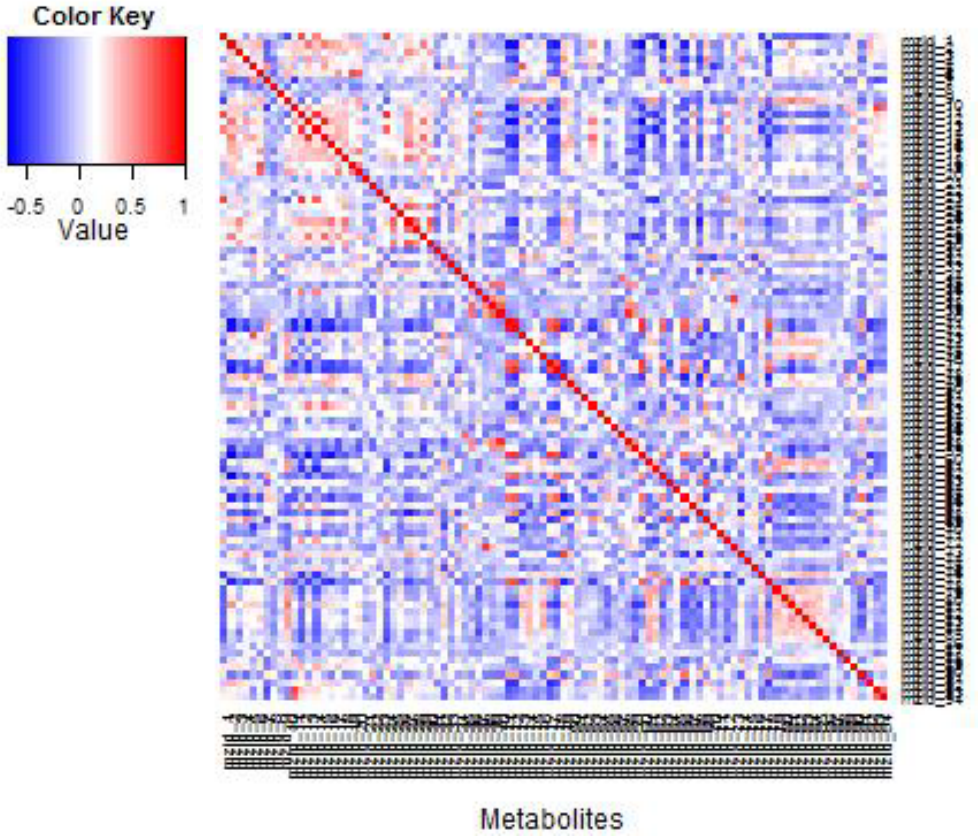
*Present >50% of samples
Significant at q=0.05

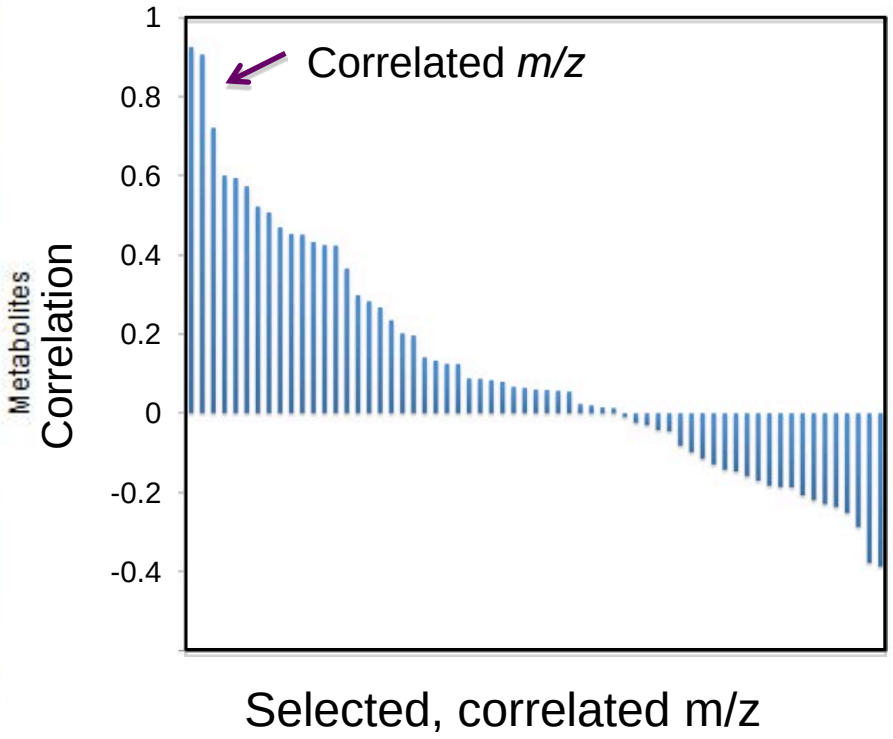


M Brantley, Y Park et al, unpublished

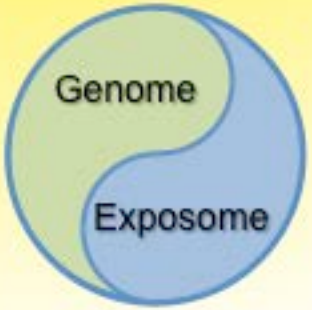


Metabolite correlations are very useful to understand redundancies of chemical detection and network associations of metabolism



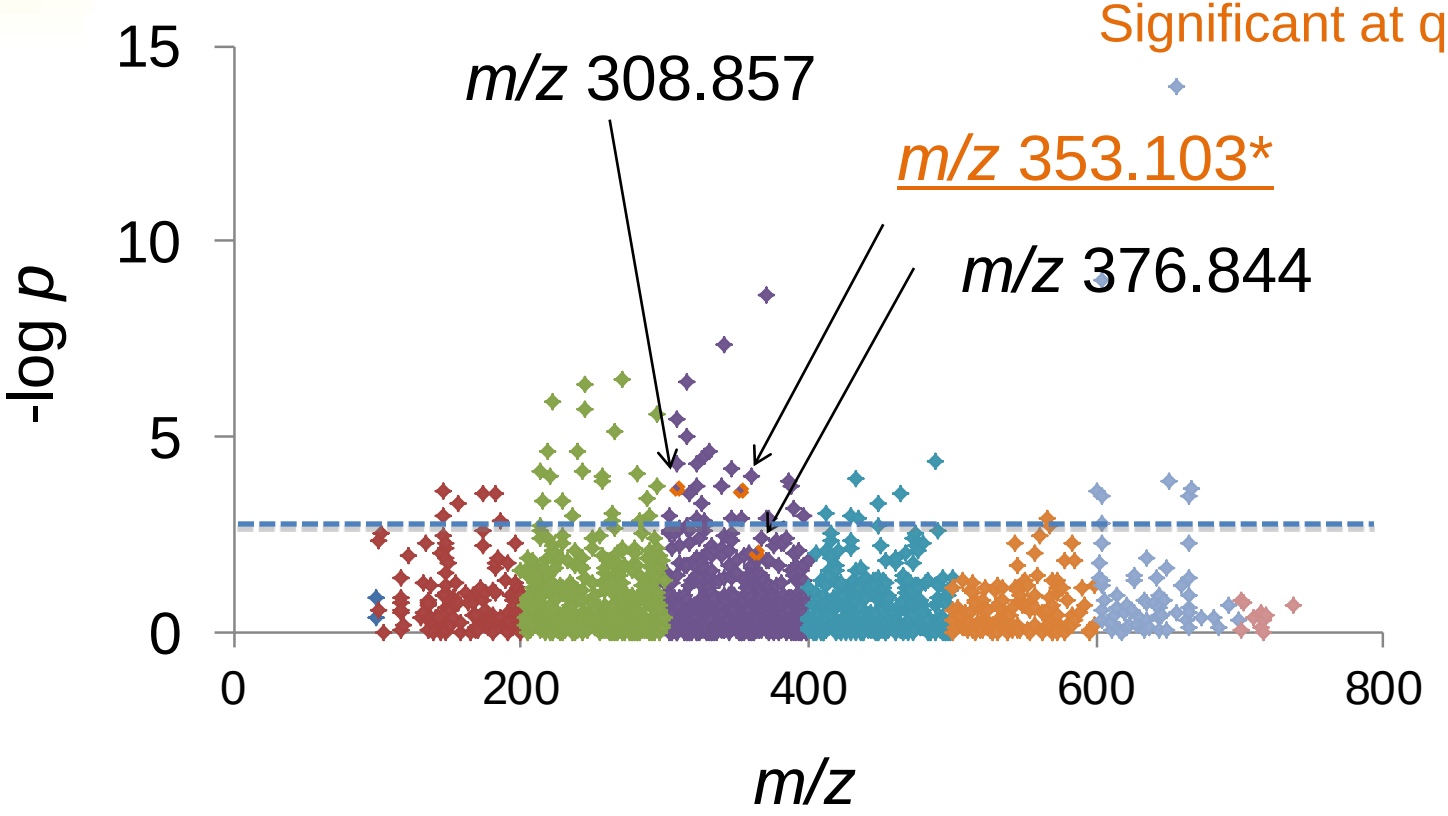


Y Park et al, unpublished



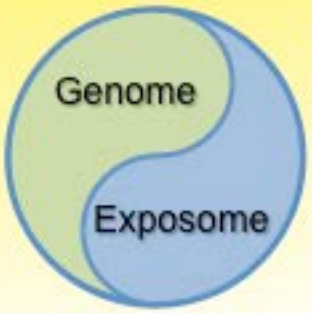
MWAS of age-related macular degeneration reveals environmental associations

*Present >50% of samples
Significant at q=0.05



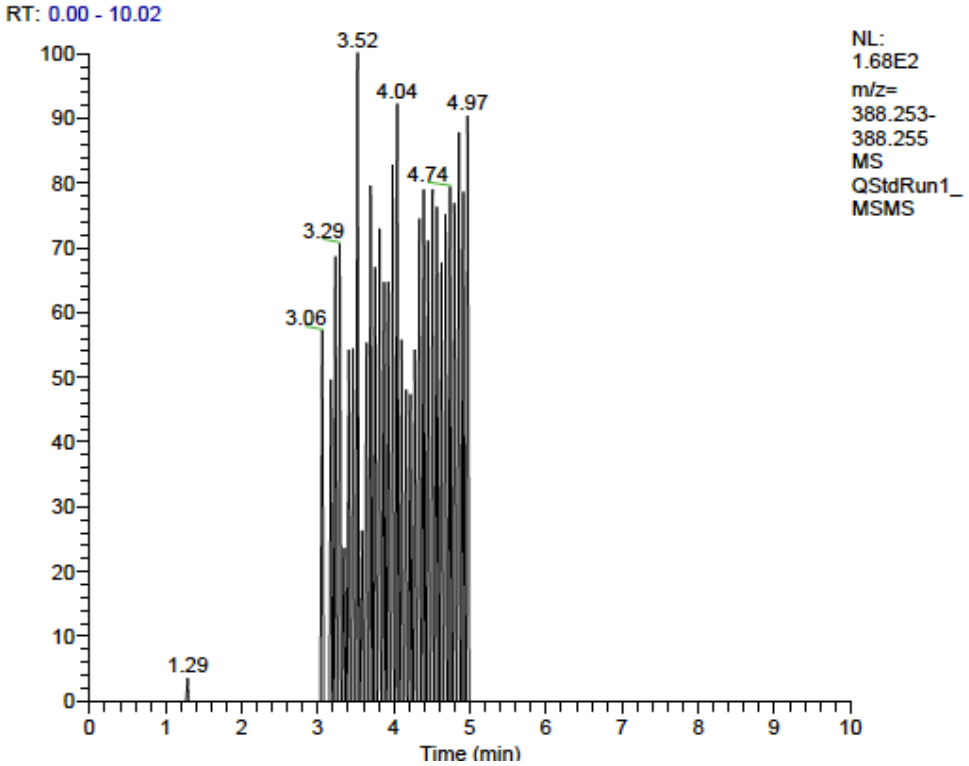
Database matches (not confirmed identities)
308.857: pentachlorocyclohexanol
376.844: pentachlorodibenzodioxin

M Brantley, Y Park et al, unpublished

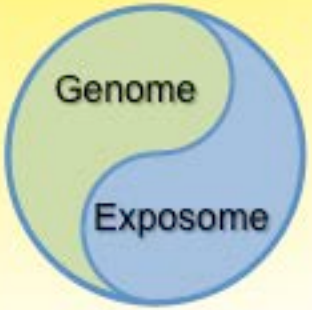


Development of Deconvolution MS/MS for Identification of Low-Abundance Ions

Elution of m/z 388.254



Upppal et al 2013 BMC Bioinformatics



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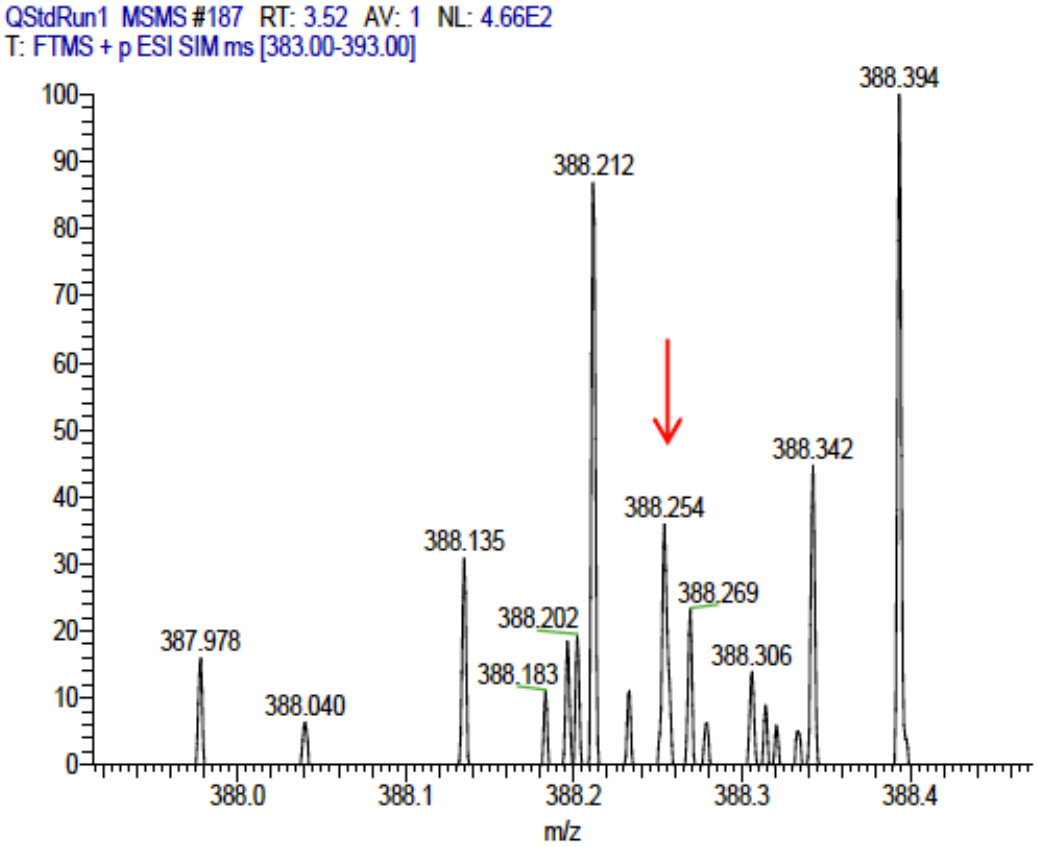
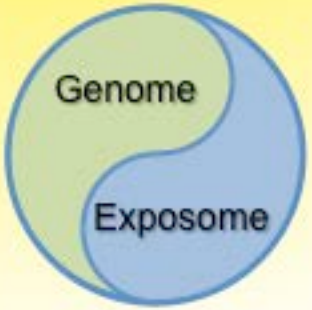
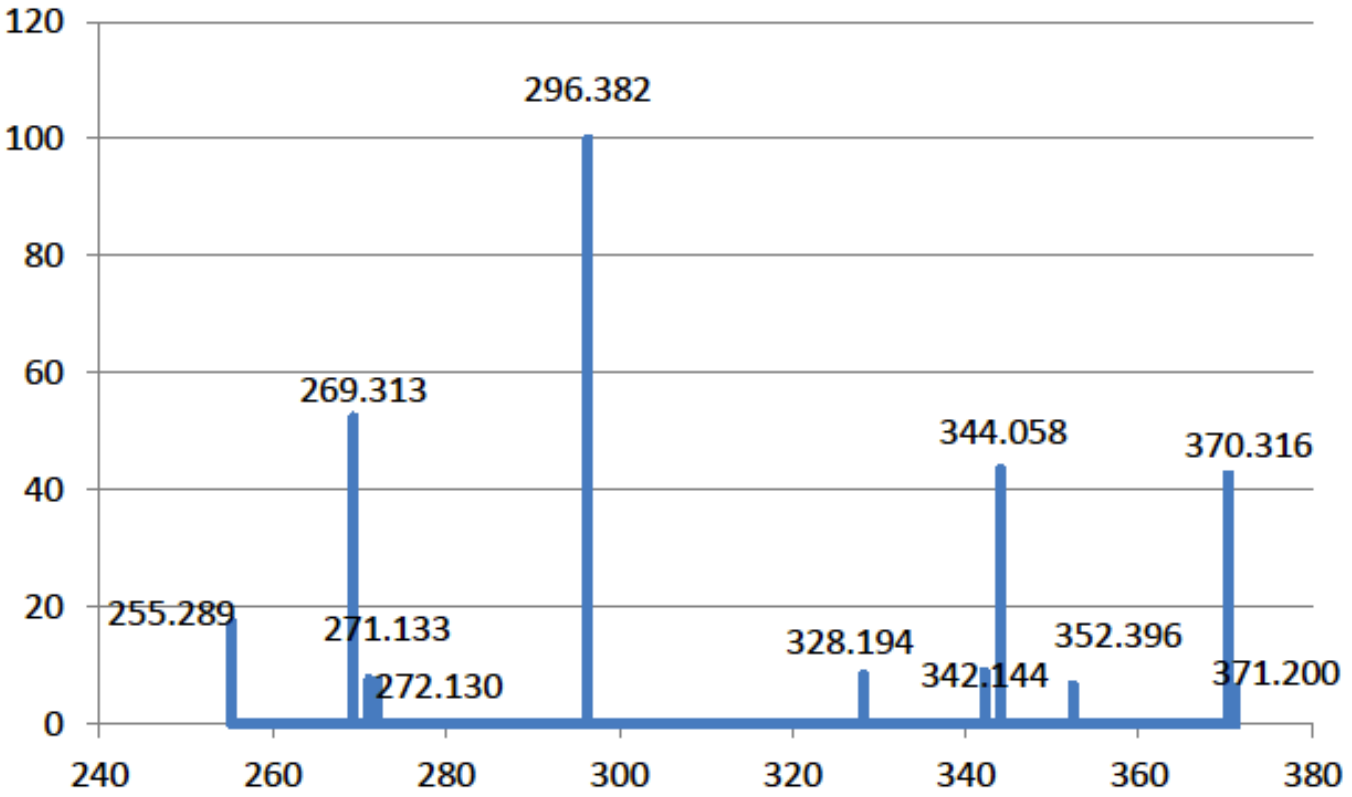


Upppal et al 2013 BMC Bioinformatics

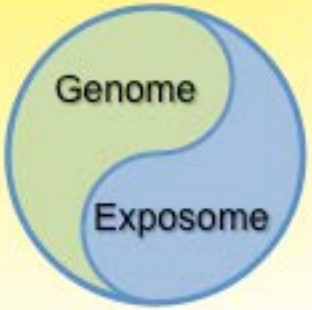


Development of Deconvolution MS/MS for Identification of Low-Abundance Ions

Predicted MS/MS

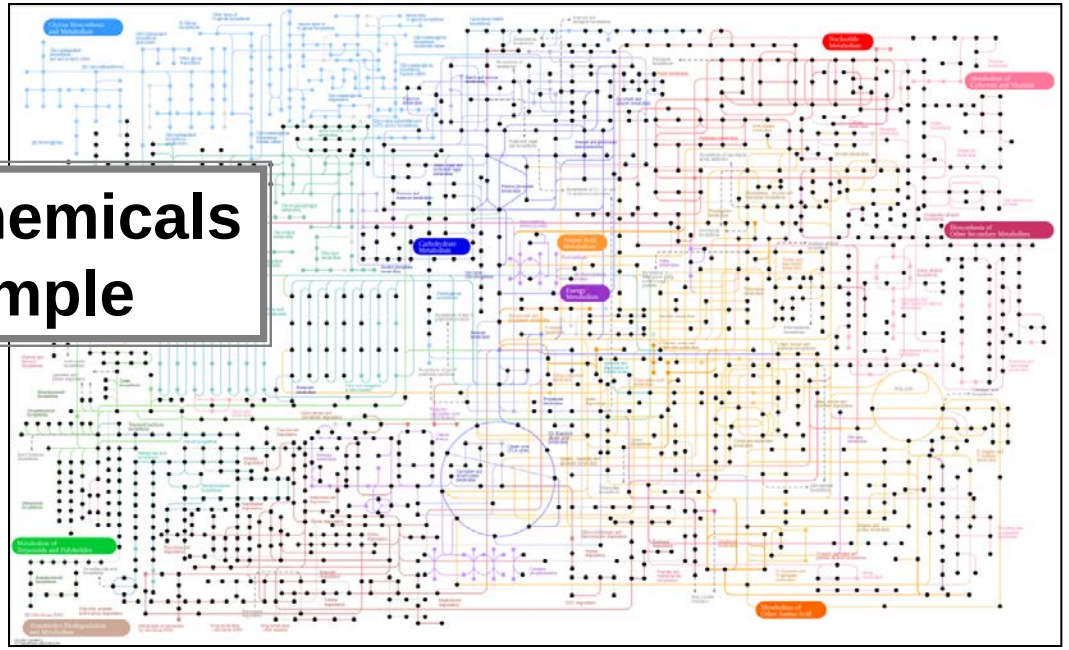


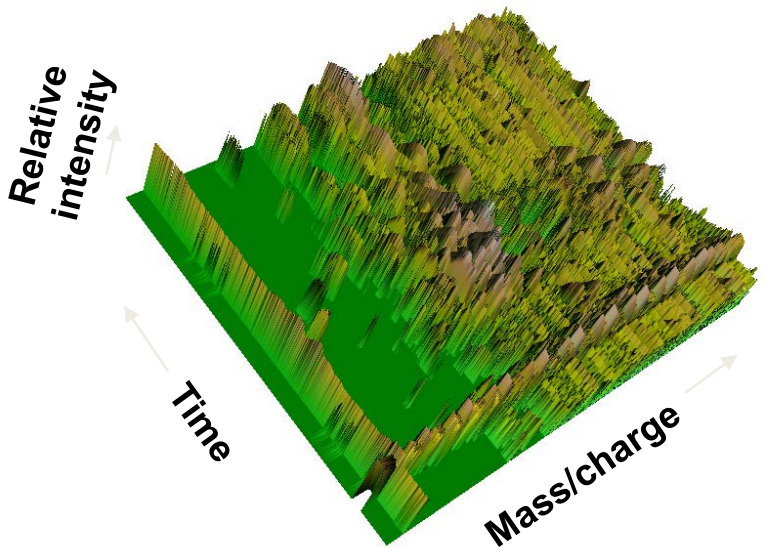
Upppal et al 2013 BMC Bioinformatics



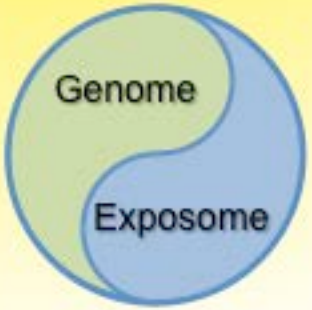
High-resolution metabolomics can provide a practical means to routinely biomonitor environmental exposures

Measure 20,000 chemicals in a plasma sample





Jones et al Annu Rev Nutr 2012



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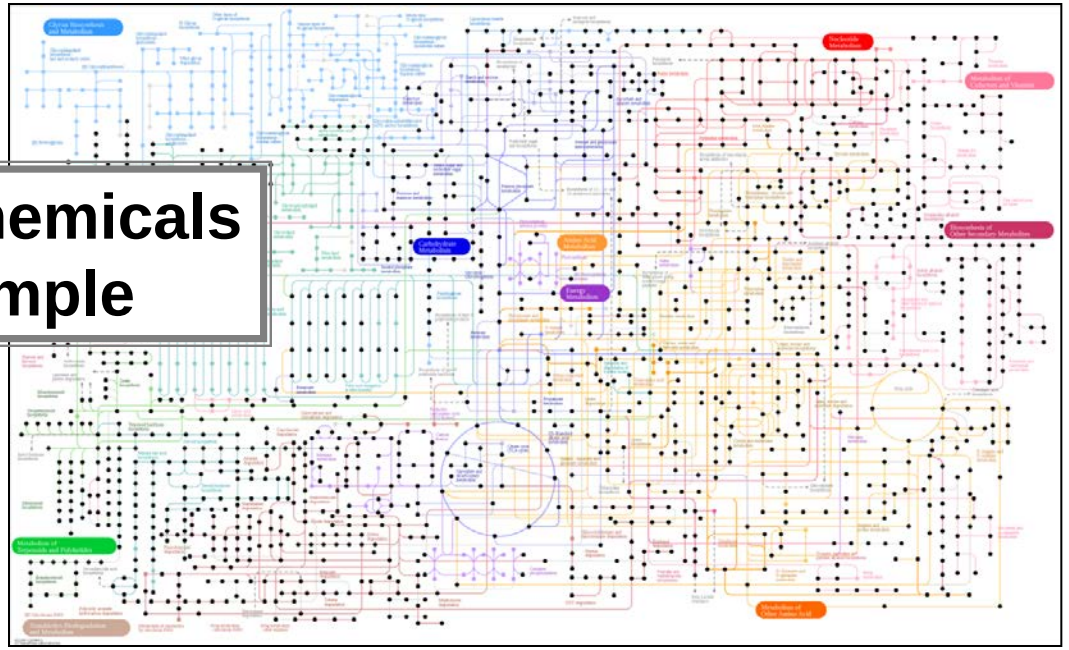
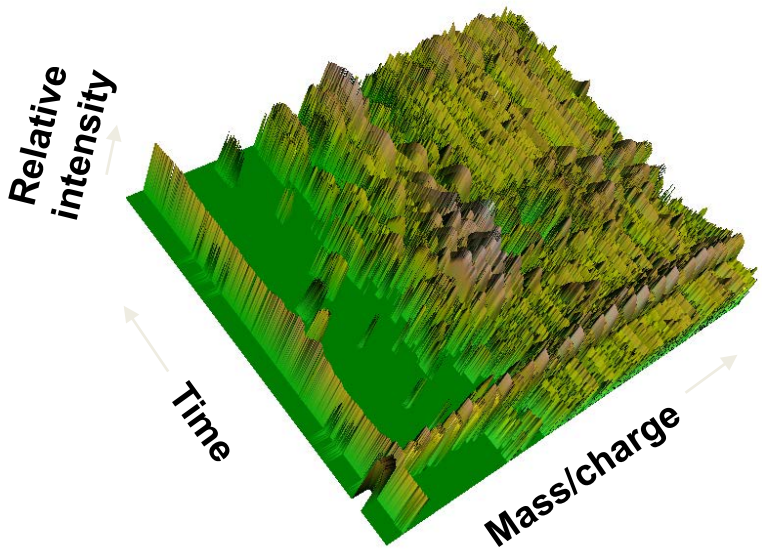
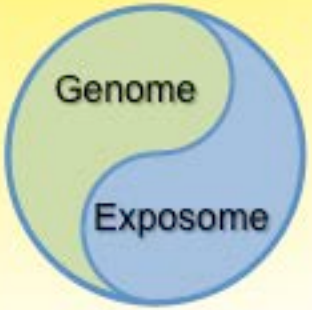




Projected cost: $125



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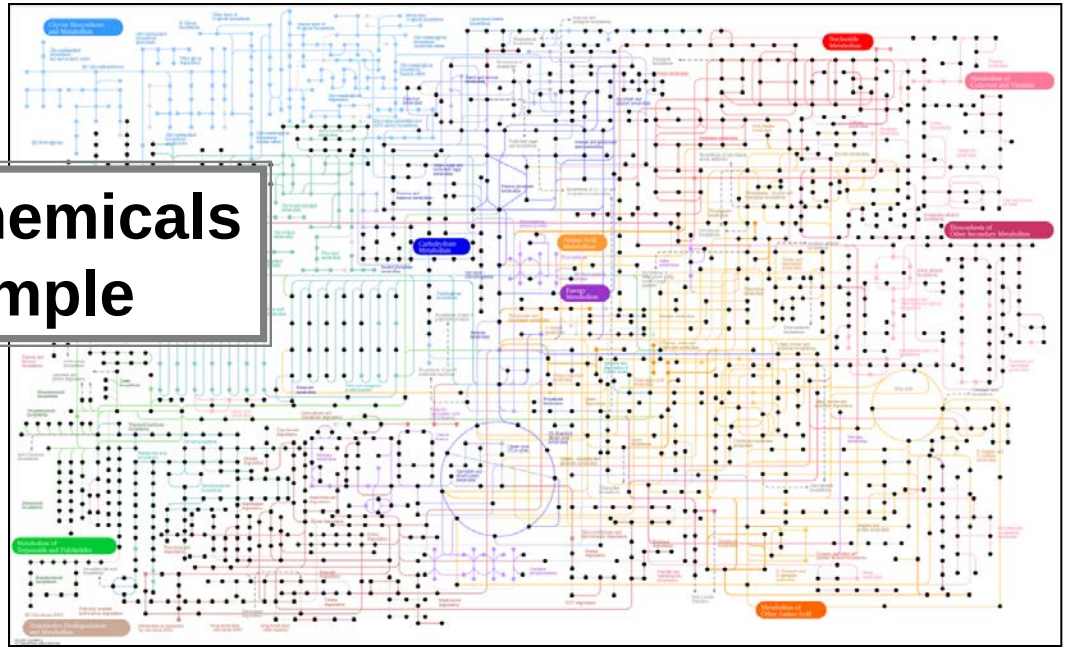
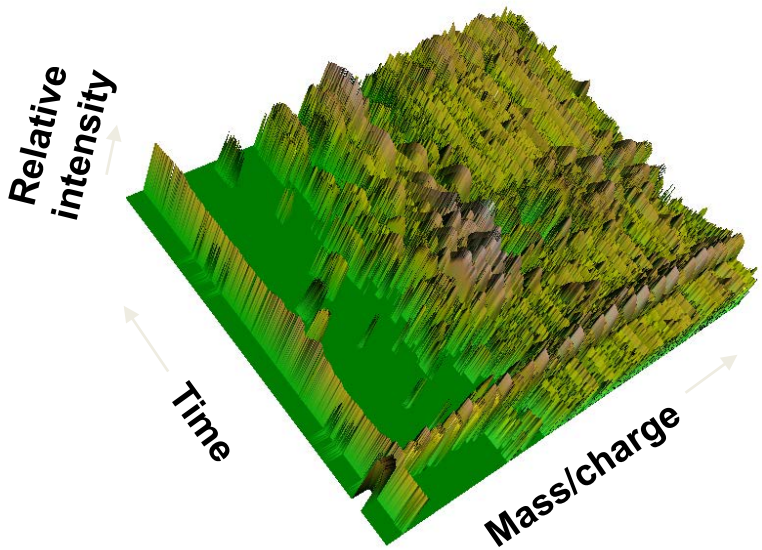
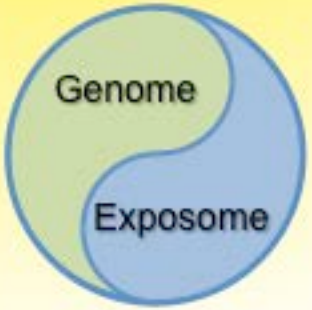




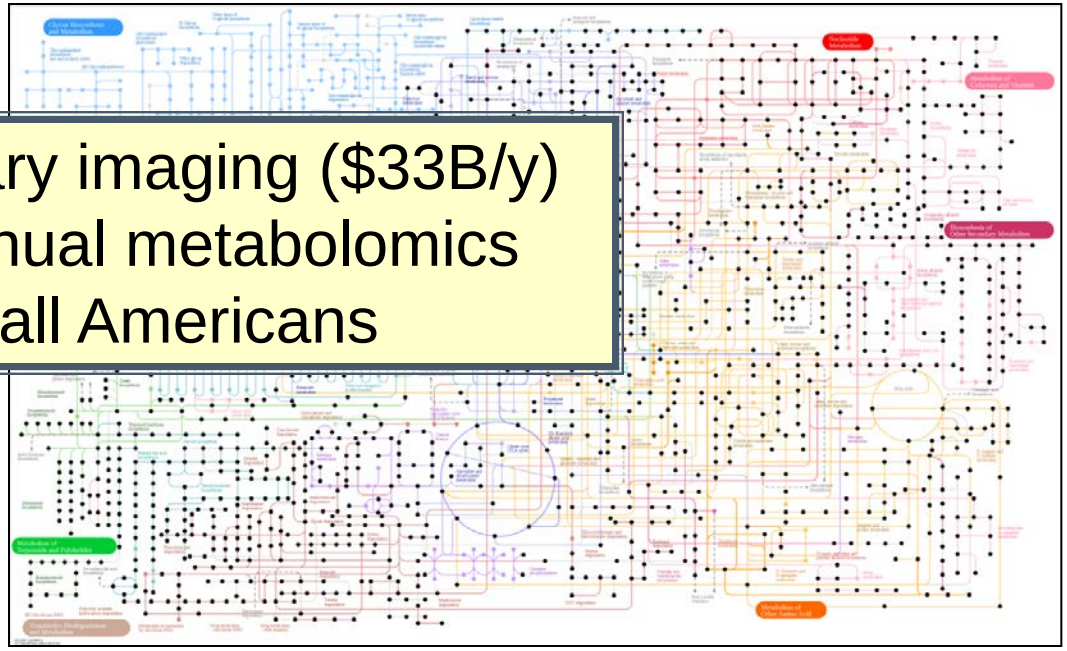
Projected cost: $125

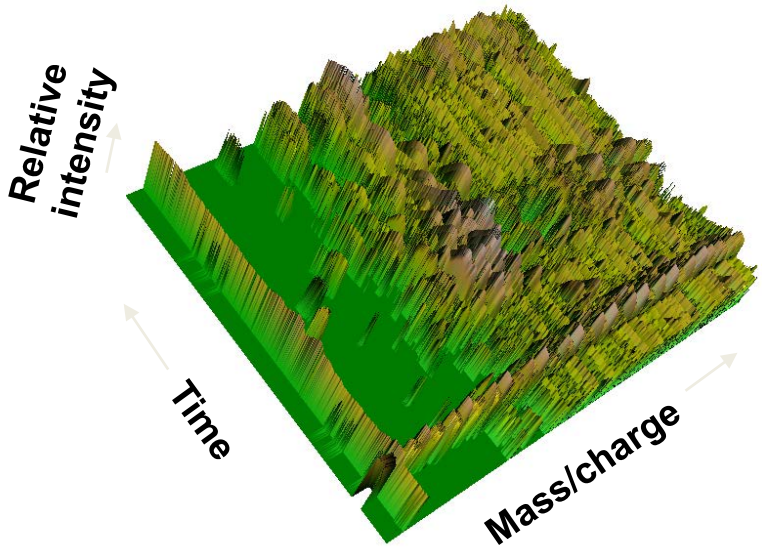
1% of healthcare costs would support annual metabolomics for all Americans



High-resolution metabolomics can provide a practical means to routinely biomonitor environmental exposures

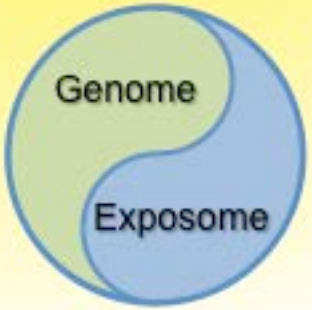
Cost of unnecessary imaging ($33B/y) would pay for annual metabolomics analysis for all Americans





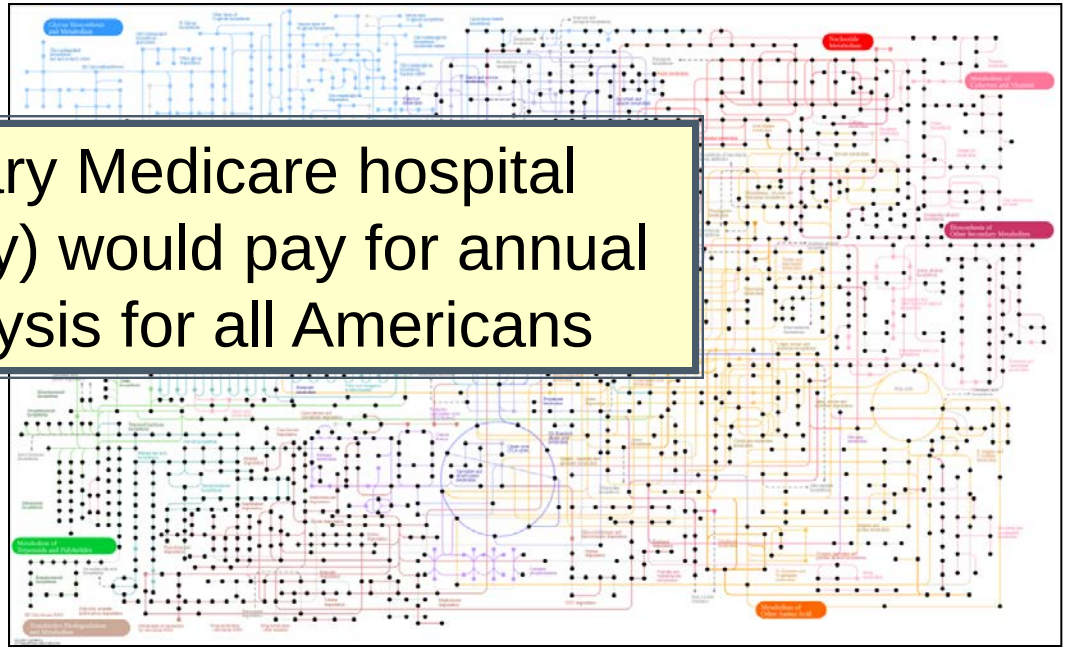
Projected cost: $125

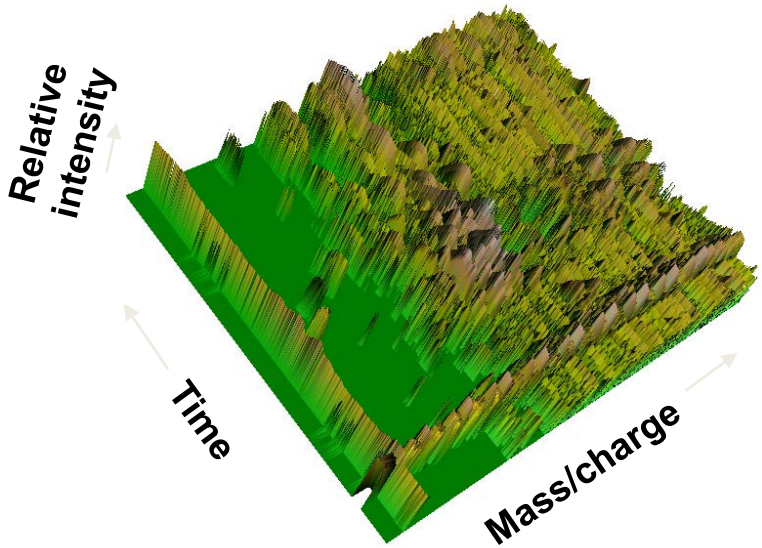
1% of annual healthcare costs



High-resolution metabolomics can provide a practical means to routinely biomonitor environmental exposures

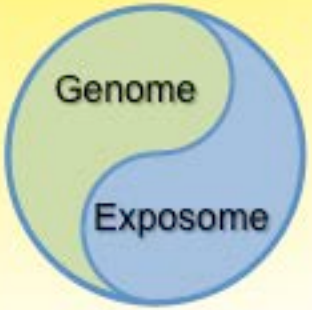
Cost of unnecessary Medicare hospital readmissions ($50B/y) would pay for annual metabolomics analysis for all Americans





Projected cost: $125

1% of annual healthcare costs



Predictive Health & Personalized Medicine

Biomonitoring Component of National Health Surveillance & Forecasting System

<u>High-resolution metabolomics can be used to develop the environmental component of the system</u>:

1. Can identify genomic associations with bioaccumulation

2. Can identify environmental exposures associated with disease

3. Can identify mechanistic associations of exposures and changes in gene expression and epigenomic modifications

4. Can track changes in exposure and bioaccumulation over time in individuals, populations and geographies

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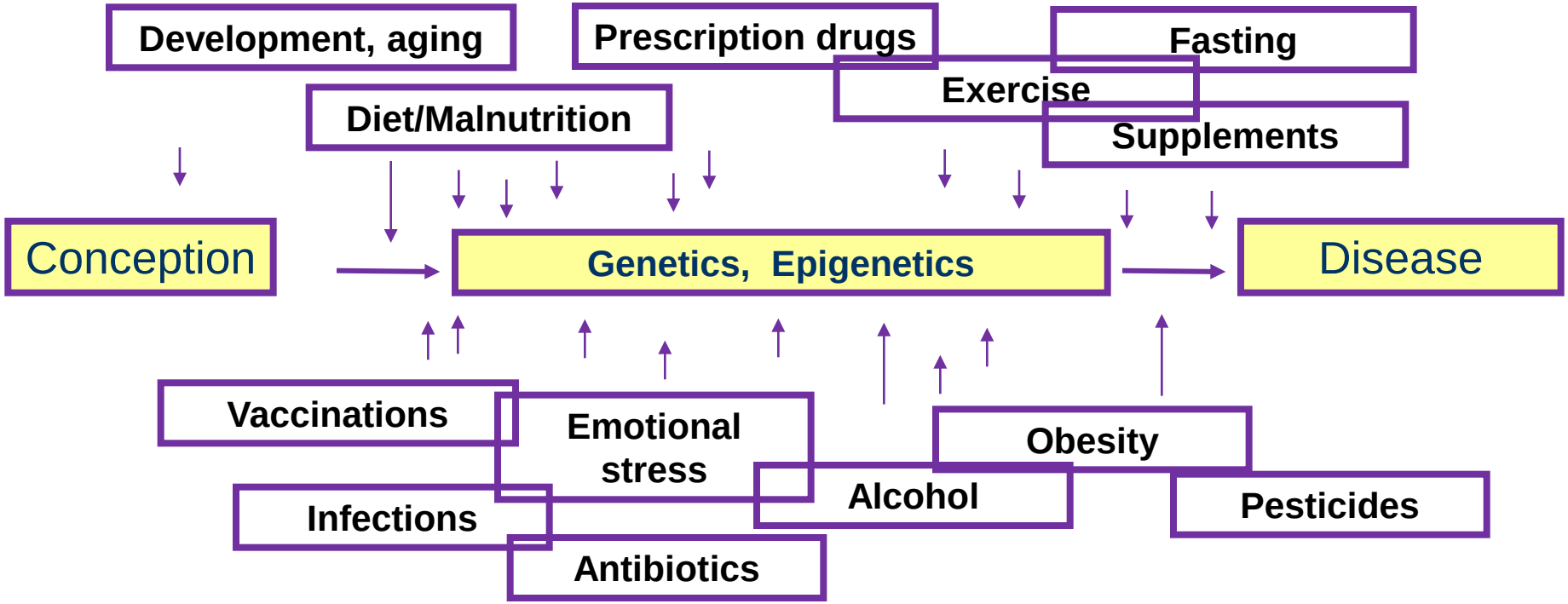


Exposome: the cumulative exposures of ones life

We accumulate chronic health conditions over the same time that we bioaccumulate persistent organic pollutants

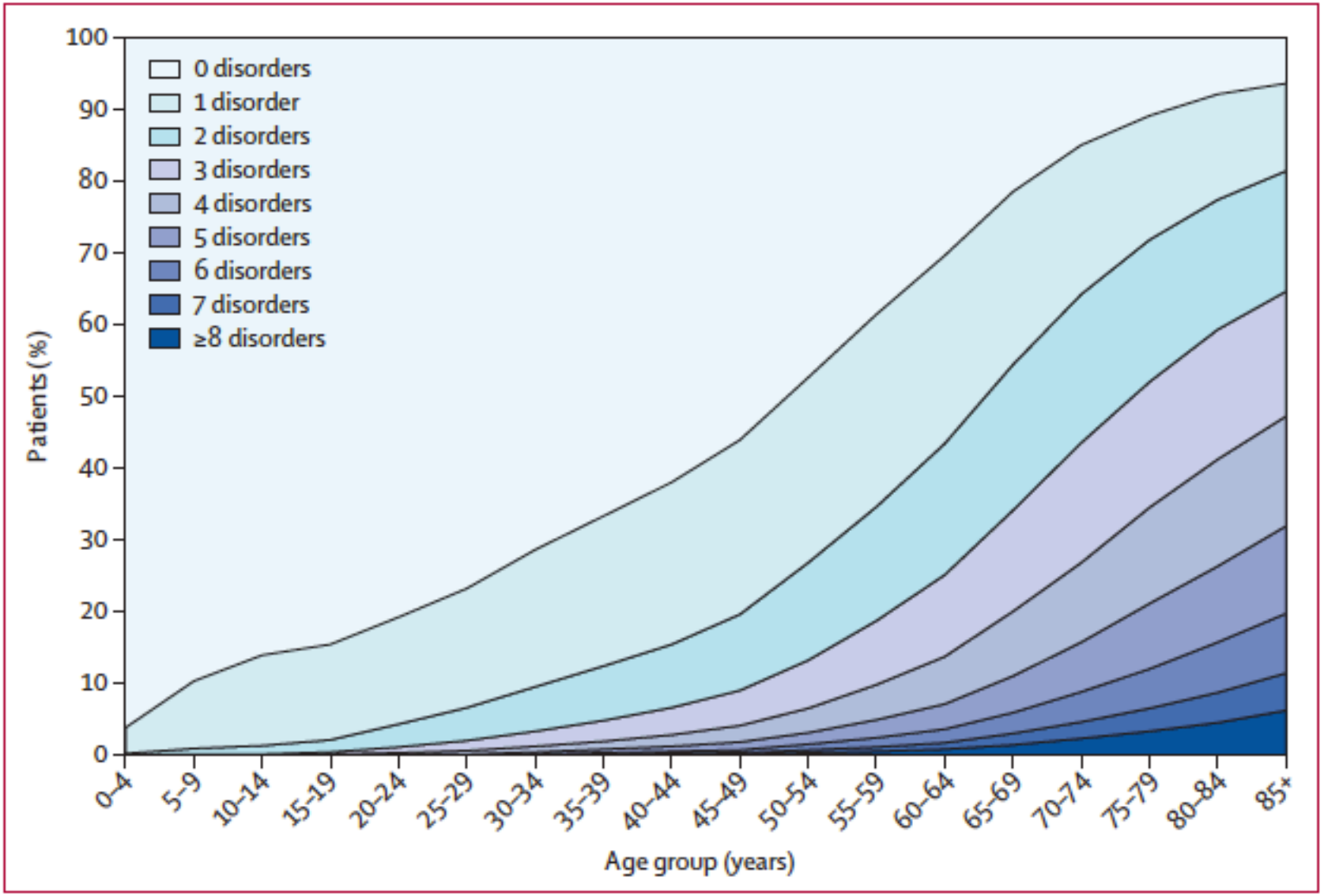
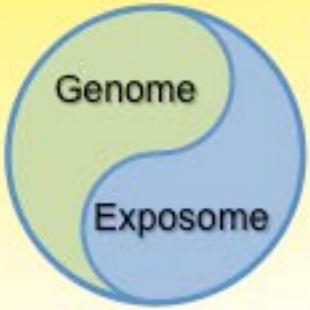
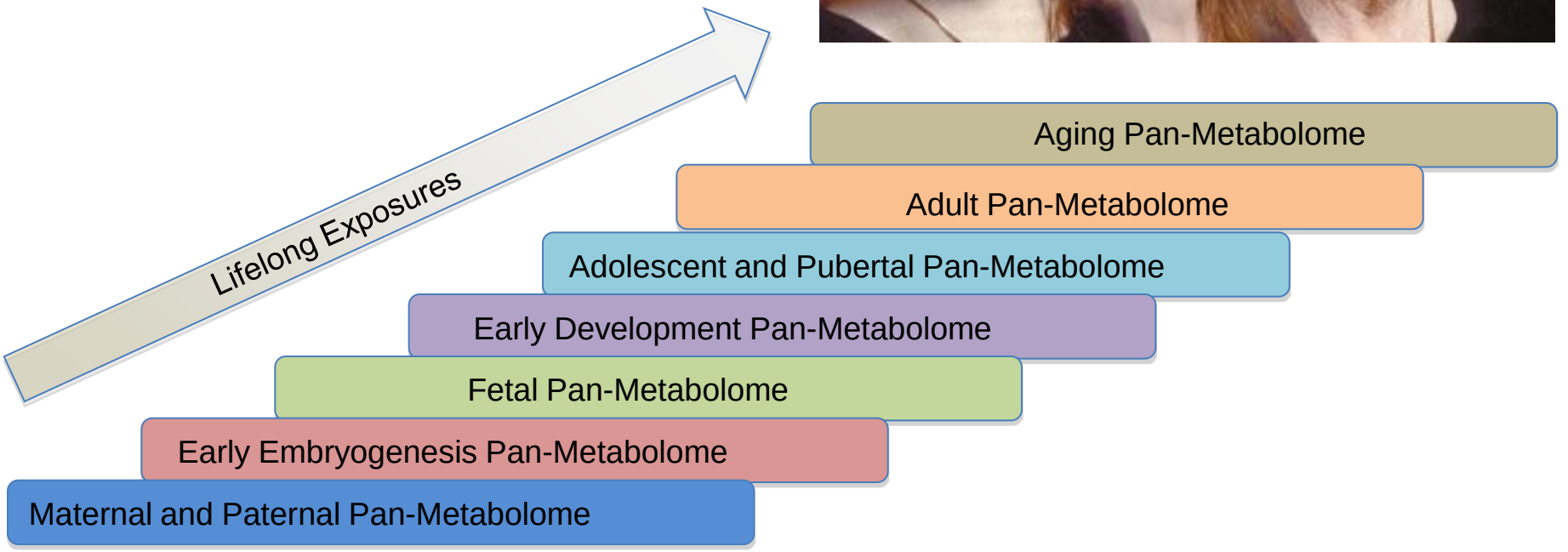


Figure 1: **Number of chronic disorders by age-group**

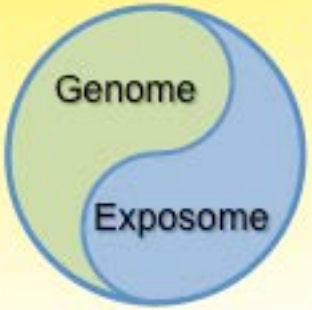
K. Barnett et al Lancet 2012 380:37



Operationalizing the Exposome



Jones et al Annu Rev Nutr 2012



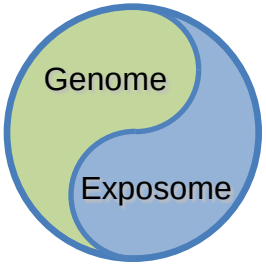
Summary

Universal Environmental Chemical Surveillance

1. High-resolution metabolomics provides an affordable platform for routine human biomonitoring

2. Systematic use on human samples, e.g., according to geography, provides means to measure low level exposures that occur variably among individuals

3. Can be used to detect complex exposures linked to risk in populations (MWAS) and provide mechanistic information (G x M, M x E, M x T)



Emory-Georgia Tech Predictive Health Institute

DOM CLINICAL BIOMARKERS LABORATORY

Acknowledgements: NIEHS, NIA, NCI, NHLBI, NIAAA, NIDDK, Emory-Georgia Tech Center for Health Discovery, Georgia Research Alliance, Emory Sch of Med, Emory Dept Med, Woodruff Research Fund

Mechanistic studies
James Roede
Bill Liang
Michael Orr
Young-Mi Go

Bioinformatics
Tianwei Yu
Shuzhao Li
Daniel Promislow
Brani Vidakovic
Greg Gibson

Metabolomics
Youngja Park
Quinlyn Soltow
Kichen (Sky) Lee
Jennifer Johnson
Karan Uppal

Mass spectrometry
ViLinh Tran
Fred Strobel
Jan Pohl

Clinical
Claudia Testa
Thomas R Ziegler
Ken Brigham
Greg Martin
Wayne Alexander
Milam Brantley
Paul Sternberg, Jr
David Guidot
Arshed Quyyumi
Nancy Kutner
Viola Vaccarino