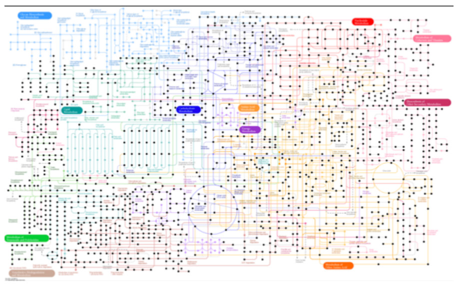
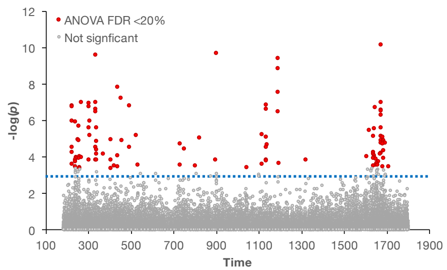
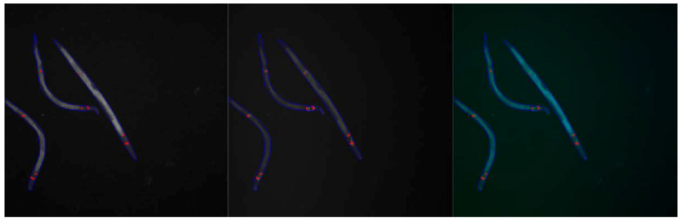
Metabolomics to measure the exposome





Gary W. Miller, Ph.D.
Emory University, Atlanta, GA USA
as of August 1, Columbia University, New York, NY USA



HERCULES
Exposome Research Center

Timeline and Goals

Tuesday:
Metabolomics as a critical tool for assessing the exposome
Examples of the types of data generated
Setting up metabolomics in *C. elegans*

Wednesday:
Practicum: initial metabolomics exploration using MetaboAnalyst
Challenges and new tools for metabolomics data
Practicum: deep dive into metabolomics analysis using R, Python, mummichog, Cytoscape, xMWAS

The Exposome: a Wild idea

Editorial

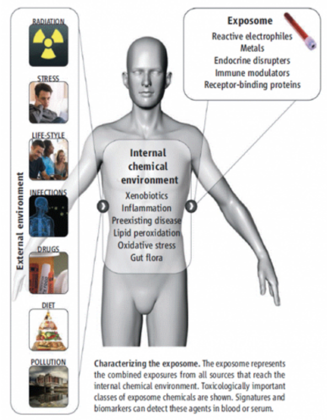
Complementing the Genome with an "Exposome": The Outstanding Challenge of Environmental Exposure Measurement in Molecular Epidemiology

Christopher Paul Wild

Molecular Epidemiology Unit, Centre for Epidemiology and Biostatistics, Leeds Institute of Genetics, Health and Therapeutics, Faculty of Medicine and Health, University of Leeds, Leeds, United Kingdom

Defined the "*Exposome*" as all exposures from conception onwards, including those from lifestyle, diet and the environment.

Rappaport and Smith

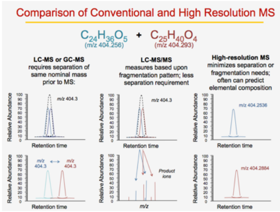


The Nature of Nurture: Refining the Definition of the Exposome

Gary W. Miller[1] and Dean P. Jones

Rollins School of Public Health, School of Medicine, Emory University, Atlanta, Georgia 30322

Exposome: The cumulative measure of environmental influences and associated biological responses throughout the lifespan, including exposures from the environment, diet, behavior, and endogenous processes



HERCULES
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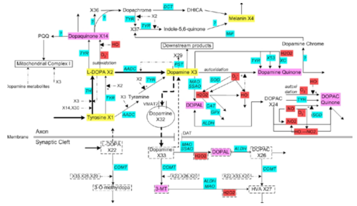
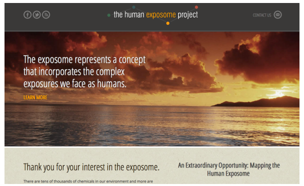
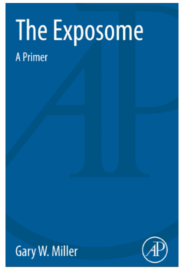
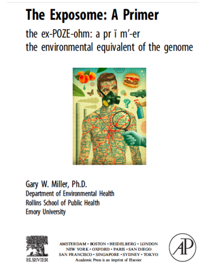


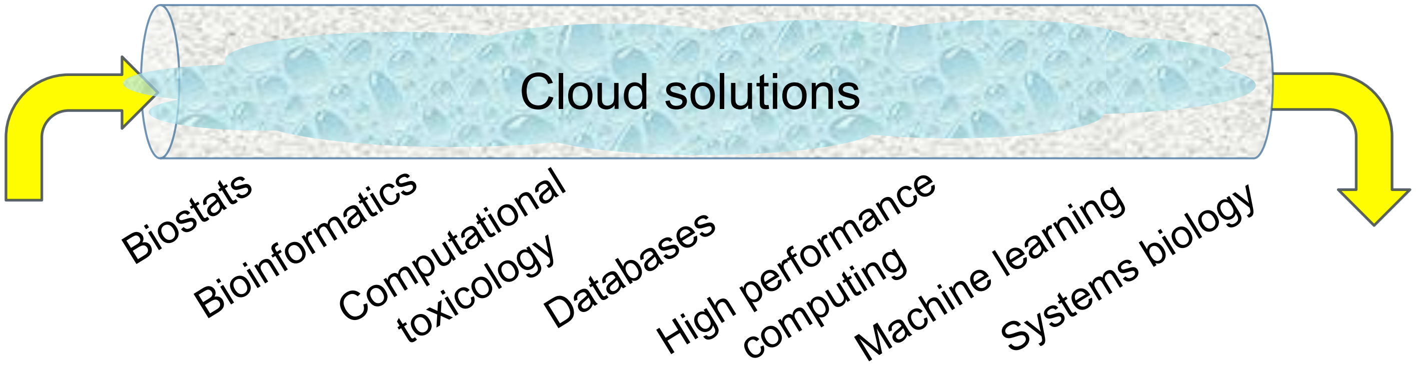
Awarded in 2013 (NIH P30-ES019776), Renewed for 5 years in 2017



administration (Miller, Tolbert)
analytical chemistry-targeted (Barr, Ryan)
metabolomics/exposomics-untargeted (Jones, Li)
pilot awards (Morgan) and patient studies (Ziegler, Marsit)
community engagement (Kegler/Pearson)
data sciences (Waller, Voit, Clifford, Qin, Li, Qiang, and Kemp)





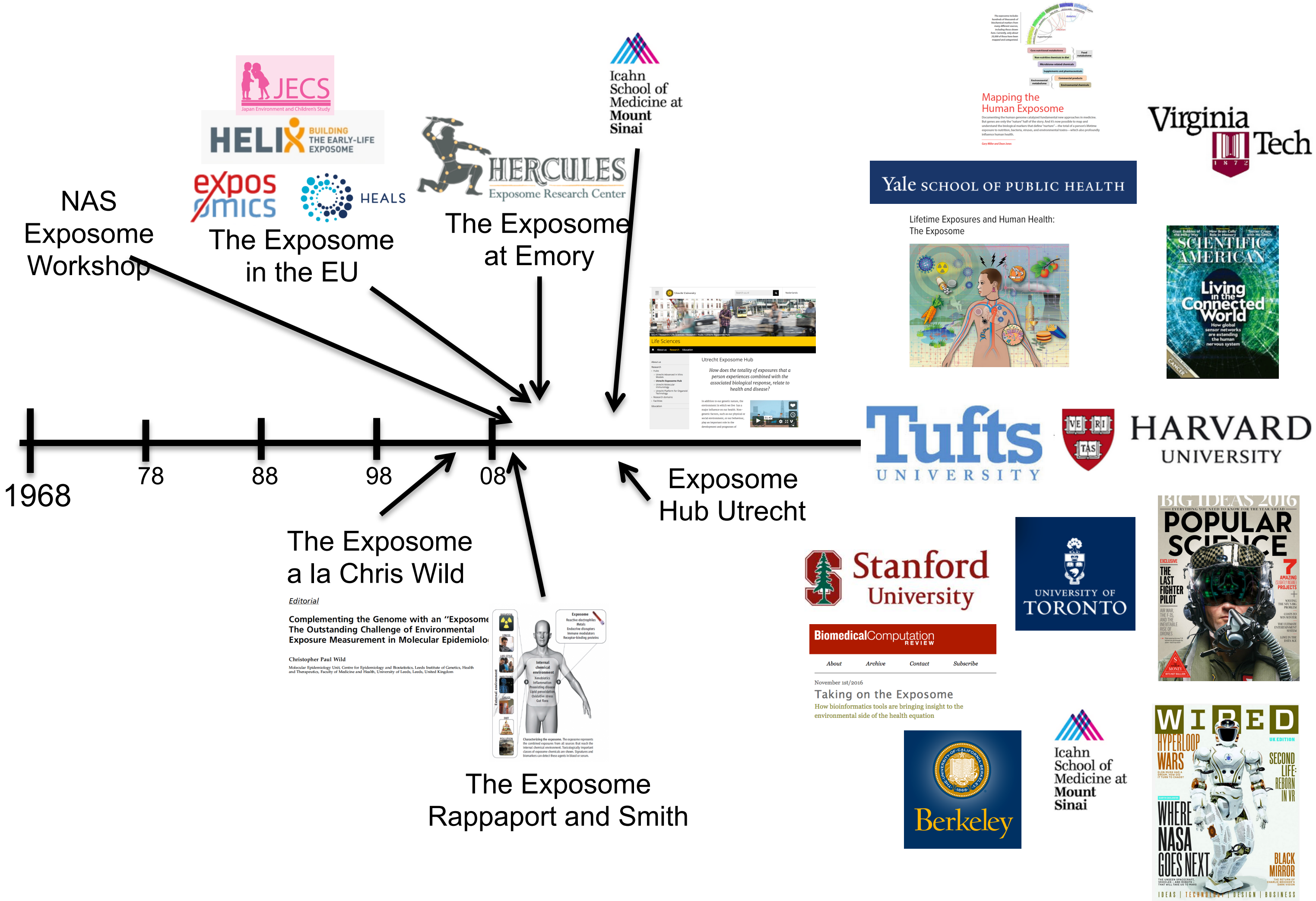


Data science and the exposome

- Data science expertise will be the bottleneck of exposome research

- There is great demand for this expertise and exposome-related research must compete with other biomedical areas, Google, Facebook, etc.

- One of the greatest contributions you can make to the field is to become *proficient in teaching* data analysis to other investigators, reserving your own time for complex problems



NAS Exposome Workshop
The Exposome in the EU
JECS
HELIX BUILDING THE EARLY-LIFE EXPOSOME
exposomics
HEALS
HERCULES Exposome Research Center
The Exposome at Emory
Icahn School of Medicine at Mount Sinai
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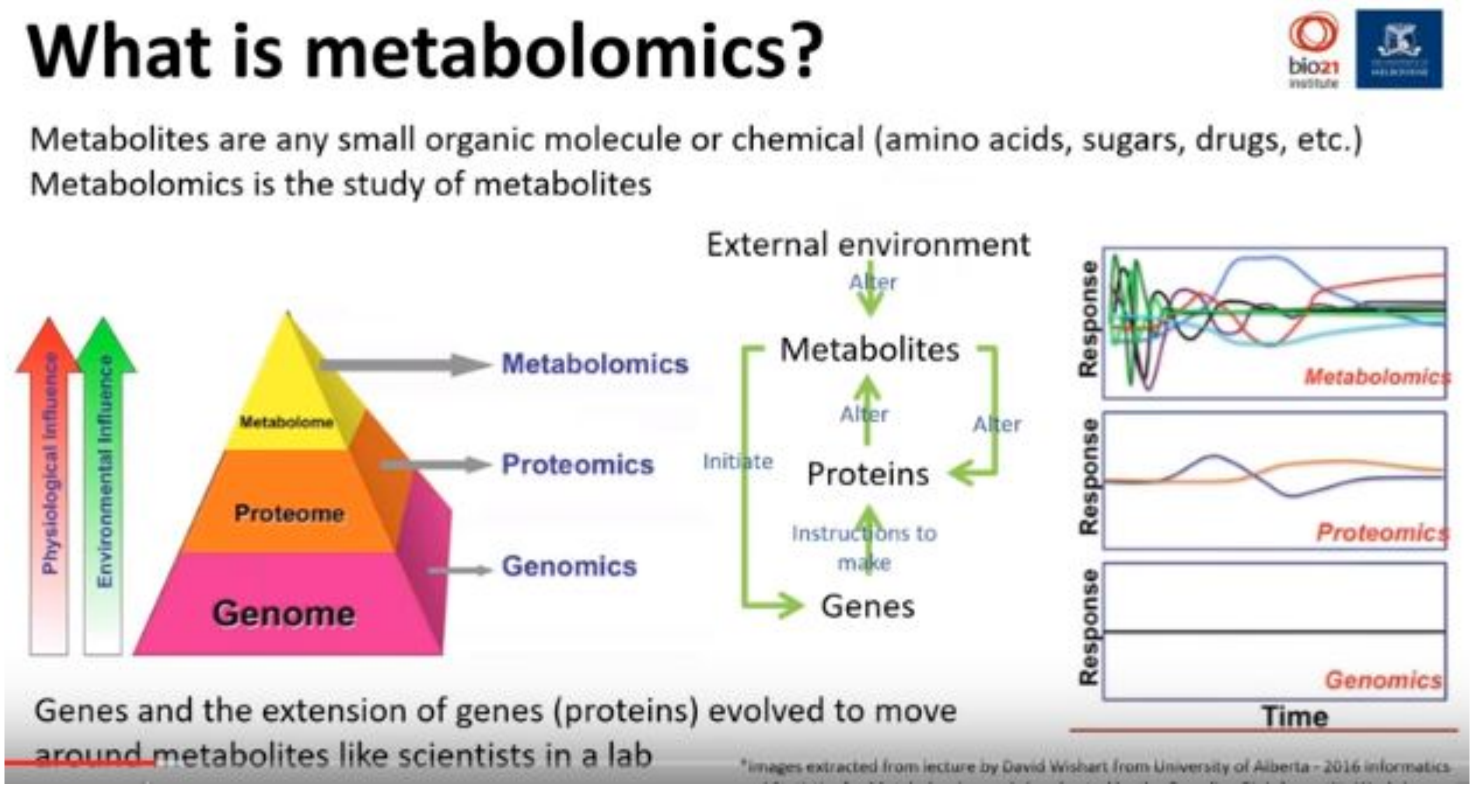
The Exposome a la Chris Wild
Editorial
Complementing the Genome with an "Exposome
The Outstanding Challenge of Environmental
Exposure Measurement in Molecular Epidemiolo
Christopher Paul Wild
The Exposome Rappaport and Smith
Exposome Hub Utrecht
Utrecht Exposome Hub
Mapping the Human Exposome
Yale SCHOOL OF PUBLIC HEALTH
Lifetime Exposures and Human Health: The Exposome
Virginia Tech
SCIENTIFIC AMERICAN
Living in the Connected World
Tufts UNIVERSITY
HARVARD UNIVERSITY
Stanford University
UNIVERSITY OF TORONTO
POPULAR SCIENCE
BIG IDEAS 2016
BiomedicalComputation REVIEW
Taking on the Exposome
Berkeley
Icahn School of Medicine at Mount Sinai
WIRED
WHERE NASA GOES NEXT

Metabolomics
Background and Applications
Day 1

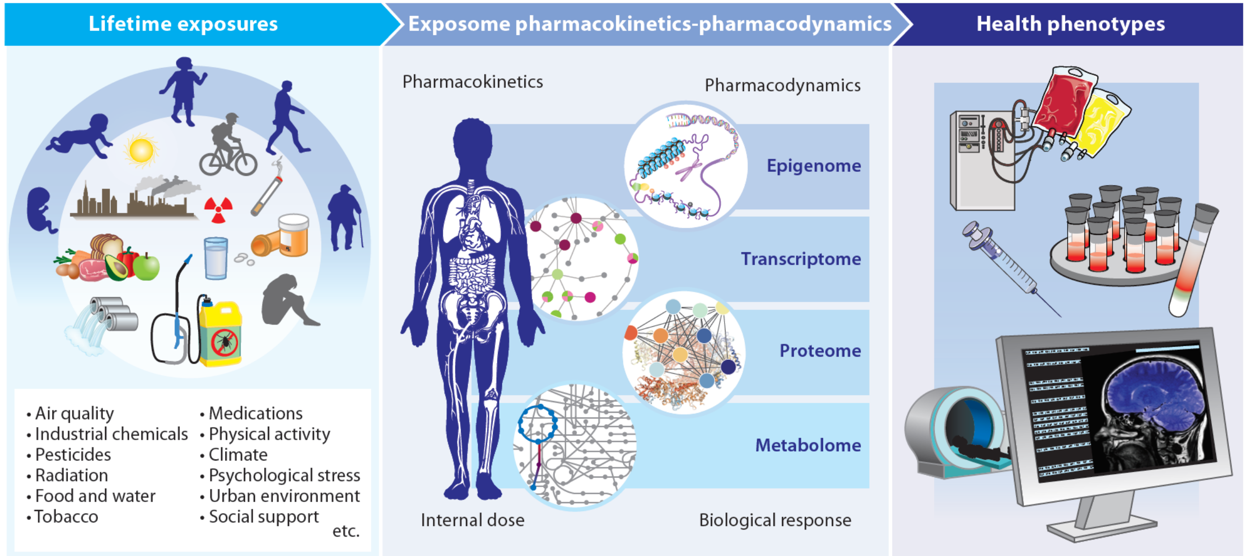
EMORY UNIVERSITY

HERCULES Exposome Research Center

Metabolomics

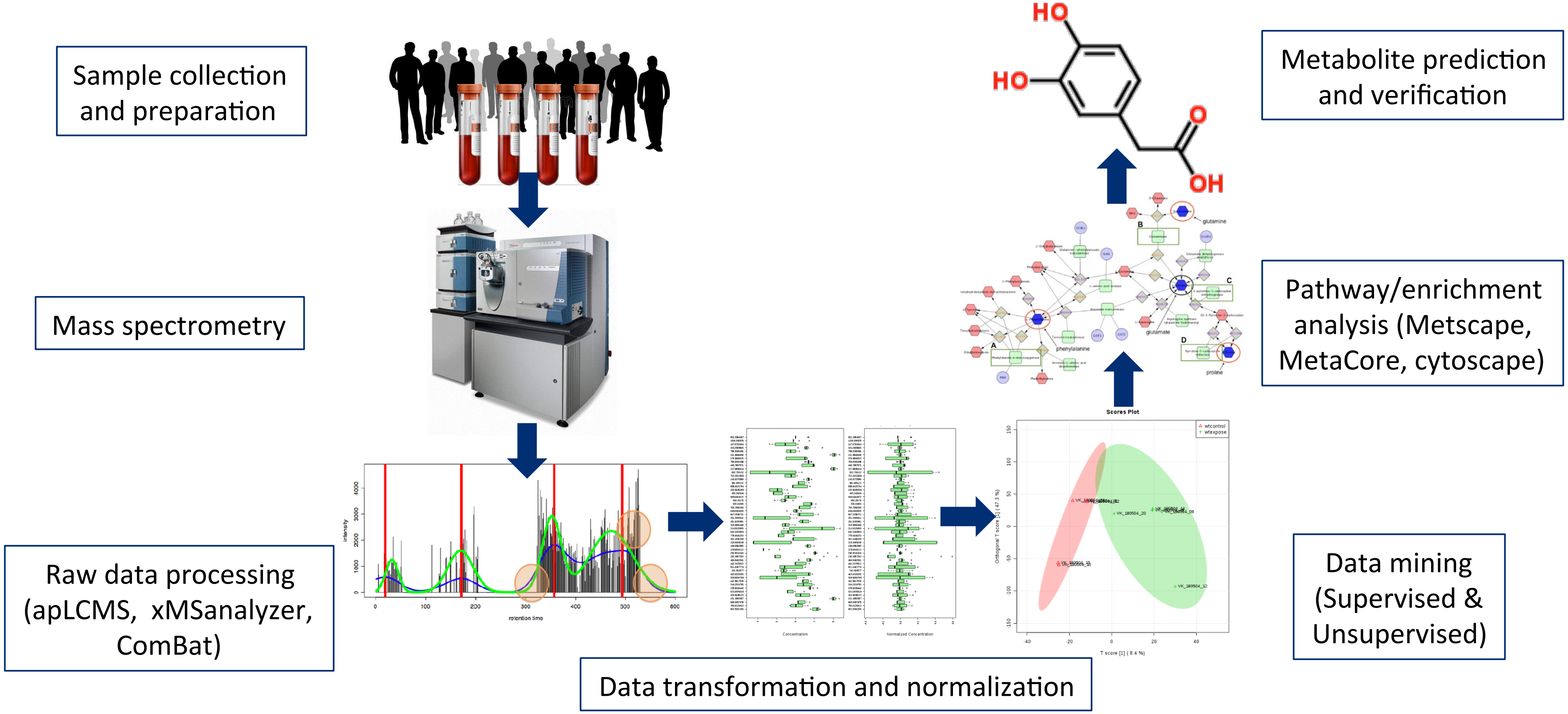


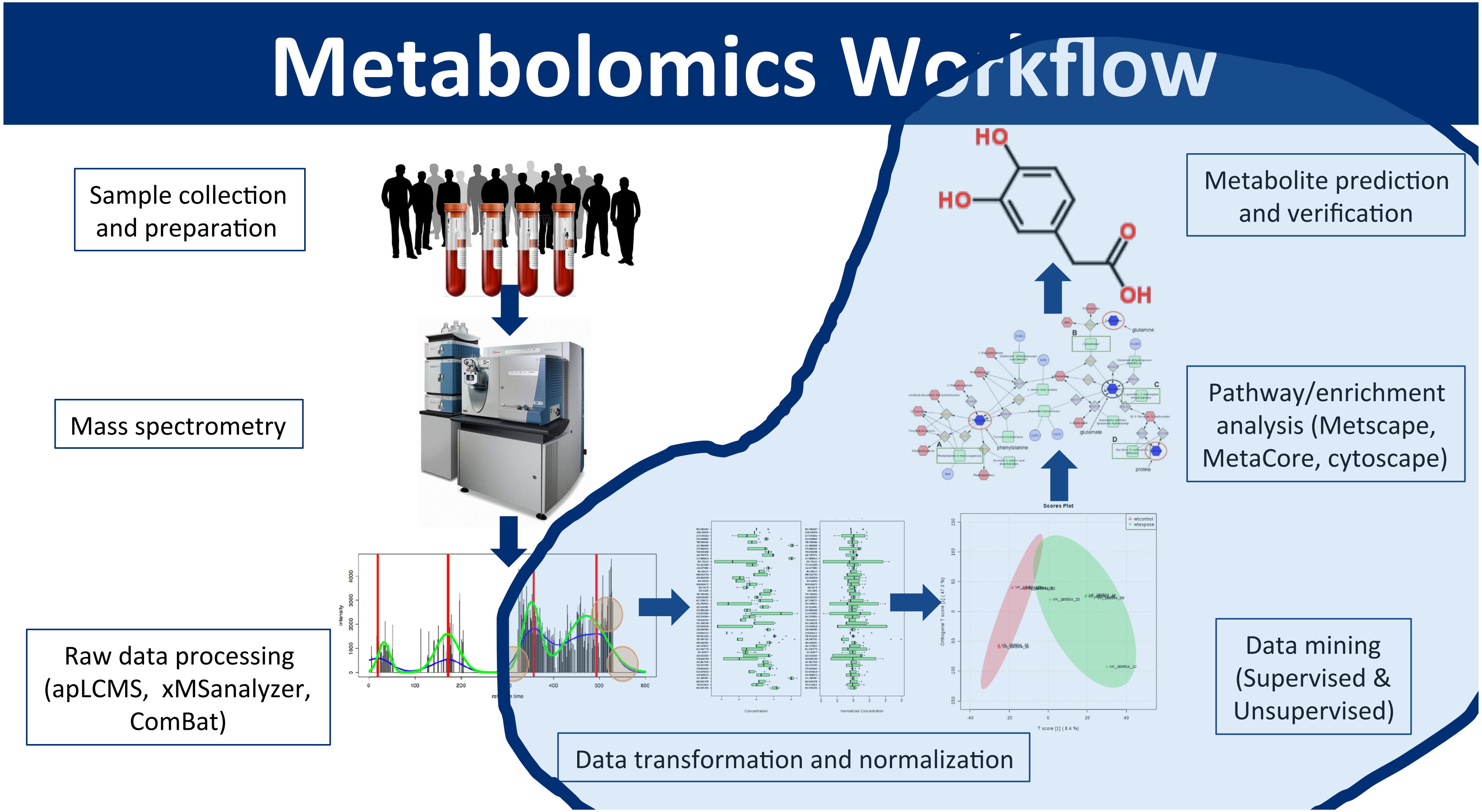
The Exposome: Molecules to Populations



Annual Reviews of Pharmacology and Toxicology. 2019. 59:X–X
https://doi.org/10.1146/annurev-pharmtox-010818-021315
MM Niedzwiecki, DI Walker, R Vermeulen, M Chadeau-Hyam, DP Jones, and GW Miller

Metabolomics Workflow





Metabolomics Workflow
Sample collection and preparation
Mass spectrometry
Raw data processing (apLCMS, xMSanalyzer, ComBat)
Data transformation and normalization
Data mining (Supervised & Unsupervised)
Pathway/enrichment analysis (Metscape, MetaCore, cytoscape)
Metabolite prediction and verification
HO
HO
O
OH

Samples, MS, and raw data

Sample prep:

-65 uL of biological sample + 130 uL acetonitrile

-pellet out proteins

-samples are run in triplicate

-2 pooled quality control standards

-randomized groups within batches

-prep depends on gas or liquid chromatography, high or low resolution

Samples, MS, and raw data

Use of dual analytical configurations increases detection of chemical space. There are multiple permutations possible:

Within chromatography, three common stationary phases:

1.) Hydrophilic interaction chromatography (dual anion/cation)

2.) Ion exchange (exclusively anion OR cation)

3.) Reverse phase (most common)

In terms of ionization at the MS interface, electrospray ionization (ESI) is most common:

-ionizes compounds with acidic or basic functional groups

-positive ionization mode: forms cation adducts with molecule, M (i.e. $[M+H]^+$, $[M+Na]^+$)

-negative ionization mode: forms anion adducts with molecule, M (i.e. $[M+Cl]^-$, $[M-H]^-$)

Samples, MS, and raw data

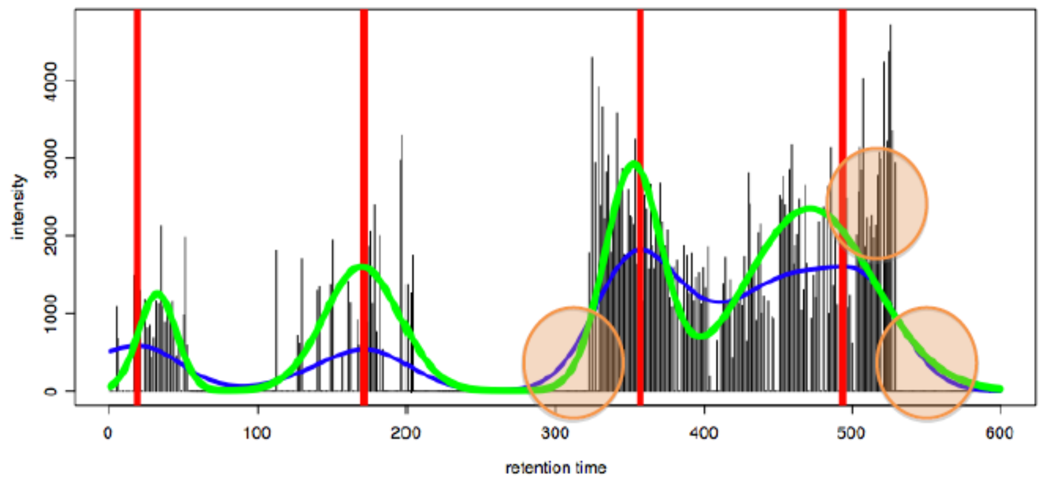
Working with raw data:

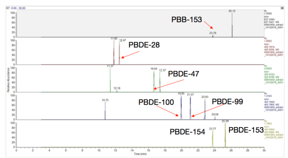
-optimizes fitting parameters for peak integration

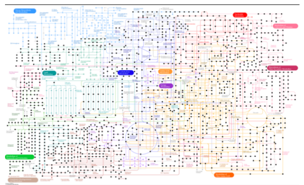
-filters noise

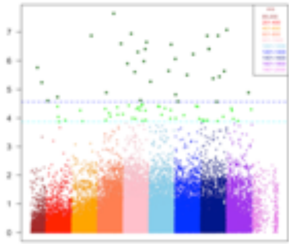
-aligns retention time and mass across all samples

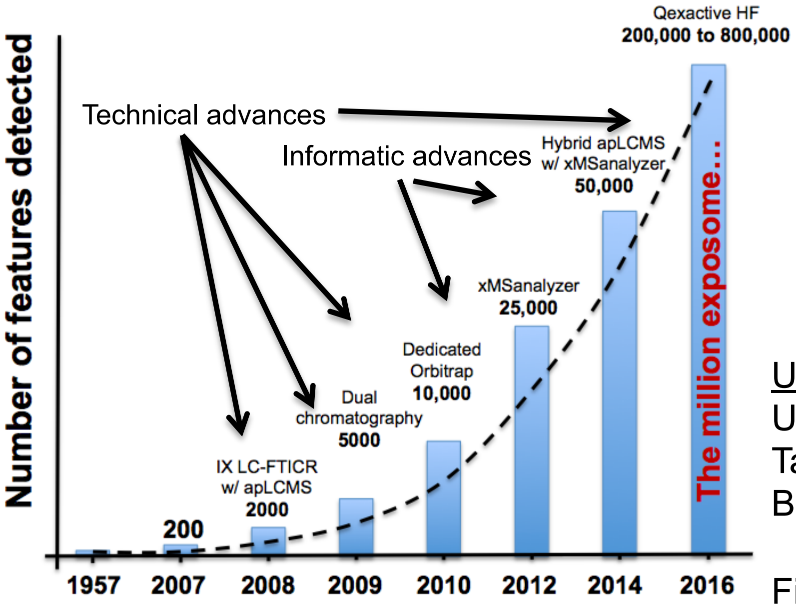
-we use apLCMS (Tianwei Yu) and xMSanalyzer (Karan Uppal) for these steps











<u>Ultra-high resolution metabolomics</u>
Untargeted metabolites
Targeted environment chemicals
Biological pathway response

Figures from Doug Walker

High-resolution **metabolomics**: Measure up to 200,000 ions in plasma by LC with high-resolution MS

ESI LC-MS

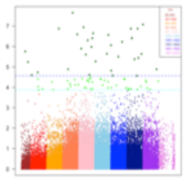
Ultra-high resolution MS
LTQ-FT
LTQ-Velos Orbitrap
Q-Exactive HF
Thermo Fusion
Agilent 6560

Data extraction

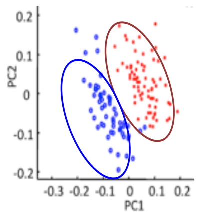
Data Table
m/z, RT, Intensity
Quality metrics

Biostatistics
Bioinformatics

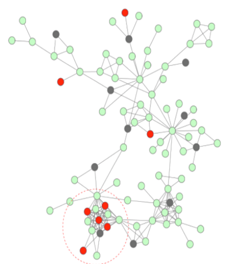
MWAS



PLS-DA



Metabolites of interest



Network and pathway analysis*: Mummichog*, MetabNet, others

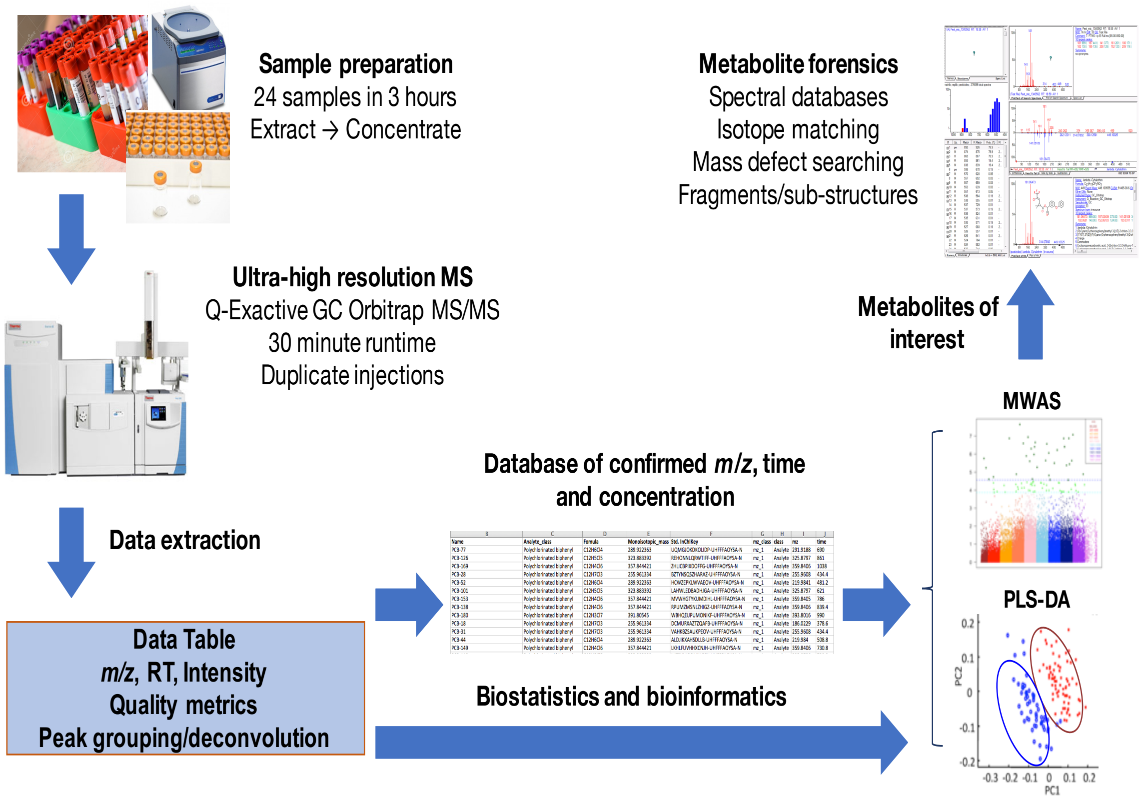
Metabolite forensics

Unequivocal Identification
MS^n; UHRAM MS; 1-D, 2-D NMR; ion Mobility; Chemical Reactivity, etc

T Yu et al 2009 Bioinformatics 25:1930-6
JM Johnson et al 2010 Analyst 135: 2864-2870
Q Soltow et al 2013 Metabolomics 9:S132–S143
T Yu et al 2013 J Proteome Res 12:1419-27
S Li et al 2013 Plos Comp Biol 9:e1003123
K Uppal et al 2013 BMC Bioinformatics 14:15
K Uppal et al 2015 Frontiers Bioeng Biotech 3:87

DP Jones 2016 *Tox Reports* **3**: 29-45

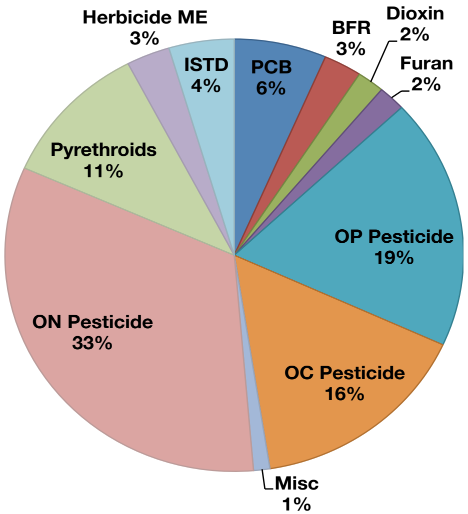
High-resolution **exposomics**: A platform for environmental chemical surveillance

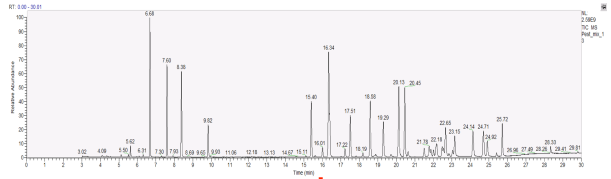


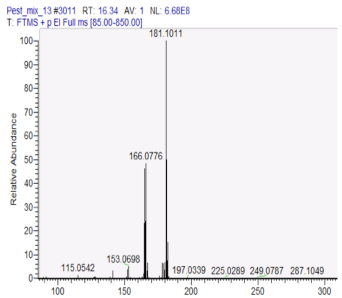
Walker, Li, Uppal, Accardi, Pennell, Miller, Jones *In preparation*

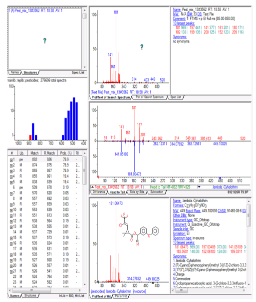
Developing a chemical database for high-resolution exposomics

- 16 standard EPA ToxCast mixtures analyzed to date
- Over 300 analytes confirmed with 4 *m/z* and time

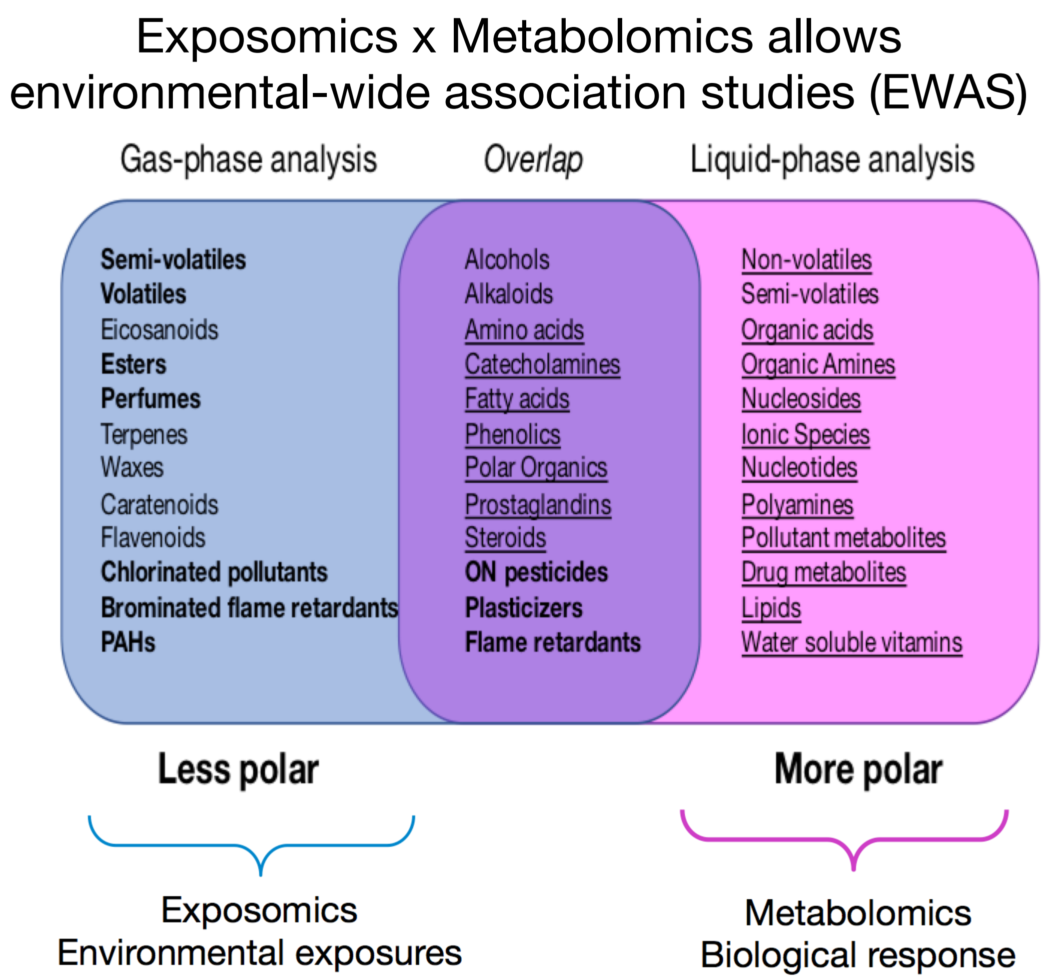








Mix number	Name	Chemical ID	Fomula	Monoisotopic_mass	Std. InChIKey	mz_1	mz_2	mz_3	mz_4	time	mz_1-mz_2	mz_1-mz_3	mz_1-mz_4
1	PCB-77	3,3',4,4'-Tetrachlorobiphenyl	C12H6Cl4	289.922363	UQMGJOKDKOLIDP-UHFFFAOYSA-N	291.9188	289.9218	219.9841	293.9158	11.5	78.18	57.06	47.59
1	PCB-126	3,3',4,4',5-Pentachlorobiphenyl	C12H5Cl5	323.883392	REHONNLQRWTIFF-UHFFFAOYSA-N	325.8797	323.8826	327.8767	253.945	14.35	64.53	63.88	47.31
1	PCB-169	3,3',4,4',5,5'-Hexachlorobiphenyl	C12H4Cl6	357.844421	ZHLICBPIXDOFFG-UHFFFAOYSA-N	359.8406	361.8376	357.8437	289.9032	17.3	77.34	52.71	49.25
2	PCB-28	2,4,4'-Trichlorobiphenyl	C12H7Cl3	255.961334	BZTYNSQSZHARAZ-UHFFFAOYSA-N	255.9608	257.9578	186.023	259.9549	7.24	94.62	74.52	30.05
2	PCB-52	2,2',5,5'-Tetrachlorobiphenyl	C12H6Cl4	289.922363	HCWZEPKLWVAEOV-UHFFFAOYSA-N	219.9841	291.9189	289.9219	221.9811	8.02	87.34	68.78	64.6
2	PCB-101	2,2',4,5,5'-Pentachlorobiphenyl	C12H5Cl5	323.883392	LAHWLEDBADHJGA-UHFFFAOYSA-N	325.8797	253.9451	255.9421	327.8767	10.35	77.66	75.47	64.3
2	PCB-153	2,2',4,4',5,5'-Hexachlorobiphenyl	C12H4Cl6	357.844421	MVWHGTYKUMDIHL-UHFFFAOYSA-N	359.8405	361.8375	289.9032	287.9061	13.1	77.19	73.83	57.65
2	PCB-138	2,2',3,4,4',5'-Hexachlorobiphenyl	C12H4Cl6	357.844421	RPUMZMSNLZHIGZ-UHFFFAOYSA-N	359.8406	289.9032	361.8376	287.9061	13.99	82.88	77.83	64.29
2	PCB-180	2,2',3,4,4',5,5'-Heptachlorobiphen	C12H3Cl7	391.80545	WBHQEUPUMONIKF-UHFFFAOYSA-N	393.8016	395.7985	323.864	325.8611	16.5	94.37	89.18	57.89
4	PCB-18	2,2',5-Trichlorobiphenyl	C12H7Cl3	255.961334	DCMURXAZTZQAFB-UHFFFAOYSA-N	186.0229	255.9606	257.9576	220.9918	6.31	68.63	65.8	50.96
4	PCB-31	2,4',5-Trichlorobiphenyl	C12H7Cl3	255.961334	VAHKBZSAUKPEOV-UHFFFAOYSA-N	255.9608	257.9578	186.023	259.9549	7.24	94.62	74.52	30.05



Exposomics x Metabolomics allows
environmental-wide association studies (EWAS)
Gas-phase analysis
Overlap
Liquid-phase analysis
Semi-volatiles
Volatiles
Eicosanoids
Esters
Perfumes
Terpenes
Waxes
Caratenoids
Flavenoids
Chlorinated pollutants
Brominated flame retardants
PAHs
Alcohols
Alkaloids
Amino acids
Catecholamines
Fatty acids
Phenolics
Polar Organics
Prostaglandins
Steroids
ON pesticides
Plasticizers
Flame retardants
Non-volatiles
Semi-volatiles
Organic acids
Organic Amines
Nucleosides
Ionic Species
Nucleotides
Polyamines
Pollutant metabolites
Drug metabolites
Lipids
Water soluble vitamins
Less polar
More polar
Exposomics
Environmental exposures
Metabolomics
Biological response

Today's unknown features… are tomorrow's discoveries

analytical chemistry

960,000 compounds

METLIN: A Technology Platform for Identifying Knowns and Unknowns

Carlos Guijas[†], J. Rafael Montenegro-Burke[†], Xavier Domingo-Almenara[†], Amelia Palermo[†], Benedikt Warth[†‡], Gerrit Hermann[§∥], Gunda Koellensperger[§], Tao Huan[†], Winnie Uritboonthai[†], Aries E. Aisporna[†], Dennis W. Wolan[⊥], Mary E. Spilker[†], H. Paul Benton[*†], and Gary Siuzdak[*†#]

Exposome-Scale Investigations Guided by Global Metabolomics, Pathway Analysis, and Cognitive Computing

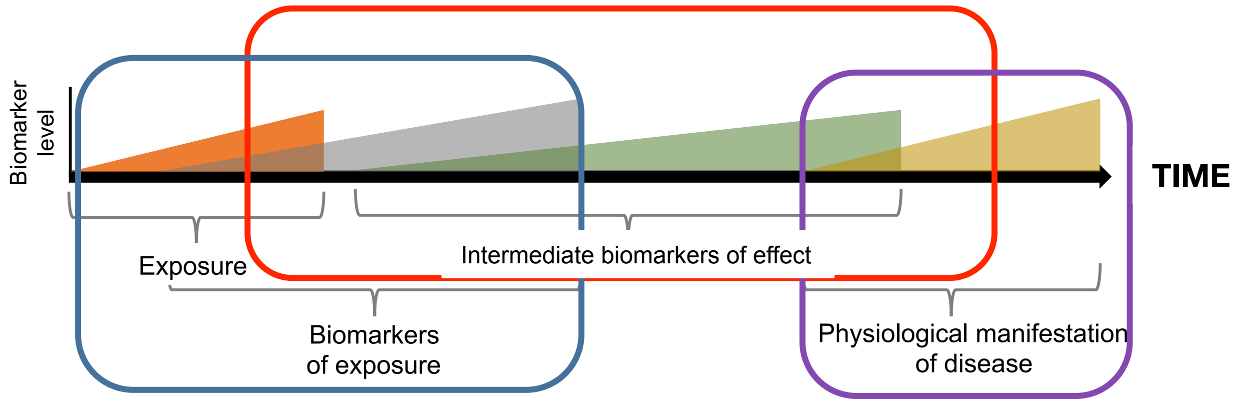
Benedikt Warth[*†§], Scott Spangler[∥], Mingliang Fang[†⊥], Caroline H. Johnson[#], Erica M. Forsberg[†], Ana Granados[†], Richard L. Martin[∥], Xavier Domingo-Almenara[†], Tao Huan[†], Duane Rinehart[†], J. Rafael Montenegro-Burke[†], Brian Hilmers[†], Aries Aisporna[†], Linh T. Hoang[†], Winnie Uritboonthai[†], H. Paul Benton[†], Susan D. Richardson[∇], Antony J. Williams[○], and Gary Siuzdak[*†‡]

mzCloud is capable of handling multiple databases containing various types of spectral trees managed by various users. We are continuously adding new records to **mzCloud** and extending the number of database types. Currently, five types of databases are available:

- Reference database – a database of spectral trees of reference compounds of the highest purity
- Putative database – a database of spectral trees with putative or elucidated structures
- Unknown database – a database of unknown compounds that may be of biological, environmental or forensic interest. In the future, you will be able to subscribe to this compound and once its identity is revealed, you will be notified.

"In the future, you will be able to subscribe to this compound and once its identify is revealed you will be notified."

Untargeted exposure analysis
metabolomics/exposomics

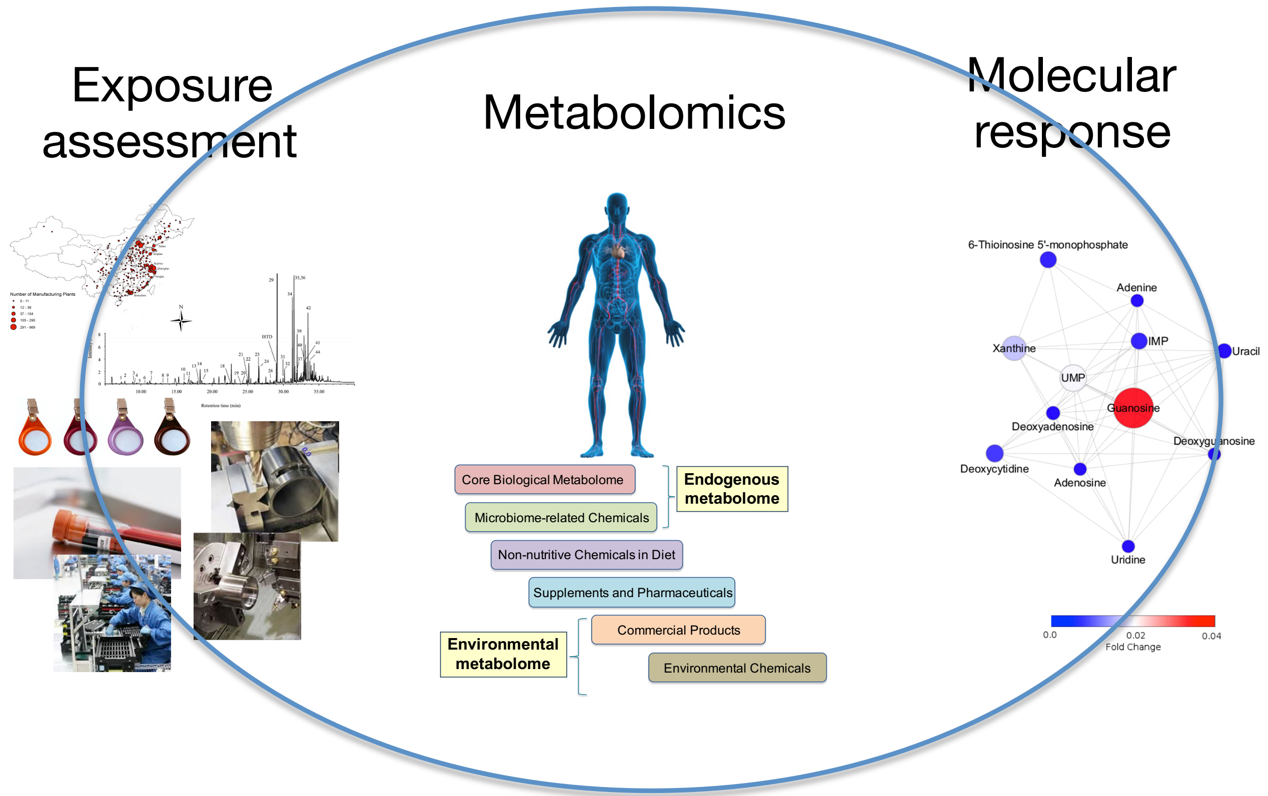


Targeted exposure analysis

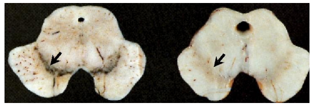
Clinical outcomes
disease processes

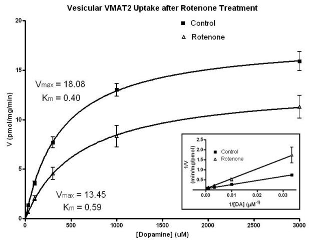
Walker, Jones

High-resolution metabolomics/exposomics is the -omic scale technique to assess environmental contributors to health and disease

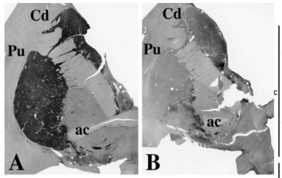


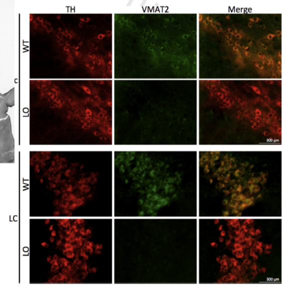
Miller, Walker, Jones

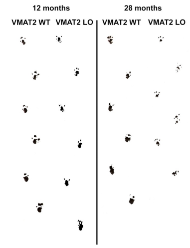


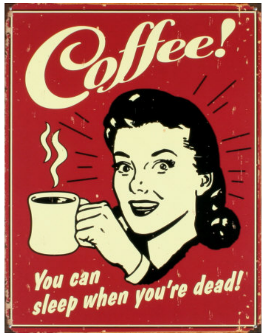


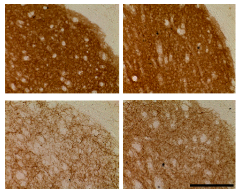
age ↑
pesticides ↑
genes ↑
head trauma ↑
smoking ↓
caffeine ↓
exercise ↓

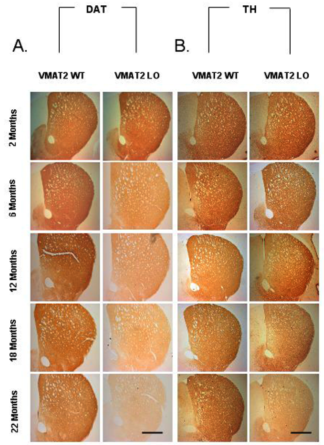


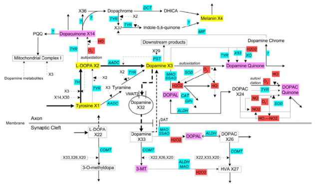


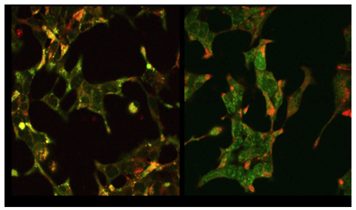


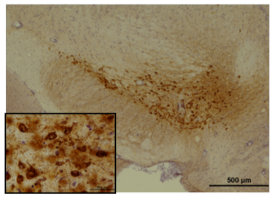


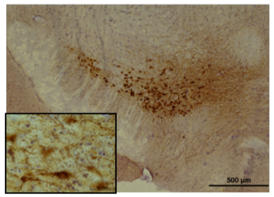








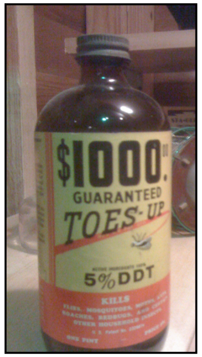




PESTICIDES

RODENTICIDES

- Zinc Phosphide
- Fluoroacetate Derivatives
- α-naphthyl thiourea
- Anticoagulants



HERBICIDES

Bipyridyls
- *Paraquat*
  Diquat

Chloroacetanilides
- *Alachlor*

Chlorophenoxy Compounds
- *2,4-dichlorophenoxyacetate*

Phosphomethyl amino acids
- *Glyphosate*

FUNGICIDES

Hexachlorobenze

Pentachlorophenol

Phthalamides
- *Captan, Folpet*

Dithiocarbamates
- *Maneb, Ziram*

FUMIGANTS

Phophine

Ethylene dibromide/
dibromochloropropane

INSECTICIDES

Organochlorines
- Cylclodienes
  -Dieldrin, Heptachlor
- Dichlorodiphenylethanes
  -DDT, methoxychlor
- Cyclohexanes
  - *Lindane, β-HCH*

Anticholinesterases
- Organophosphates
  - *Parathion, Chlorpyrifos*
- Carbamates
  - Aldicarb, Methomyl

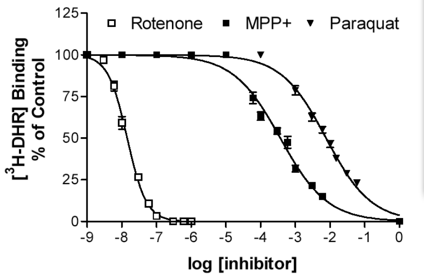
Pyrethroids
- Type I
  - *Phenothrin*
- Type II
  - *Cypermethrin, Deltamethrin*

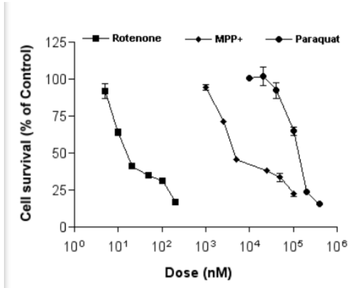
Avermectins
- *Ivermectin*

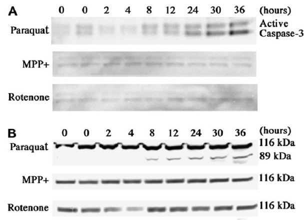
Botanicals
- Nicotine
- Rotenoids
  - *Rotenone, Deguelin*

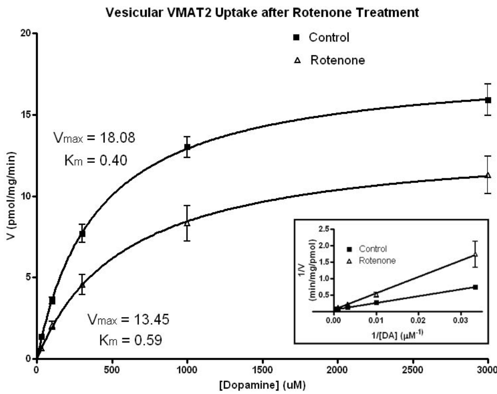
Other
- Nitromethylene
- Chlor-onicotinyl
- Phenylpyrazole

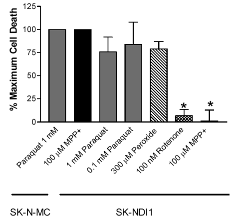
Paraquat, rotenone, and MPTP are different

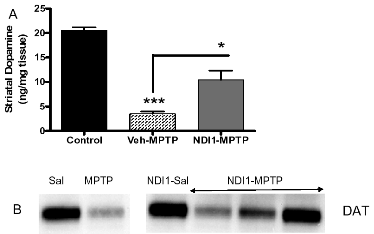


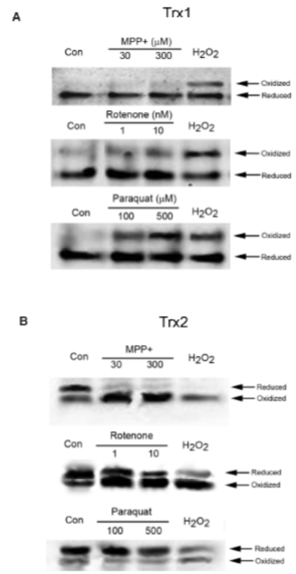










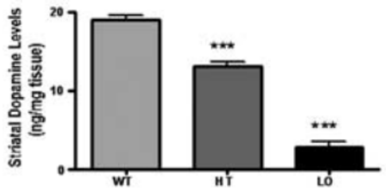


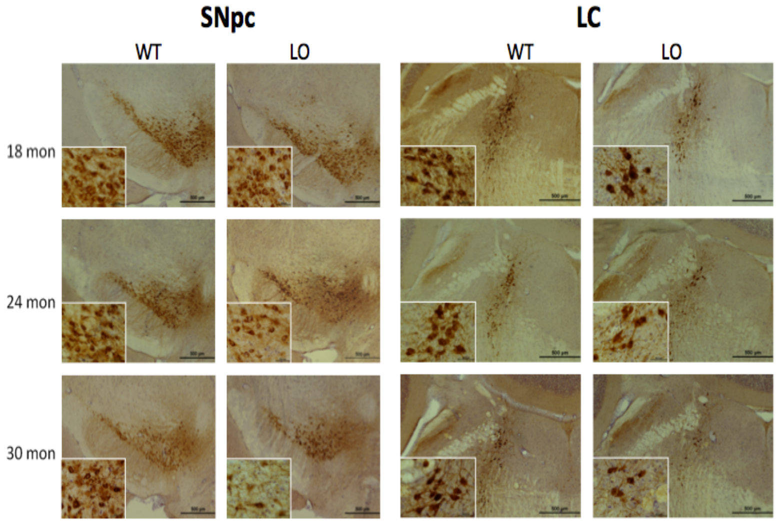
8138 • The Journal of Neuroscience, July 25, 2007 • 27(30):8138 – 8148

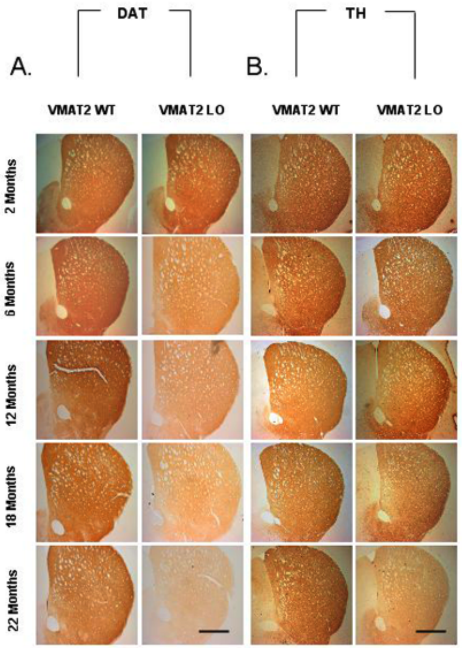
Neurobiology of Disease

Reduced Vesicular Storage of Dopamine Causes Progressive Nigrostriatal Neurodegeneration

W. Michael Caudle,[1,2] Jason R. Richardson,[1,2,3] Min Z. Wang,[1,2] Tonya N. Taylor,[1,2] Thomas S. Guillot,[1,2] Alison L. McCormack,[4] Rebecca E. Colebrooke,[5] Donato A. Di Monte,[4] Piers C. Emson,[5] and Gary W. Miller[1,2]





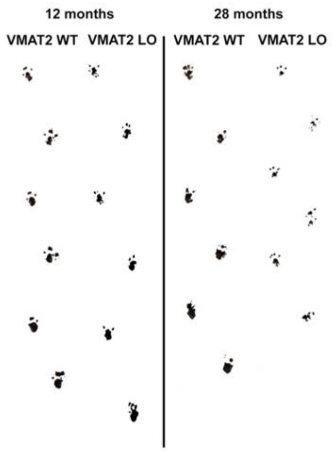


The Journal of Neuroscience, June 24, 2009 • 29(25):8103–8113 • 8103

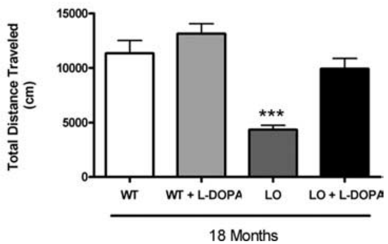
Behavioral/Systems/Cognitive

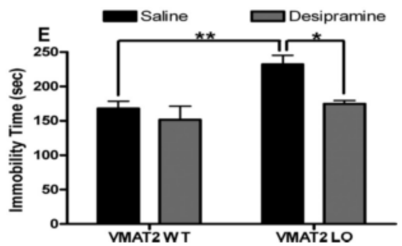
Nonmotor Symptoms of Parkinson's Disease Revealed in an Animal Model with Reduced Monoamine Storage Capacity

Tonya N. Taylor,[1,2] W. Michael Caudle,[7] Kennie R. Shepherd,[1,2] AliReza Noorian,[1,5] Chad R. Jackson,[4] P. Michael Iuvone,[4,5] David Weinshenker,[6] James G. Greene,[1,3,4] and Gary W. Miller[1,2,3,4]



olfactory discrimination deficit
decreased gastric motility
depressive symptoms
sleep disturbances

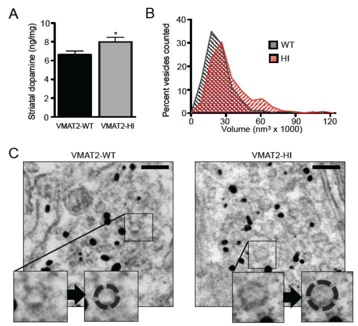


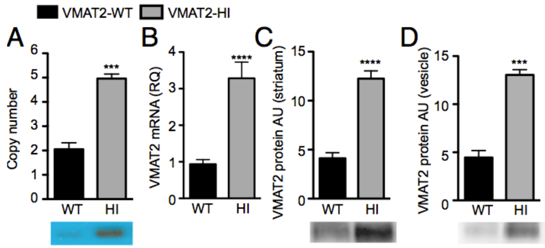


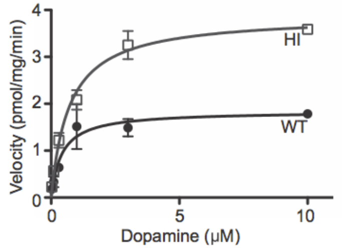
Increased vesicular monoamine transporter enhances dopamine release and opposes Parkinson disease-related neurodegeneration in vivo

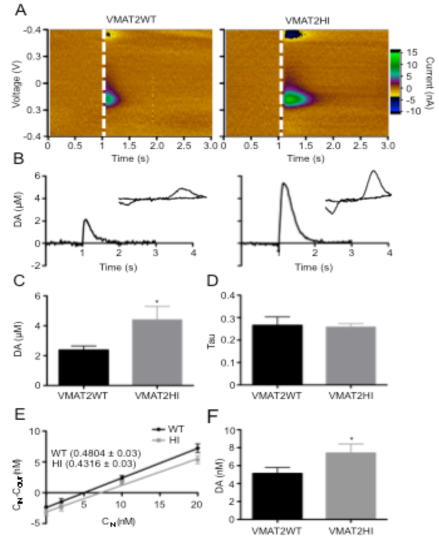
Kelly M. Lohr[a,1], Alison I. Bernstein[a,1], Kristen A. Stout[a], Amy R. Dunn[a], Carlos R. Lazo[a], Shawn P. Alter[a], Minzheng Wang[a], Yingjie Li[a], Xueliang Fan[b], Ellen J. Hess[b,c], Hong Yi[d], Laura M. Vecchio[e], David S. Goldstein[f], Thomas S. Guillot[a], Ali Salahpour[e], and Gary W. Miller[a,b,c,g,2]

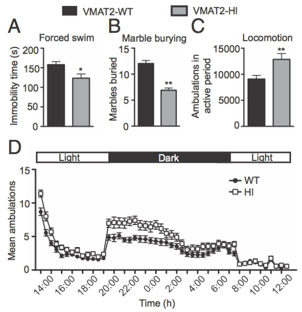
[a]Department of Environmental Health, Rollins School of Public Health, [b]Department of Pharmacology, [c]Department of Neurology, [d]Robert P. Apkarian Integrated Electron Microscopy Core, and [g]Center for Neurodegenerative Diseases, Emory University, Atlanta, GA 30322; [e]Department of Pharmacology and Toxicology, University of Toronto, Toronto, ON, Canada M5S 1A8; and [f]National Institute of Neurological Disorders and Stroke, Bethesda, MD 20824

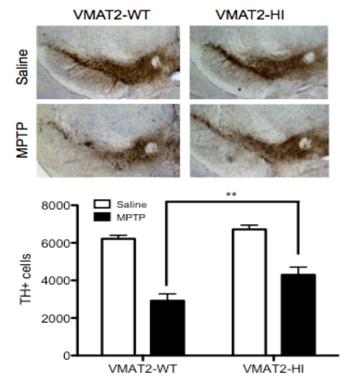






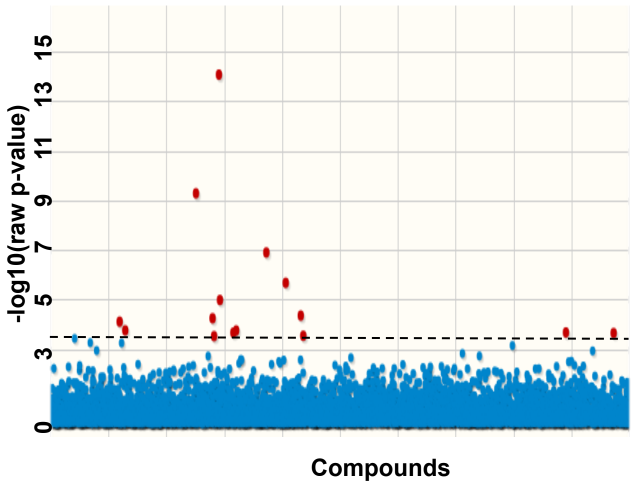


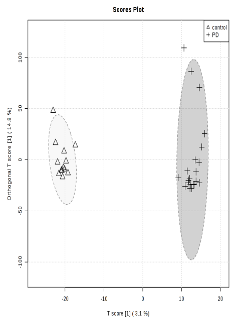




Untargeted MWAS for Parkinson's disease

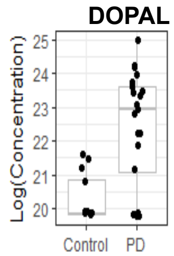
Characteristic	PD (n=21)	Control (n=13)	p-value
Sex			0.73
Male	10 (47.6%)	5 (38.5%)	
Female	11 (52.4%)	8 (61.5%)	
Age (mean ± sd)	61.7 ± 8.0	71.3 ± 5.4	0.001 **
Race			0.048 *
Caucasian	21 (100%)	10 (76.9%)	
African American	0 (0%)	3 (23.1%)	
Educational level			
Years of education	15.7	17.8	0.035 *
Less than high school	1 (4.8%)	0 (0%)	
High school	3 (14.3%)	1 (7.7%)	
Some college	3 (14.3%)	0 (0%)	
Associate/ vocational	1 (4.8%)	0 (0%)	
Bachelor's	8 (38.1%)	5 (38.5%)	
Master's	5 (23.8%)	5 (38.5%)	
Doctorate	0 (0%)	2 (15.4%)	
PD characteristics	2.6 ± 2.14	10.9 ± 6.5	0.0001 ***
UPDRS-III score	*N/A*	7.7 ± 3.4 years	
Disease duration	*N/A*	817.8 ± 372.8	
L-dopa equivalent	26.6 ± 3.7	27.6 ± 2.1	0.35
MOCA score	9.2 ± 3.8	3.7 ± 2.5	<0.0001 ***
NMSQ score			

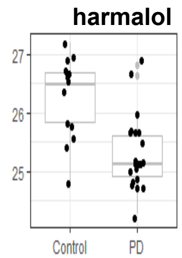




Branco, Ellsworth, Niedzwiecki, Butkovich, Walker, Huddleston, Jones, Miller, under review

m/z	retention time	Putative identity	Function	Higher in	p-value	FDR
212.092	71.06078	3-methoxytyrosine	PD drug metabolite	PD	8.33E-15	7.56E-11
195.0653	71.42926	3-methoxytyrosine	PD drug metabolite	PD	5.17E-10	2.34E-06
256.0555	66.04632	3-methoxytyrosine	PD drug metabolite	PD	1.27E-07	0.000383
278.0327	141.87809	DOPA sulfate	PD drug metabolite	PD	2.13E-06	0.004821
213.0951	72.66672	3-methoxytyrosine	PD drug metabolite	PD	1.05E-05	0.019062
292.0489	143.37057	Unknown	Unknown	PD	4.46E-05	0.067475
207.1104	68.90621	Unknown	Unknown	Control	5.74E-05	0.074343
149.0599	74.33799	Unknown	Unknown	PD	7.87E-05	0.089226
153.0548	75.71757	DOPAL	DA metabolite	PD	0.000177	0.15867
225.0826	70.12920	Unknown	Unknown	Control	0.000178	0.15867
916.6712	55.28919	glycerophospolipids	Lipid membrane	PD	0.000215	0.15867
223.0846	68.65464	Harmalol	Antioxidant	Control	0.000219	0.15867
1427.752	132.30322	Ganglioside GM2	Lipid membrane	Control	0.000227	0.15867
294.846	64.13191	gylcerophospholipids	Lipid membrane	Control	0.000292	0.18283
208.1141	69.02394	Unknown	Unknown	Control	0.000302	0.18283





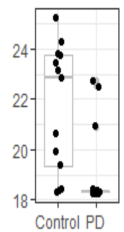
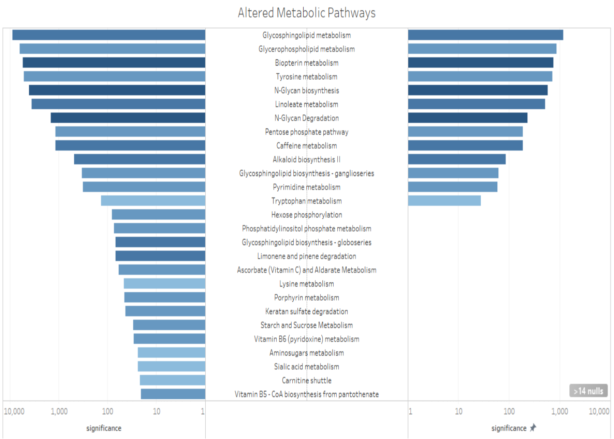
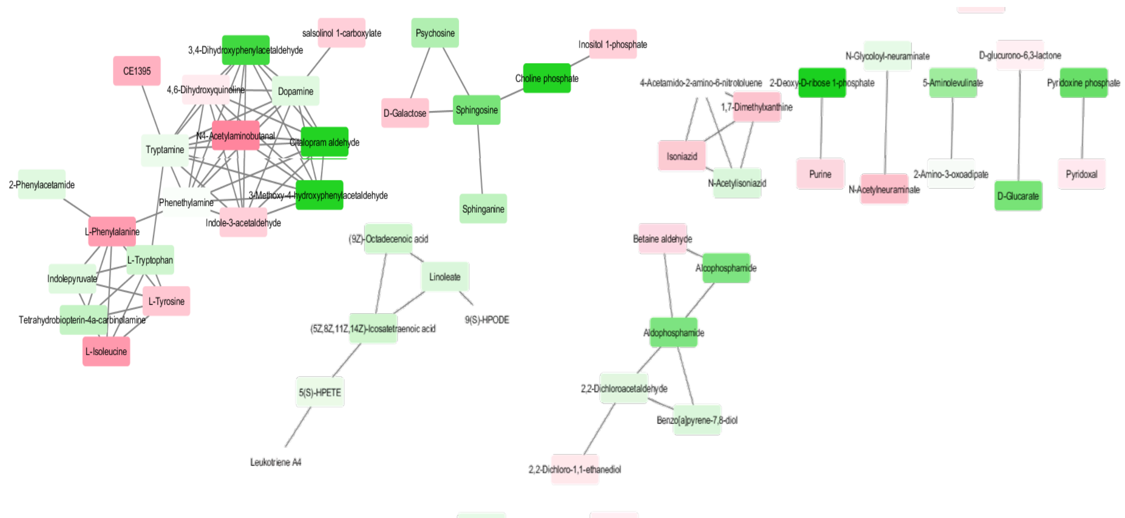


Table 1. Results of untargeted metabolomics (LC) in human PD

Majority of changes are correlated to L-DOPA levels





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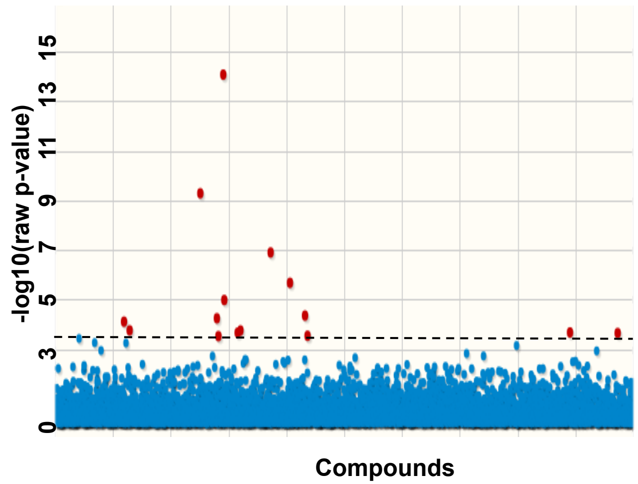
New Results

Levodopa and dopamine dynamics in Parkinson's disease metabolomics

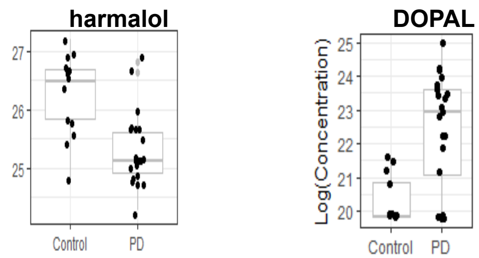
Rachel C Branco, William Ellsworth, Megan M Niedzwiecki, Laura M Butkovich, Doug I Walker, Daniel E Huddleston, Dean P Jones, Gary W Miller

doi: https://doi.org/10.1101/306266

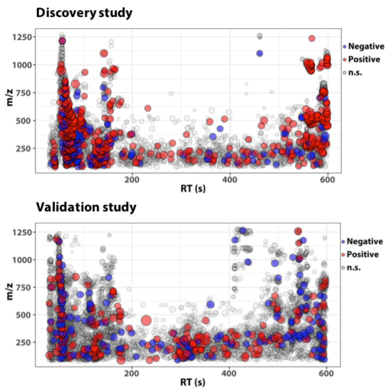
This article is a preprint and has not been peer-reviewed [what does this mean?].



Detected therapeutic drug metabolites
Toxic dopamine intermediates
Natural antioxidants
GM2 ganglioside
21 patients



Alzheimer's disease,
Mild cognitive impairment



	Control	AD	MCI	Ctrl vs. AD
Discovery study	*n* = 41	*n* = 43	*n* = 45	*p*[c]
Demographics				
Male	11 (27%)[a]	16 (37%)	22 (49%)	0.43
Age (y)	67.5 ± 7.3[b]	65.9 ± 8.8	69.4 ± 6.6	0.36
CSF protein biomarkers				
Aβ42 (pg/mL)	340 ± 137	203 ± 76	218 ± 90	<0.0001
t-Tau (pg/mL)	44 ± 24	117 ± 70	76 ± 67	<0.0001
p-Tau$_{181}$ (pg/mL)	32 ± 15	75 ± 32	51 ± 25	<0.0001
t-Tau/Aβ42	0.14 ± 0.09	0.64 ± 0.39	0.39 ± 0.33	<0.0001
APOE genotypes				
Subjects with data (*n*)	*n* = 18	*n* = 13	*n* = 21	0.003
No ε4 alleles	11 (61%)	1 (8%)	10 (48%)	
One ε4 allele	7 (39%)	8 (62%)	7 (33%)	
Two ε4 alleles	0 (0%)	4 (31%)	4 (19%)	
Validation study	*n* = 18	*n* = 50		*p*
Demographics				
Male	8 (44%)	22 (44%)		1.00
Age (y)	61.2 ± 12.6	65.1 ± 9.3		0.24
CSF protein biomarkers				
Aβ42 (pg/mL)	218 ± 112	143 ± 73		0.01
t-Tau (pg/mL)	43 ± 30	114 ± 54		<0.0001
p-Tau$_{181}$ (pg/mL)	27 ± 16	56 ± 27		<0.0001
t-Tau/Aβ42	0.23 ± 0.13	0.93 ± 0.53		<0.0001
APOE genotypes				
Subjects with data (*n*)	*n* = 16	*n* = 40		0.19
No ε4 alleles	10 (63%)	15 (38%)		
One ε4 allele	5 (31%)	17 (43%)		
Two ε4 alleles	1 (6%)	8 (20%)		

a. N (%) (all such values); b. mean ± SD (all such values); c. *p*-values for control vs. AD comparisons from t-tests (continuous variables) or chi-square tests (categorical variables)

Niedzwiecki, Walker, Howell, Watts, Jones, Miller, Hu under review

Alzheimer's disease (93), Mild cognitive impairment (50), controls (59)
APOEgentoype, CSF (AB42, pTau)

Table 4. Putative compound identification of plasma features from MWAS.

m/z	RT	Change in AD	Putative compound(s)	Predicted adduct	ID level[a]	Notes
129.0661	89	Higher	Glutamine (2 ppm)	$-H_2O+H$	1	--
231.1205	211	Higher	5S,6S-epoxy-15R-hydroxy-ETE (+Na, 0 ppm)	--	3	--
246.9550	127	Higher	Numerous database matches	$-H_2O+H$	--	Contains halogen (Cl and/or Br)
334.1410	86	Lower	Piperettine (1 ppm)	+Na	4	--
349.1515	80	Lower	Piperine (1 ppm)	+ACN+Na	4	--
386.8946	61	Higher	1,1-Dichloro-2-(dihydroxy-4'-chlorophenyl)-2-(4'-chlorophenyl)ethylene (9 ppm)	+K	2	Contains halogen (Cl and/or Br)
662.0933	158	Higher	GDP-D-mannuronate (+ACN+H [M+1], 0 ppm); Chaetocin ($-2H_2O+H$ [M+1], 8 ppm); Blighinone (+H [M+1], 9 ppm)	[M+1] isotope	4	--
663.4524	36	Higher	Lipid A-disaccharide-1-P (+2H, 2 ppm); Aluminium dodecanoate (+K, 2 ppm)	--	4	--

[a]ID level indicates annotation confidence: 1, *m/z* and retention time confirmed with MS^2, 2: Multiple/isotopes present; 3: *m/z* matched single adduct mass within 10 ppm mass error, 4: *m/z* matched adduct mass of multiple isobaric species, probable identifications listed.

Niedzwiecki, Walker, Howell, Watts, Jones, Miller, Hu under review

Alzheimer's disease (93), Mild cognitive impairment (50), controls (59) APOEgentoype, CSF (AB42, pTau)

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334.1410	86	Lower	Piperettine (1 ppm)			
349.1515	80	Lower	Piperine (1 ppm)	+ACN+Na	4	--
386.8946	61	Higher	1,1-Dichloro-2-(dihydroxy-4'-chlorophenyl)-2-(4'-chlorophenyl)ethylene (9 ppm)	+K	2	Contains halogen (Cl and/or Br)
662.0933	158	Higher	GDP-D-mannuronate (+ACN+H [M+1], 0 ppm); Chaetocin ($-2H_2O+H$ [M+1], 8 ppm); Blighinone (+H [M+1], 9 ppm)	[M+1] isotope	4	--
663.4524	36	Higher	Lipid A-disaccharide-1-P (+2H, 2 ppm); Aluminium dodecanoate (+K, 2 ppm)	--	4	--

Hydroxyated metabolite of DDE

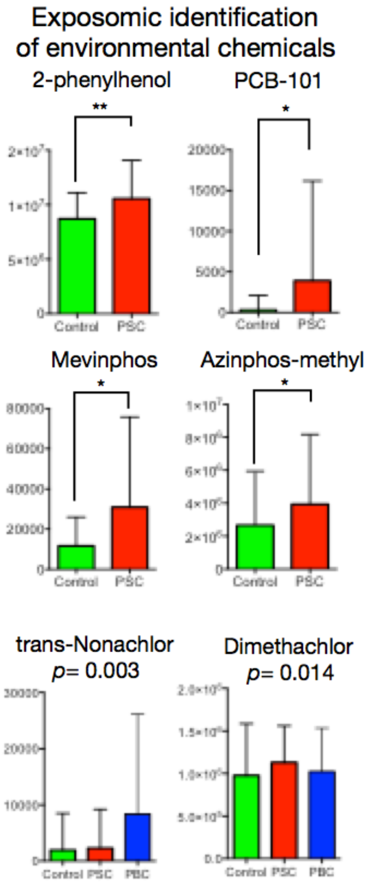
[a]ID level indicates annotation confidence: 1, *m/z* and retention time confirmed with MS^2, 2: Multiple/isotopes present; 3: *m/z* matched single adduct mass within 10 ppm mass error, 4: *m/z* matched adduct mass of multiple isobaric species, probable identifications listed.

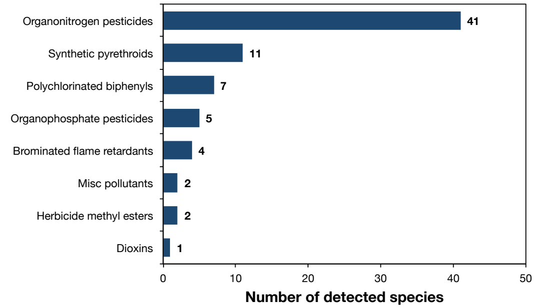
Niedzwiecki, Walker, Howell, Watts, Jones, Miller, Hu under review

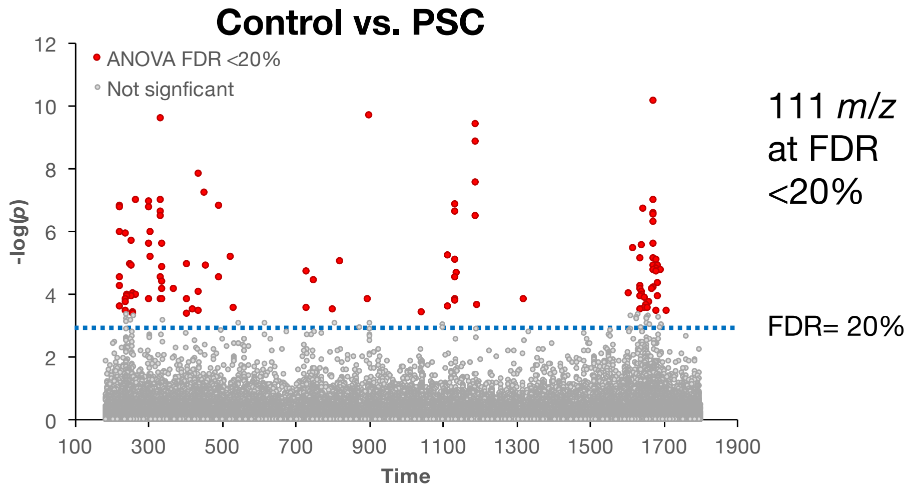
Recent collaboration with the Mayo Clinic

- ***Primary sclerosing cholangitis (PSC)***
- US prevalence: 13.6/100,000 (0.014%)
- Average age of diagnosis: 41 years
- Transplant free survival: 12 years
- Only treatment: Liver transplant (OLT)
- Outcomes: malignancy, liver failure
- Mayo sees 5% of all U.S. patients with disease
- ~70% of cases have inflammatory bowel disease

GC-based exposomics







309 *m/z* spectral signals matching 94 unique pollutant biomarkers were identified by comparison to an in-house library of 262 compounds

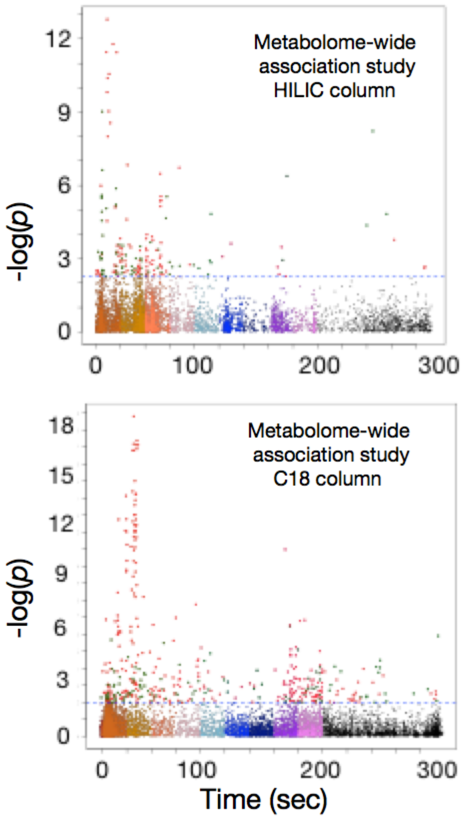
EWAS reveals altered levels of environmental pollutants

Chemical	*p-value*	Regression Coefficient	Odds ratio , IQR (95% Confidence interval)
Fenpropimorph	0.012	2.05	7.78 (2.47, 82.22)
Nonylphenol	0.002	1.04	2.84 (1.55, 5.89)
Protocatechuic acid	0.003	1.01	2.75 (1.53, 5.75)
Aldicarb sulfone/Acetamiprid	0.012	0.75	2.13 (1.28, 4.19)
Ethyl paraben	0.014	0.63	1.87 (1.22, 3.33)
Chlorthiophos	0.078	0.49	1.63 (0.97, 2.89)
Terbutylazine	0.056	0.48	1.62 (1.01, 2.75)
Fenvalerate	0.063	0.44	1.56 (1.00, 2.58)
Triclocarban	0.076	-0.24	0.79 (0.59, 1.02)
Anthraquinone	0.093	-0.37	0.69 (0.44, 1.05)
Perfluorooctanoic acid	0.084	-0.38	0.68 (0.44, 1.05)
Diphenamid	0.012	-0.40	0.67 (0.47, 0.9)
Diphenamid	0.052	-0.41	0.66 (0.43, 0.99)
Dimethachlor	0.049	-0.45	0.64 (0.40, 0.98)
Monocrotophos	0.030	-0.46	0.63 (0.41, 0.94)
Thiabendazole	0.062	-0.59	0.55 (0.29, 1.02)
Perfluorooctanesulfonic acid	0.007	-0.65	0.52 (0.31, 0.79)
Fenobucarb/Promecarb	0.019	-0.76	0.47 (0.24, 0.87)
Carbaryl	2.6E-05	-0.97	0.38 (0.23, 0.57)

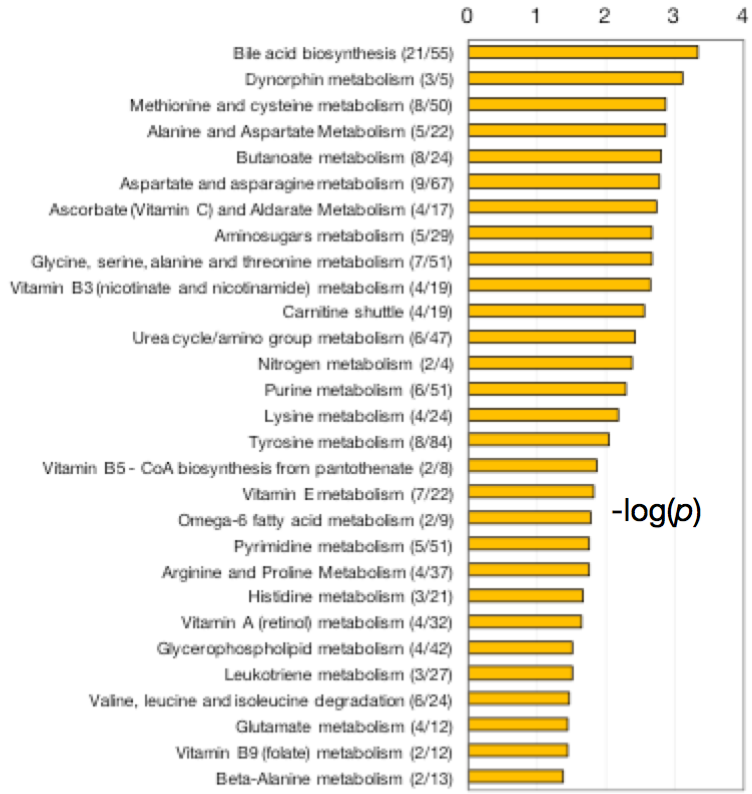
- 205 environmental chemical biomarkers identified in PSC, PBC and control population. Each was tested for association with disease status using logistic regression.

Chemical	Class	Known effects
Fenpropimorph	Fungicide	Inhibits sterol pathways in plants
Nonylphenol	Personal care product	
Protocatechuic acid	Personal care product	
Acetamiprid/ Aldicarb sulfone	Insecticide	Nicotinic agonist that reacts with nicotinic acetylcholine receptors
Ethyl paraben	Personal care product	
Chlorthiophos	Insecticide	Neurological toxin
Terbutylazine	Herbicide	Endocrine, reproductive and oxidative stress
Fenvalerate	Insecticide	Central nervous system pathway
Perfluorooctanonic acid	Commercial chemical	Liver toxicant, a developmental toxicant, and an immune system toxicant
Perfluorosulfonic acid	Commercial chemical	Liver and immune toxicant

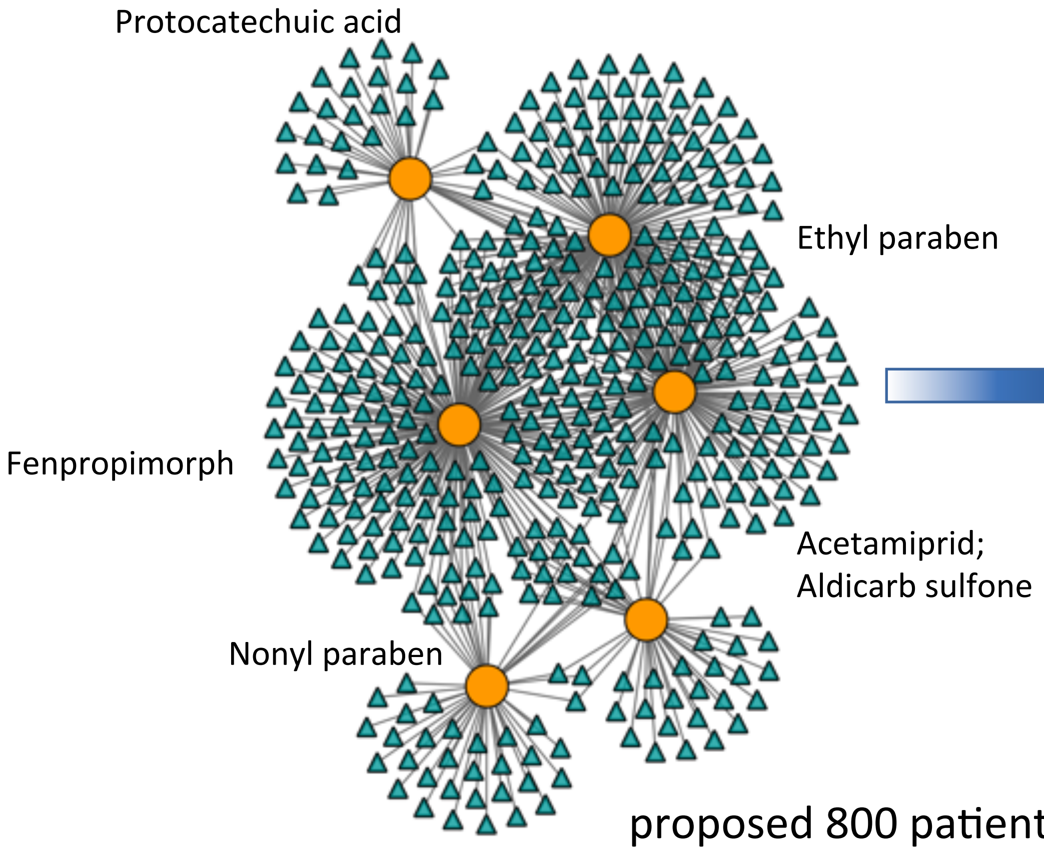
LC-based metabolomics

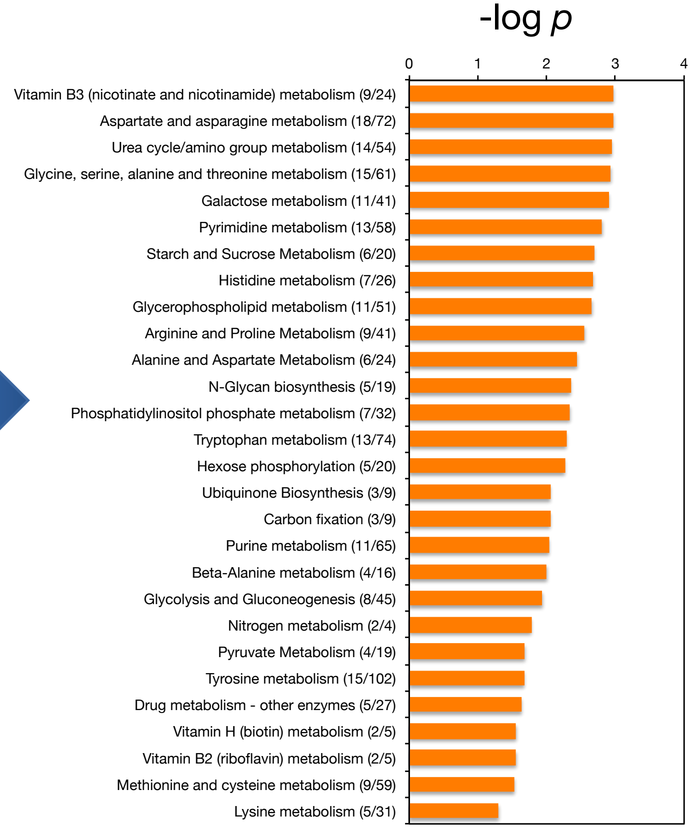


Mummichog-based analysis of biological pathways



EWAS x MWAS of PSC: Linking environmental chemicals to metabolic pathway alterations





proposed 800 patients per group

High Impact, Interdisciplinary Science in NIDDK Research Areas (RC2)

- Dissecting the Pathogenesis an Outcomes of PSC using Multi-omics by Studying the Exposome and Genome. Kostas Lazaridis, P.I.

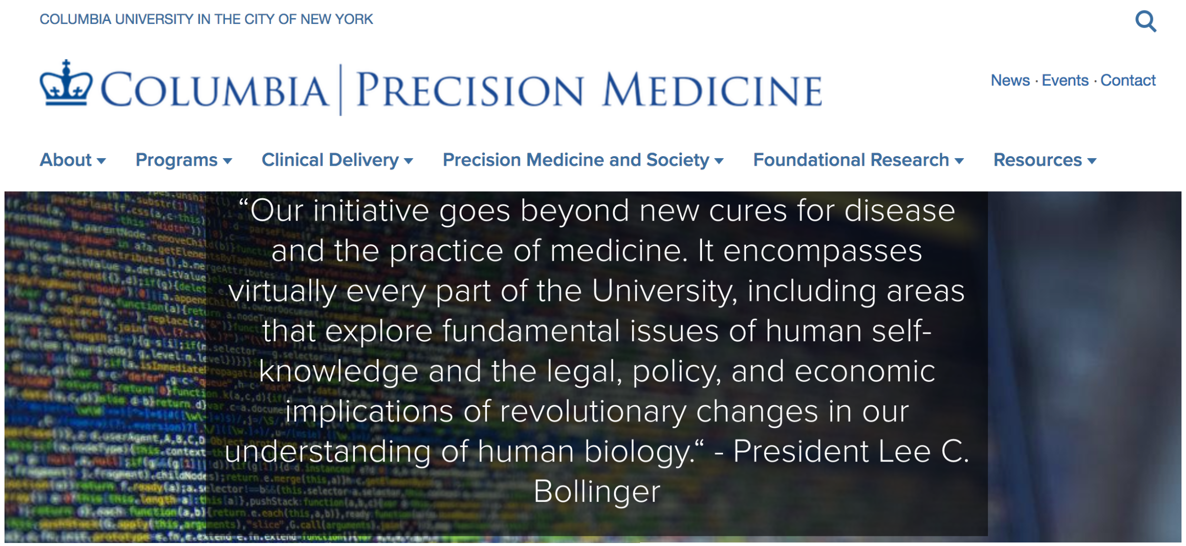
- "*The major goal of this application is to generate the first, multi-omics translational study capturing the sum of environmental exposures (**the exposome**) and comprehensive data resource for primary sclerosing cholangitis (PSC), a chronic, progressive liver disease without effective medical therapy.*"

When it comes to the exposome, we do not need to measure everything. We need a reproducible system that captures “E” in an unbiased manner and can be used in conjunction with existing studies that employ genomics, epigenomics, proteomics, etc. Providing a means for non-environmental health scientists to incorporate “E” into their studies, even if at a single time point, *should be considered a prime deliverable.*

The *All of Us* Research Program

- The cornerstone of the larger PMI – led by the NIH
- One million or more volunteers, reflecting the broad diversity of the U.S.
- Opportunities for volunteers to provide data on an ongoing basis
- Data will inform a variety of research studies

All of Us RESEARCH PROGRAM | The Precision Medicine Initiative



The Irving Institute for Clinical and Translational Science

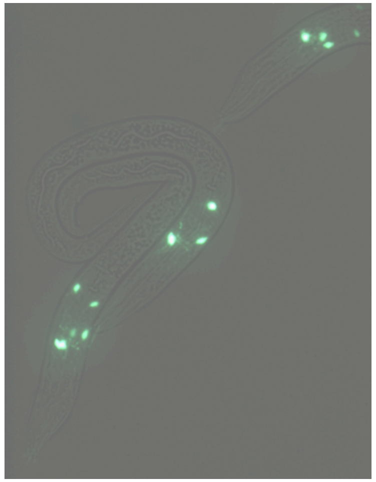
The Irving Institute for Clinical and Translational Research, funded by a National Institutes of Health Clinical and Translational Science Award (CTSA), serves as the cornerstone of translational science for the Columbia Precision Medicine Initiative.

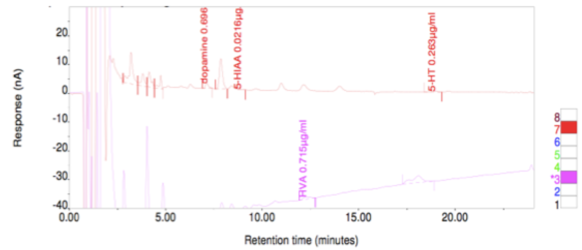
Under the leadership of Director Designate Dr. Muredach Reilly, the Irving Institute centralizes all activities in non-cancer biobanking, genome-based clinical phenotyping and "omics", and clinical trials that apply to human genetics to improve patient care at the Columbia University Medical Center and NewYork-Presbyterian Hospital. The Institute for Genomic Medicine will be a critical partner for genomic analysis in this collaborative and synergistic initiative.

U.S. Exposome Consortium

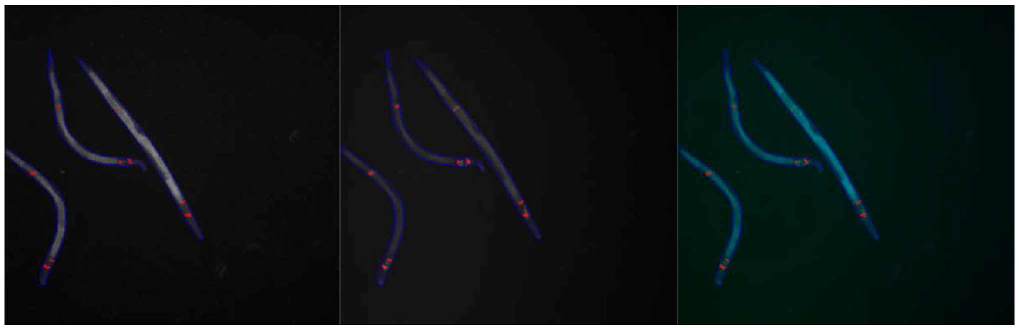
Inter-laboratory validation*
Sharing of pooled standards*
Standard operating procedures*
Shared bioinformatic platforms*
Harmonization of exposome measures*

The model organism *C. elegans*





959 cells
302 neurons
8 dopamine neurons



The Lifespan Machine: automated assessment

nature methods
Techniques for life scientists and chemists
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NATURE METHODS | ARTICLE

The *Caenorhabditis elegans* Lifespan Machine

Nicholas Stroustrup, Bryne E Ulmschneider, Zachary M Nash, Isaac F López-Moyado, Javier Apfeld & Walter Fontana

nature
International journal of science

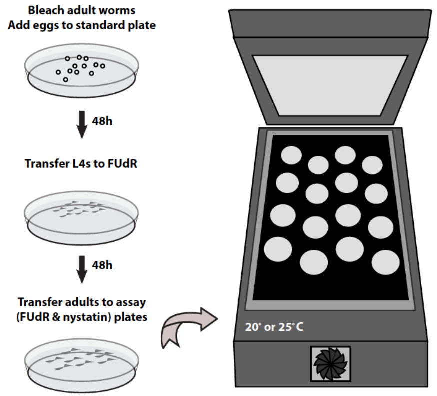
Altmetric: 120 Citations: 24 More detail »

Letter

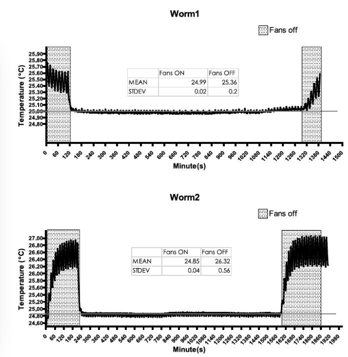
The temporal scaling of *Caenorhabditis elegans* ageing

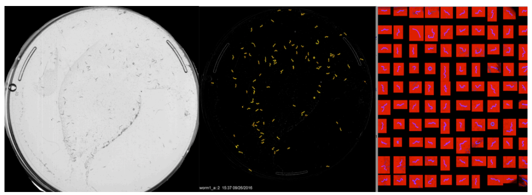
Nicholas Stroustrup, Winston E. Anthony, Zachary M. Nash, Vivek Gowda, Adam Gomez, Isaac F. López-Moyado, Javier Apfeld & Walter Fontana

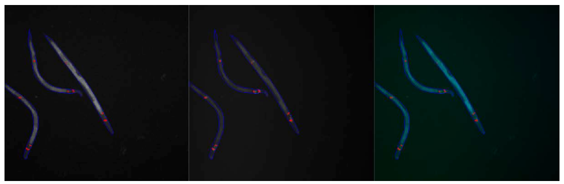
Workflow



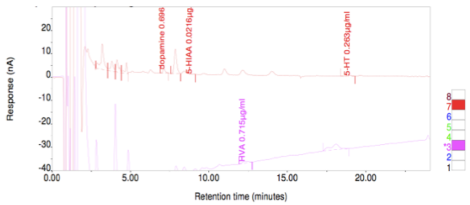
Temperature Stability

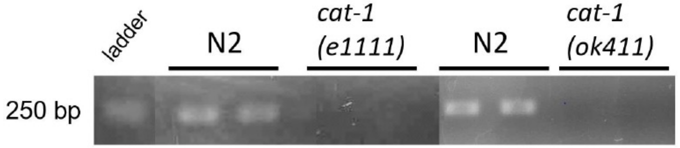


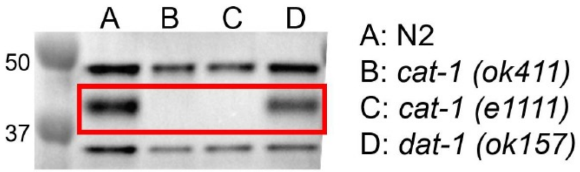


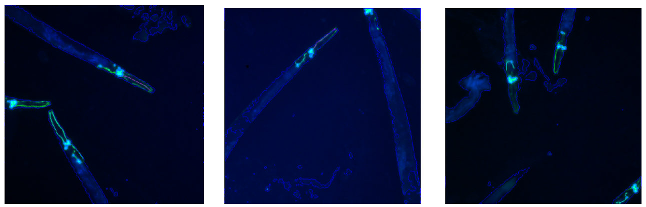


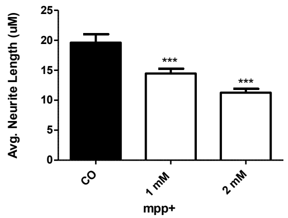
Josh Bradner

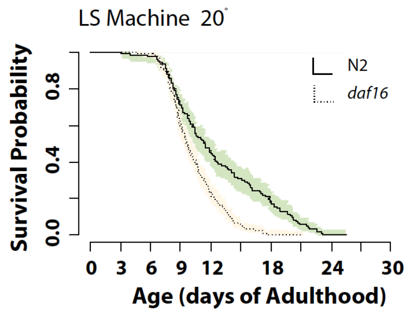

Response (nA)
Retention time (minutes)

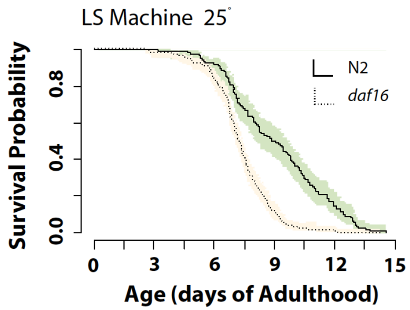

ladder
N2
cat-1 (e1111)
N2
cat-1 (ok411)
250 bp

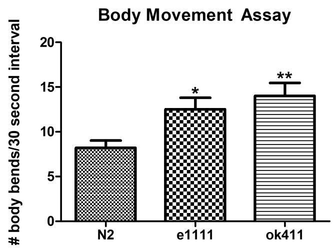

A B C D
50
37
A: N2
B: cat-1 (ok411)
C: cat-1 (e1111)
D: dat-1 (ok157)

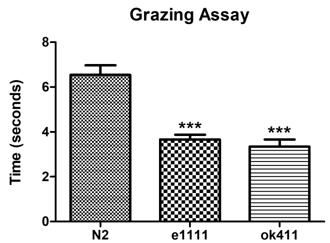

control
1mM
2mM


Avg. Neurite Length (uM)
CO
1 mM
2 mM
mpp+

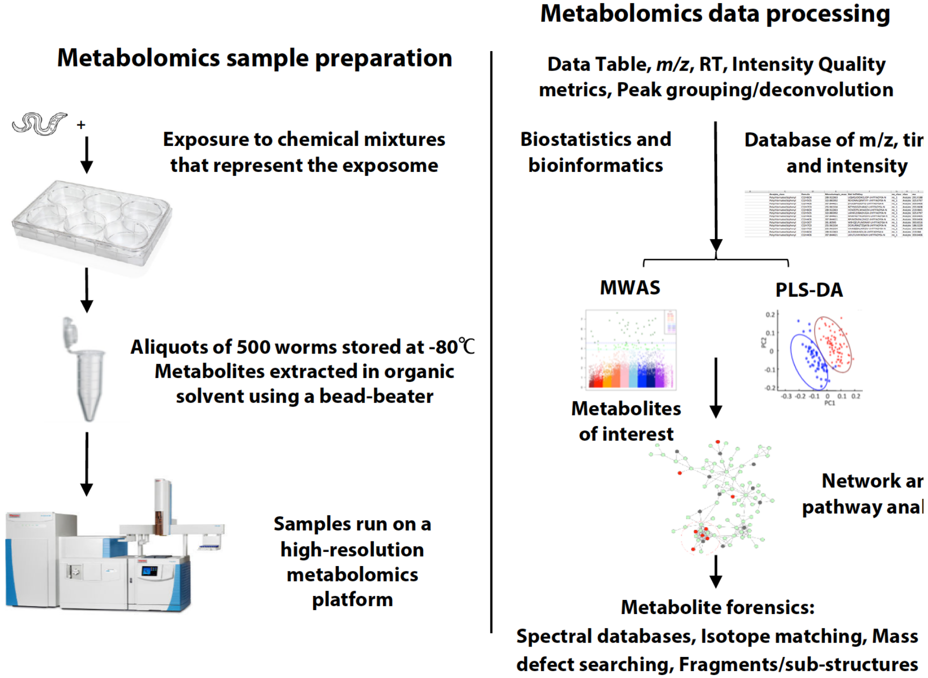

LS Machine 20°
Survival Probability
N2
daf16
Age (days of Adulthood)

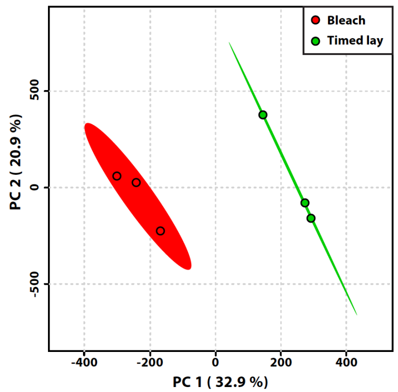

LS Machine 25°
Survival Probability
N2
daf16
Age (days of Adulthood)

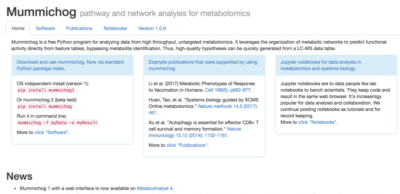

Body Movement Assay
body bends/30 second interval
N2
e1111
ok411


Grazing Assay
Time (seconds)
N2
e1111
ok411

Our entire exposomic and metabolomic workflow can be performed in as few as 500 worms

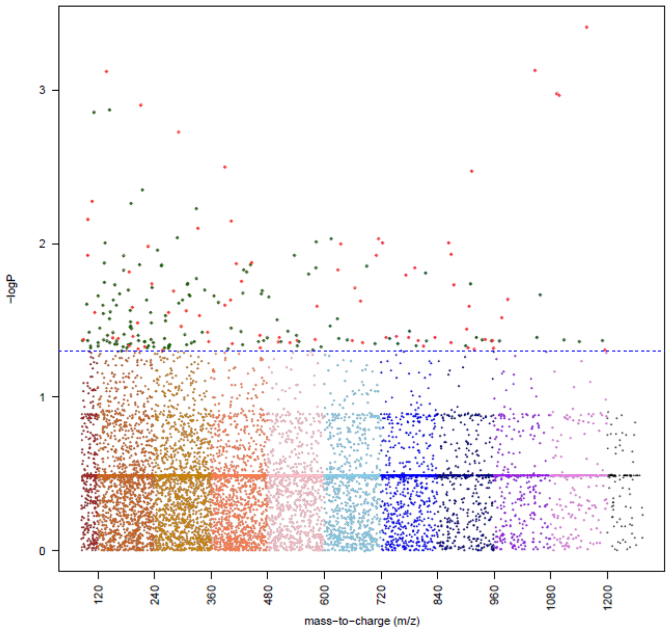


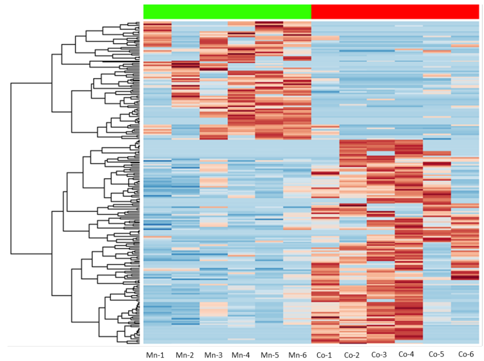




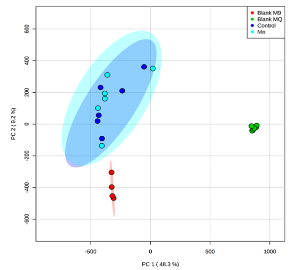
Kalia, Bradner, Niedzwiecki, Li, Walker, Elsworth, Jones, Miller

Metabolomic profile of manganese exposure

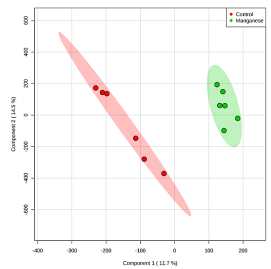




Above: a manhattan plot shows features that were higher in controls (green) and lower in controls (red), Top right: hierarchical clustering of features detected

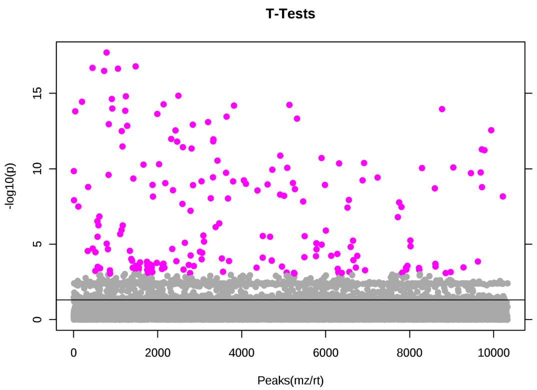


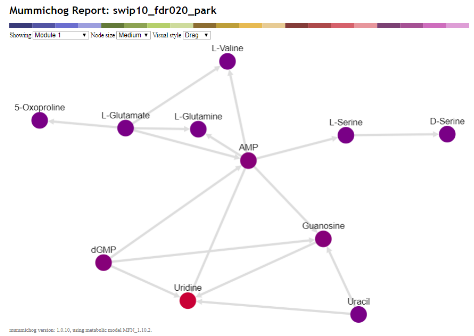
PCA



PLS-DA

Swip 10

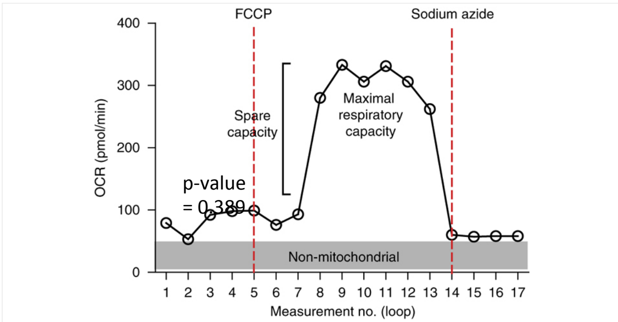




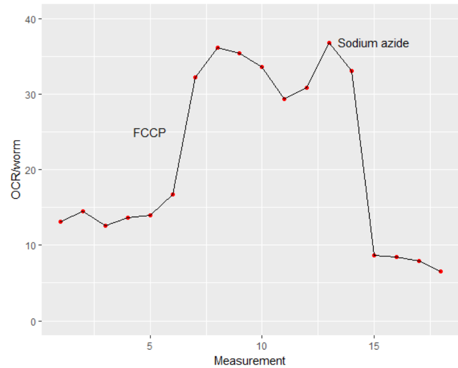
Top pathways

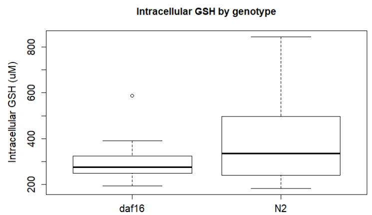
Pathways	overlap_size	pathway_size	p-value (raw)	p-value
Pyrimidine metabolism	7	14	0.0458	0.00141
Butanoate metabolism	3	3	0.01796	0.00177
Valine, leucine and isoleucine degradation	3	3	0.01796	0.00177
Purine metabolism	6	12	0.06488	0.00184
Methionine and cysteine metabolism	6	15	0.17645	0.00466
Lysine metabolism	4	8	0.132	0.00468
Aminosugars metabolism	4	8	0.132	0.00468
Vitamin B3 (nicotinate and nicotinamide) metabolism	3	5	0.11785	0.00593
Tyrosine metabolism	7	21	0.30616	0.0099
Glutamate metabolism	2	3	0.17261	0.01958
Arachidonic acid metabolism	2	3	0.17261	0.01958
Glutathione Metabolism	2	3	0.17261	0.01958
Selenoamino acid metabolism	2	4	0.28711	0.03766
Nitrogen metabolism	2	4	0.28711	0.03766
Vitamin B9 (folate) metabolism	2	4	0.28711	0.03766
Histidine metabolism	2	4	0.28711	0.03766

Seahorse and glutathione

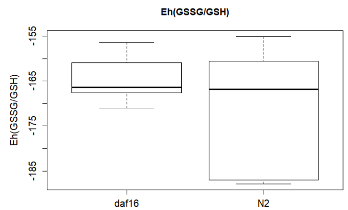


Starting with the basal respiration, the spare capacity and maximal (mitochondrial) respiratory capacity (plateau phase) can be estimated by injecting FCCP. Non-mitochondrial respiration can be distinguished from mitochondrial respiration by injecting sodium azide. Dashed red lines indicate injections of compounds.





Intracellular GSH measured in wild type (N2) and mutant (daf16) worms



GSSG/GSH, normalized to protein concentration
Caveat: Measurement of GSSG was around limit of detection

Koopman, Mandy, et al. "A screening-based platform for the assessment of cellular respiration in Caenorhabditis elegans." Nature protocols 11.10 (2016): 1798.

Metabolomics measures dynamic biological systems. As such there is a high level of variability. However, the information is very relevant to health and disease conditions and we must exert great effort to capture it.