



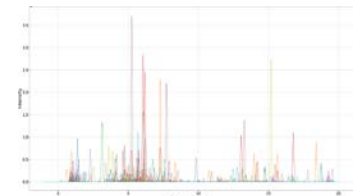
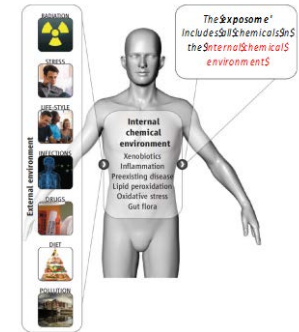
Emerging Biomarker Approaches and Technologies for Exposure Assessment

William E. Funk, Ph.D.
Department of Preventive Medicine
Northwestern University



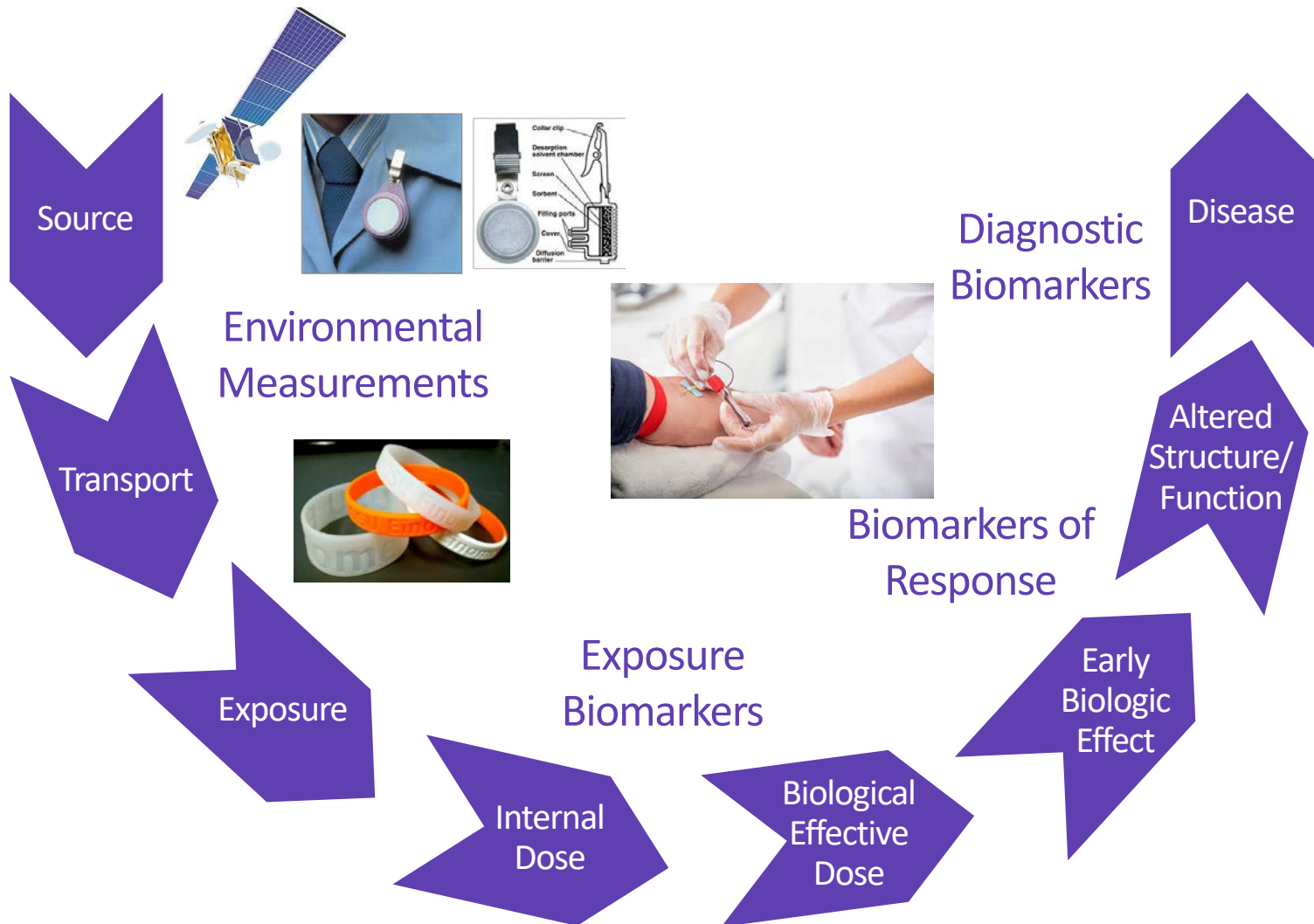
Overview

1. Environmental measurements, biomarkers of exposure, and biomarkers of response
2. The exposome
3. Untargeted versus targeted biomarker approaches
4. Field-friendly biospecimen collection

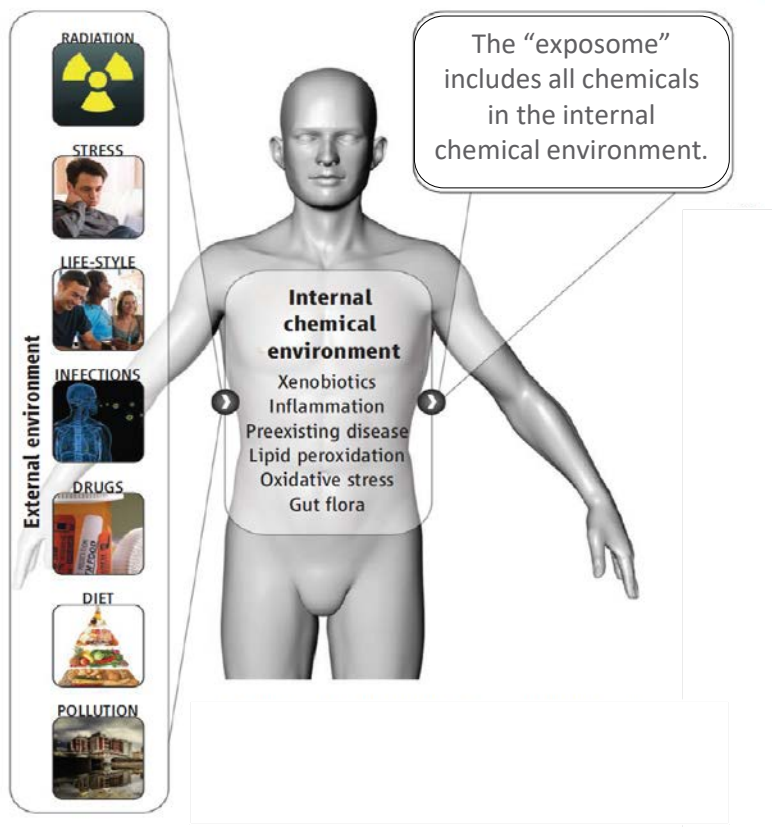


Disclosure: I have a financial interest in EnMed MicroAnalytics, Inc. which could potentially benefit from the outcomes of this research.

Exposure-Disease Continuum



The Exposome



S.M. Rappaport & M.T. Smith, Science, 2010



The Exposome:

A Powerful Approach for Evaluating Environmental
Exposures and Their Influences on Human Disease

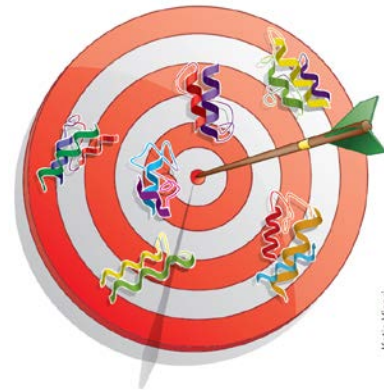
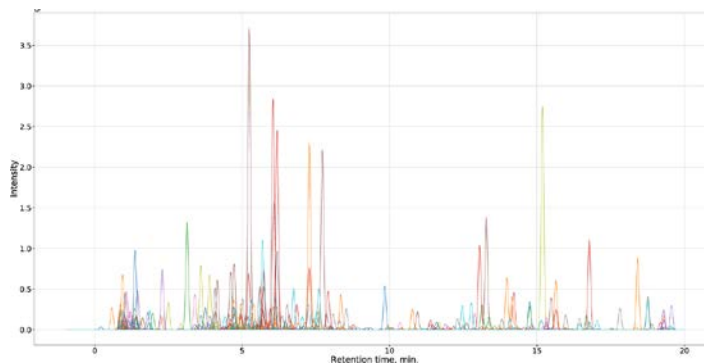
FEBRUARY 25-26, 2010 • WASHINGTON, DC



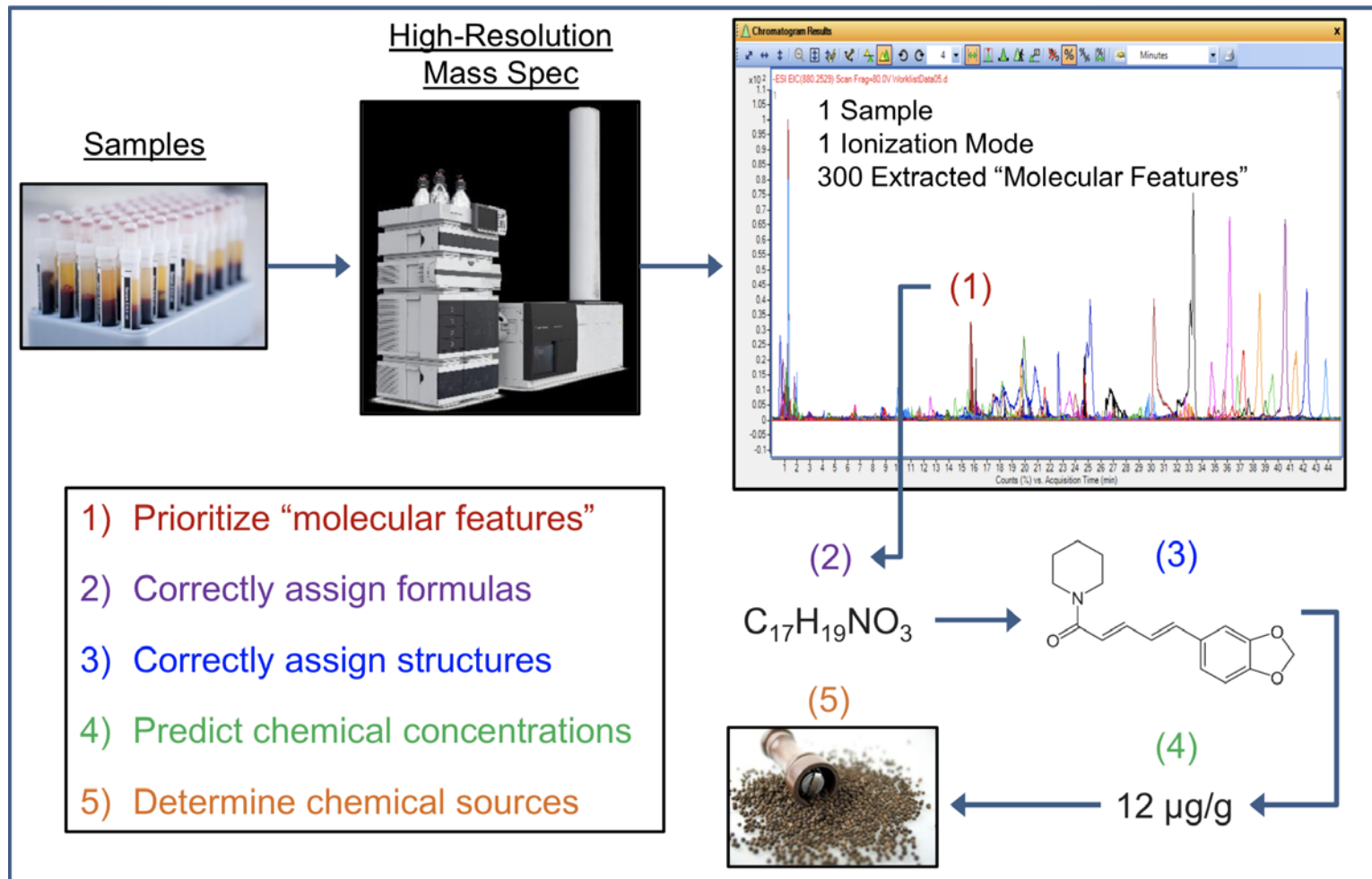
Emerging Technologies for Measuring Individual Exposomes

DECEMBER 8-9, 2011 • THURSDAY, 8:30-5:00, FRIDAY, 8:30-NOON*

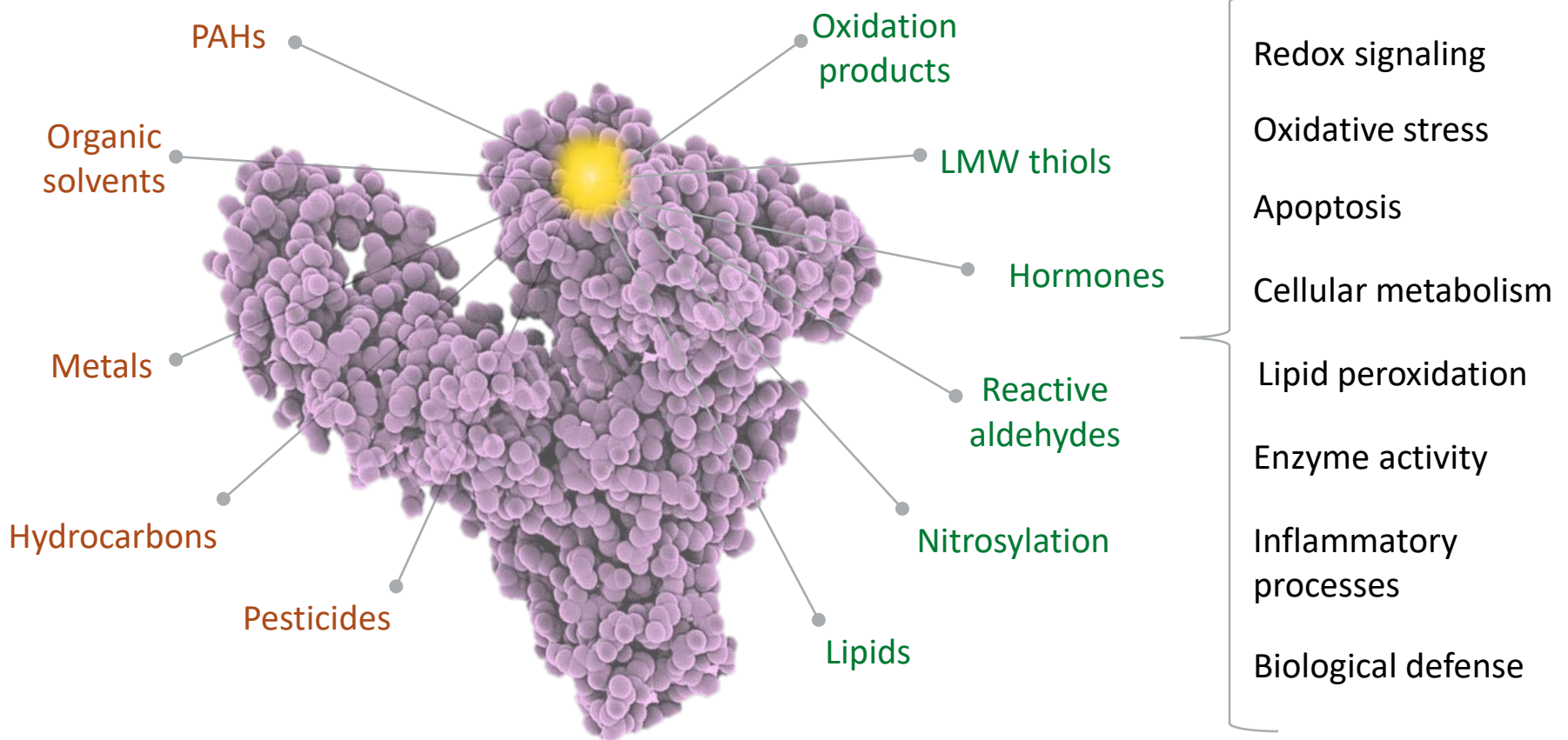
Untargeted Versus Targeted Biomarker Approaches

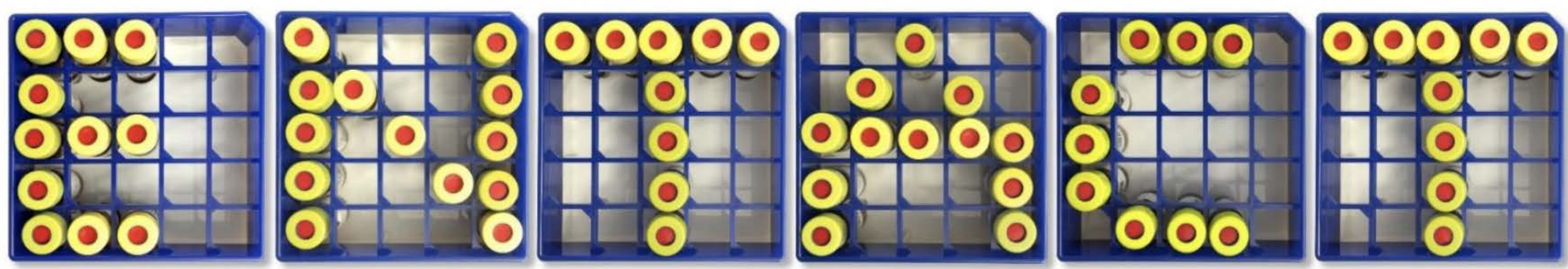


Untargeted Biomarker Analyses for Biomarker Discovery

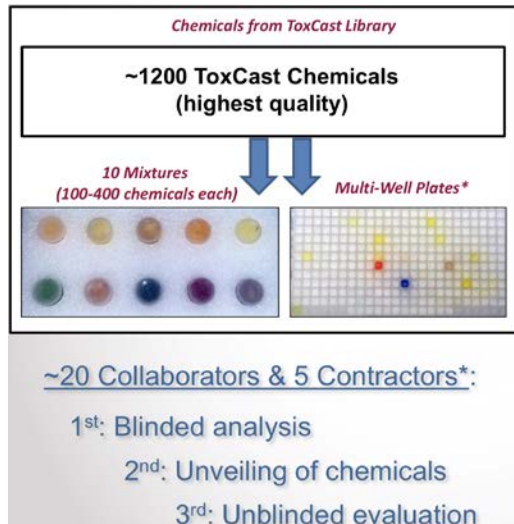


HSA-Cys³⁴ Adductomics





EPA's Non-Targeted Analysis Collaboration Trial

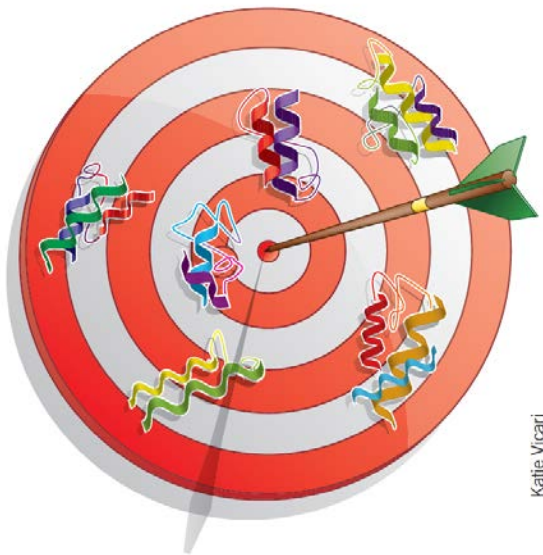


	<u>ToxCast Mixtures</u>										<u>Spiked Matrices</u>		
	Mix1	Mix2	Mix3	Mix4	Mix5	Mix6	Mix7	Mix8	Mix9	Mix10	Dust	Serum	Band
Lab	95	95	95	95	185	185	365	365	95	365	365	95	185
A	93	114	116	106	182	201	360	374	73	330	236	92	124
B	337	372	303	365	321	363	466	505	510	463	259	222	313
C	135	130	125	154	188	195	284	295	100	153	270	54	101
D	595	486	571	630	746	669	899	910	588	792	1009	614	NR
E	66	170	51	41	272	116	214	101	163	404	861	145	557
F	51	37	35	39	74	59	124	109	42	105	124	52	76
G	137	65	45	74	68	234	413	408	120	317	389	178	88

Blue = under reported; Green = near actual; Red = over reported

Targeted proteomics

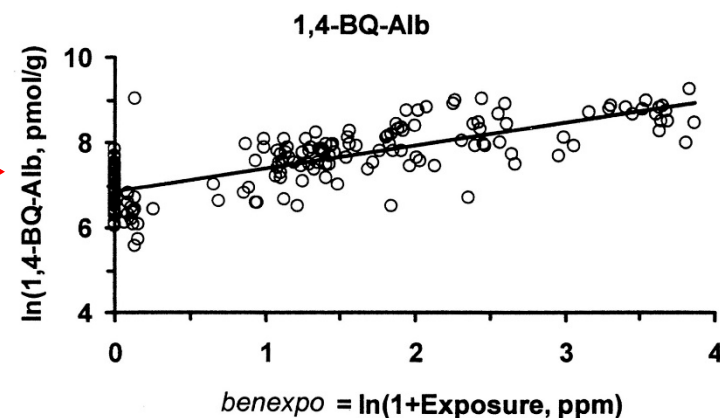
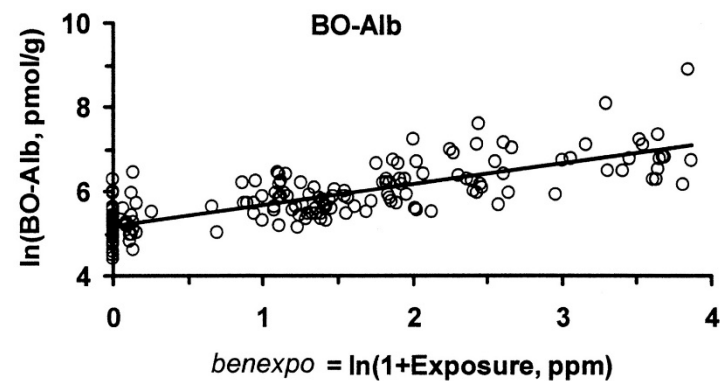
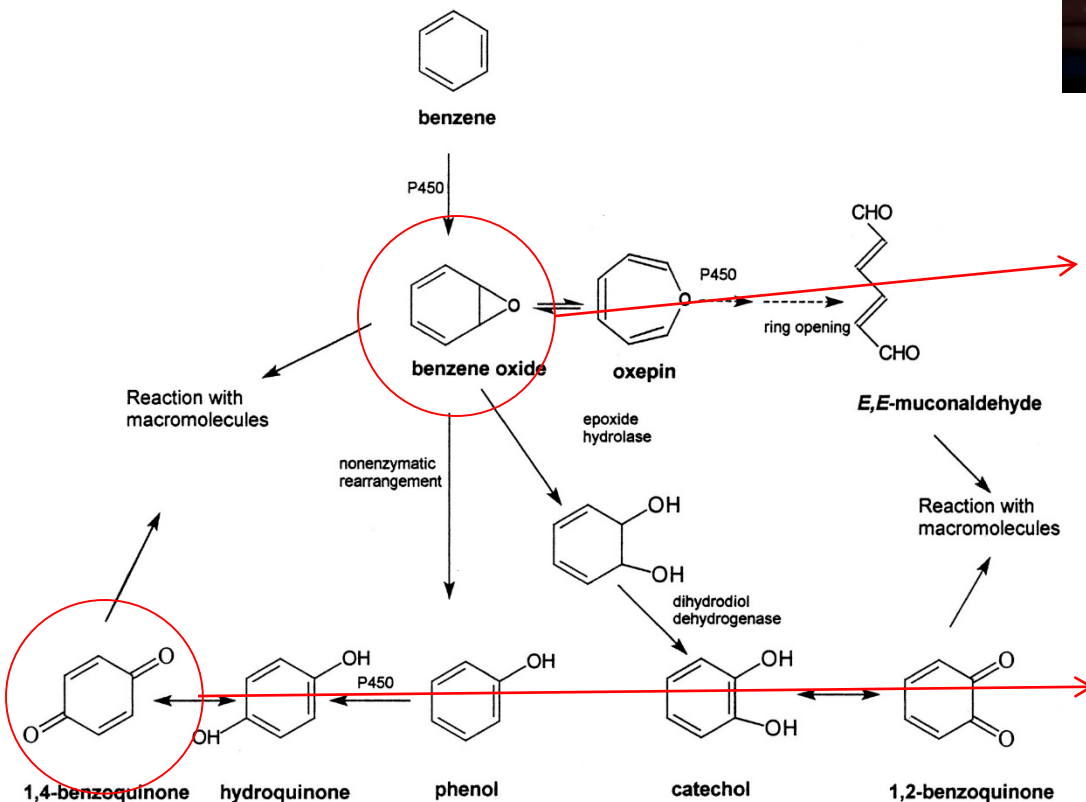
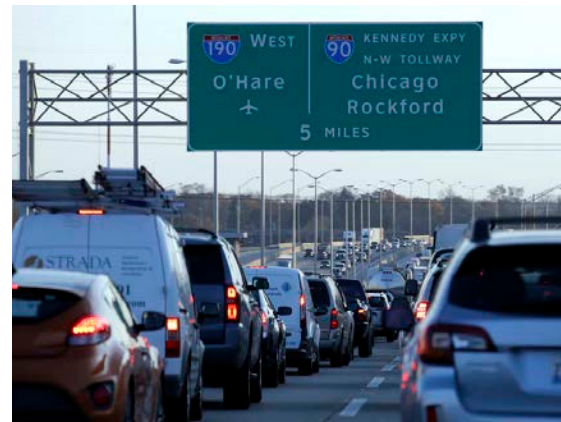
Analysis of a preselected group of proteins delivers more precise, quantitative, sensitive data to more biologists. Vivien Marx reports.



“Targeted proteomics using mass spectrometry promises to deliver data to help address specific biological questions in a way that makes it fundamentally unlike discovery proteomics”

Targeted proteomics detects proteins of interest with high sensitivity, quantitative accuracy and reproducibility.

Benzene-Related Adducts



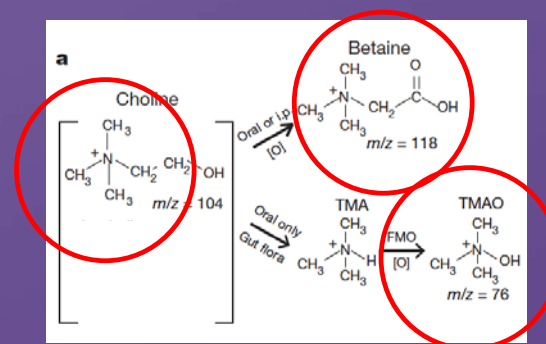
Gut flora metabolism of phosphatidylcholine promotes cardiovascular disease

Zeneng Wang^{1,2}, Elizabeth Klipfell^{1,2}, Brian J. Bennett³, Robert Koeth¹, Bruce S. Levison^{1,2}, Brandon DuGar¹, Ariel E. Feldstein^{1,2}, Earl B. Britt^{1,2}, Xiaoming Fu^{1,2}, Yoon-Mi Chung^{1,2}, Yuping Wu⁴, Phil Schauer⁵, Jonathan D. Smith^{1,6}, Hooman Allayee⁷, W. H. Wilson Tang^{1,2,6}, Joseph A. DiDonato^{1,2}, Aldons J. Lusis³ & Stanley L. Hazen^{1,2,6}

7 APRIL 2011 | VOL 472 | NATURE | 57

Untargeted: Biomarker Discovery

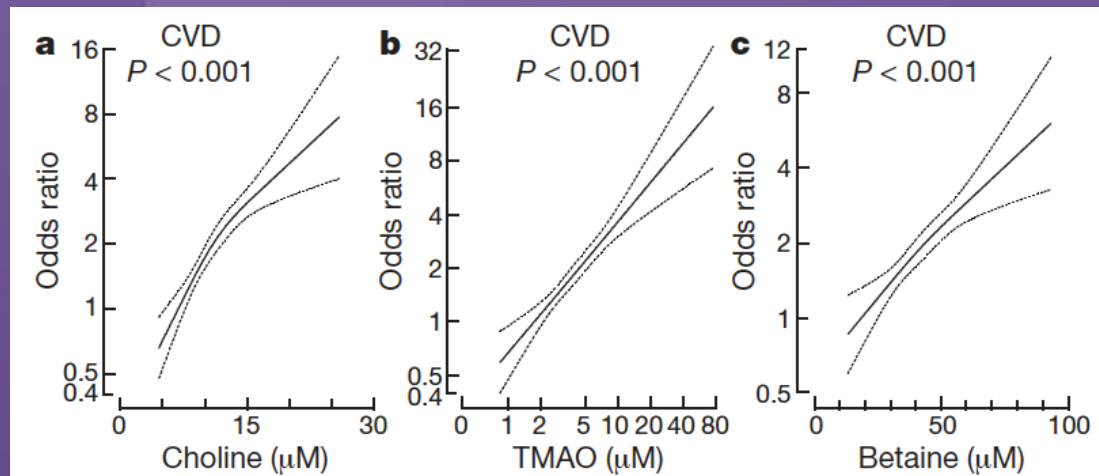
- From serum metabolomics, identified 18 unique features related to CVD
- Of these, 3 were highly correlated suggesting a common biochemical pathway
- Identified 3 metabolites of a dietary phospholipid



Targeted: Molecular Epidemiology



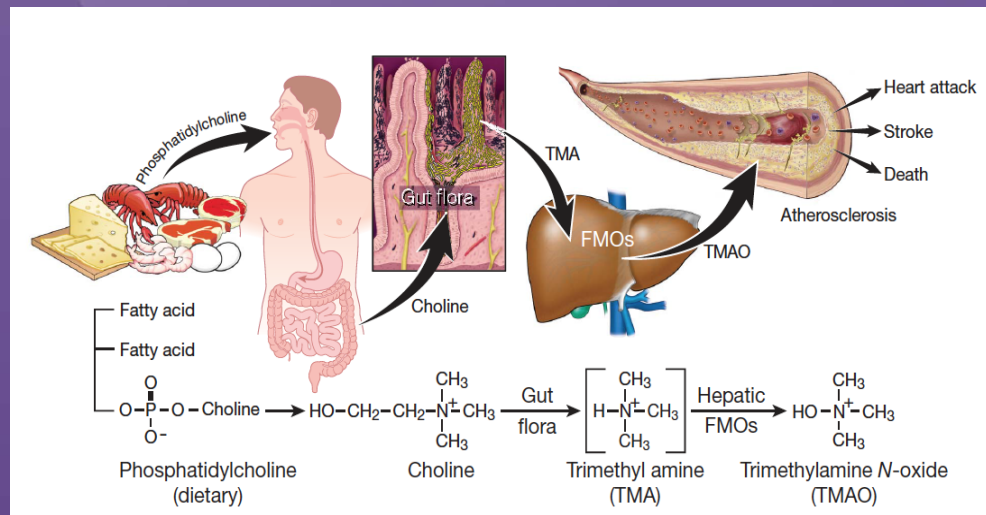
- Identified a large independent cohort of 1,867 individuals
- Quantified TMAO, choline, and betaine in fasting serum



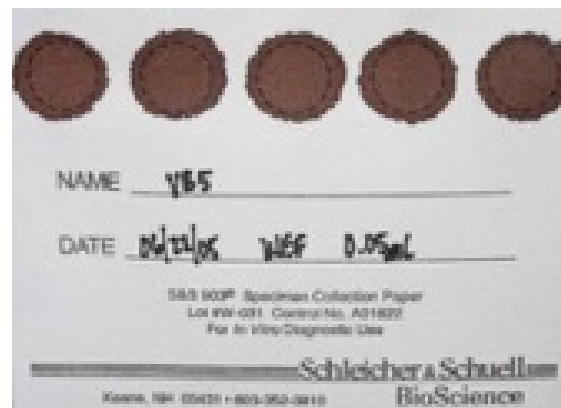
Exploring the Underlying Biology



- Metabolism of dietary phosphatidylcholine is gut flora-mediated
- Suppression of intestinal microflora inhibited this process



Dried Blood Spot (DBS) Sampling



Advantages of DBS Sampling

- Collection does not require a phlebotomist
- Less invasive than venipuncture
- Specimens do not need to be processed in the field
- Easier to transport and store
- Cost

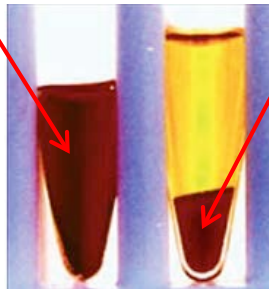


Challenges of DBS Sampling

- Minimum blood volume
- Some biomarkers may not be stable in DBS samples
- More complex biological matrix

Extracted DBS

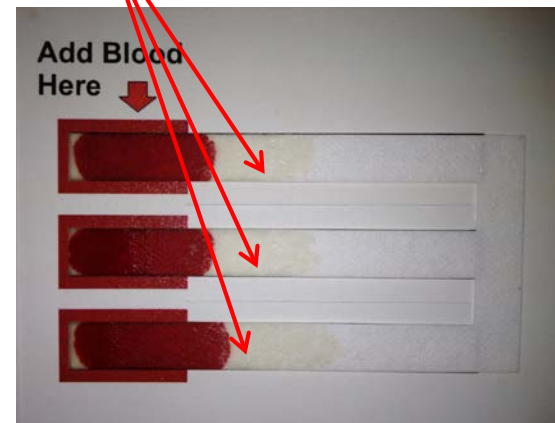
Hb bound &
removed



Hemoglobind™

(Retrospective DBS Studies)

Plasma



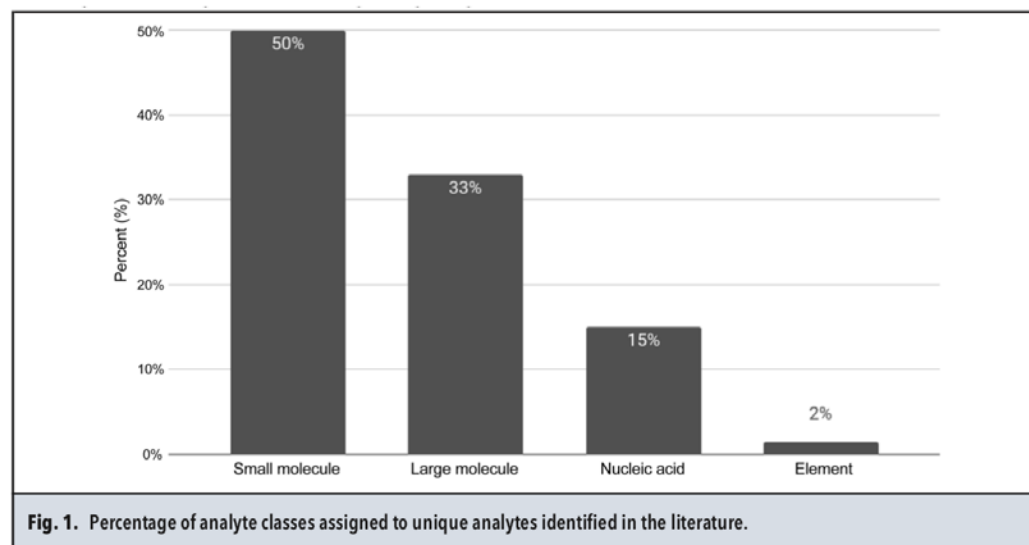
Protein separator cards

(Prospective DBS Studies)

State of the Science in Dried Blood Spots

Jeffrey D. Freeman,^{1*} Lori M. Rosman,² Jeremy D. Ratcliff,³ Paul T. Strickland,⁴ David R. Graham,⁵ and Ellen K. Silbergeld⁴

CONTENT: A scoping review of reviews methodology was used for characterizing the state of the science in DBS. We identified 2018 analytes measured in DBS and found every common analytic method applied to traditional liquid samples had been applied to DBS samples. Ana-



Exposure Biomarkers in DBS

Pollutant	Amount Required	Methodology	Selected References
Nicotine	1x4.8 mm or 3x3.2 mm punches (Murphy 2013)	LCMS	Murphy 2013, Yang 2013
	1x6.4 mm punch (Yang 2013)		
Benzene	1 DBS (50 uL)	GCMS	Funk 2008
Organophosphate and carbamate	1 DBS punch (15 uL) (Hilborn 2004, Quandt 2010)	Cholinesterase Assay	Trudeau 2007, Quandt 2010
Organochlorine pesticides	1 DBS (50 uL) (Batterman 2014)	GCMS	Batterman 2014
DTT	1 DBS (20 uL) (L'Homme 2015)		L'Homme 2016
Chlorinated persistent organic pollutants	12.5 mm diameter spot, 50uL (Lehner,2018)	GC-ECD (Lehner,2018)	Lehner,2018
Organofluorine pesticide	LOD 0.1 ng/mL (Raju 2016)	LCMS	Raju 2016
Polychlorinated biphenyls	1 DBS (50 uL) (Batterman 2014, Lu 2012)	GCMS	Batterman 2014
	1 DBS (20 uL) (L'Homme 2015)		L'Homme 2016
Polybrominated diphenyl esters	1 DBS (50 uL) (Batterman 2014, Lu 2012)	GCMS	Batterman 2014
			L'Homme 2016
Perfluoroalkyl compounds	1 DBS (50 uL) (Ma 2013b, Shlosberg 2012), 16-mm diameter discs LOD 0.03ng/L PFOS, LOD 0.05 ng/L PFOA (Ghassabian 2018)	LCMS(Ma), HPLC–MS/MS (Ghassabian 2018)	Batterman 2014 Ma 2013a, Shlosberg 2011/2012, Kato 2009
Polyaromatic Hydrocarbons (PAHs)	1 DBS (15 uL) (Provatas 2017)	LCMS with ultraviolet–visible detection	Provatas 2017
Bisphenol A	50 uL DBS (Ma 2013a), 16-mm diameter discs, (Ghassabian 2018)	LCMS(Ma), HPLC–MS/MS (Ghassabian 2018)	Ma 2013
Perchlorate	10x3.2 mm punches (Otero-Santos 2009)	GCMS	Otero-Santos 2009
Domoic Acid	LOD 0.01 ng/mL	cELISA	Maucher 2005
Brevetoxins	LOD 0.31 nM	Radioimmunoassay, c-ELISA	Woofter 2003, Maucher 2007, Fairey 2001
Ciguatoxin	LOD 0.006 ng/mL	Cytotoxicity Assay	Decharoui 2005
Ochratoxin	1 8.8 mm punch (Osteresch 2016)	LCMS	Osteresch 2016, Cramer 2015
Fumonisin	1x6 mm or 1x8 mm punch (Riley 2015)	LCMS	Riley 2015
Mycotoxins	50 µl, using 12.7 mm diameter hole-puncher cutting out the whole spot,LOD 10 pg/mL(Xue,2016)	Agilent 1100 HPLC-fluorescence detection system	Xue 2016
Pb	1 DBS (20 uL) (Vacchina 2014)	ICPMS	Vacchina 2014, Funk 2013, Lehner 2013
As	1 DBS (20 uL) (Vacchina 2014)	ICPMS	Vacchina 2014, Funk 2013, Lehner 2013
Cd	1 DBS (20 uL) (Vacchina 2014)	ICPMS	Vacchina 2014, Funk 2013, Lehner 2013
Hg	1/2 DBS (30 uL) (Funk 2013)	ICPMS	Funk 2013, Lehner 2013

Use of Dried Blood Spots for Estimating Children's Exposures to Heavy Metals in Epidemiological Research

William E Funk^{1*}, Joachim D Pleil², Dana J Sauter³, Thomas W McDade⁴ and Jane L Holl¹

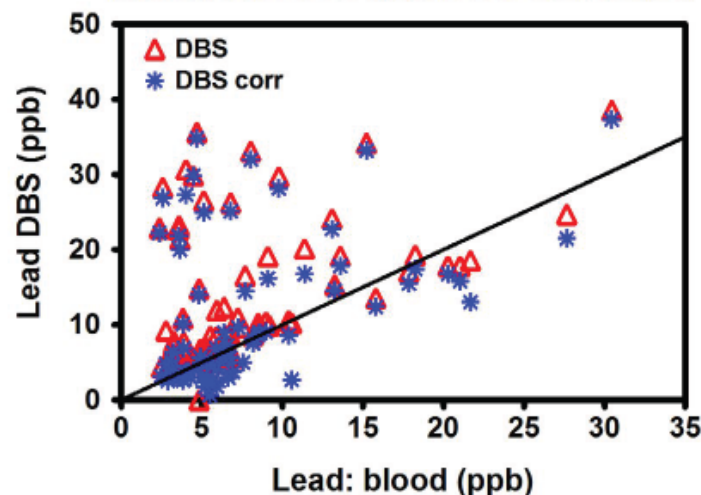
¹Department of Preventive Medicine, Northwestern University, Chicago, IL, USA

²United States Environmental Protection Agency, National Exposure Research Laboratory/ORD, Research Triangle Park, NC, USA

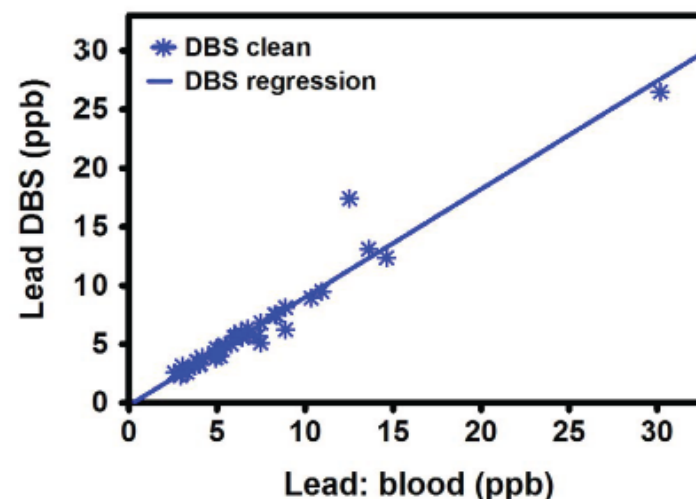
³Cabot Microelectronics, Aurora, IL USA

⁴Department of Anthropology and Institute for Policy Research, Northwestern University, Evanston, IL, USA

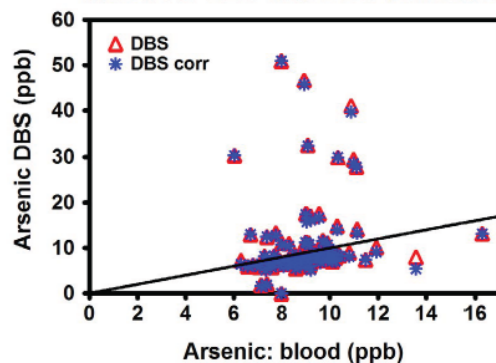
**Lead scatterplot:
blood vs. DBS and DBS corrected**



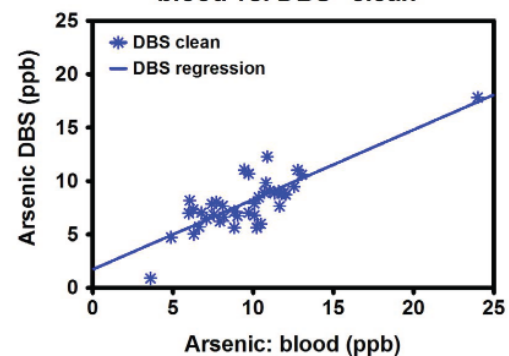
**Lead scatterplot:
blood vs. DBS "clean"**



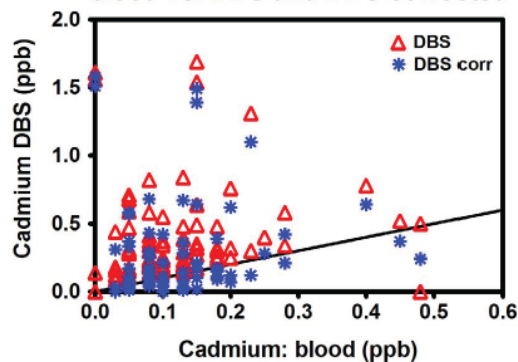
**Arsenic scatterplot:
blood vs. DBS and DBS corrected**



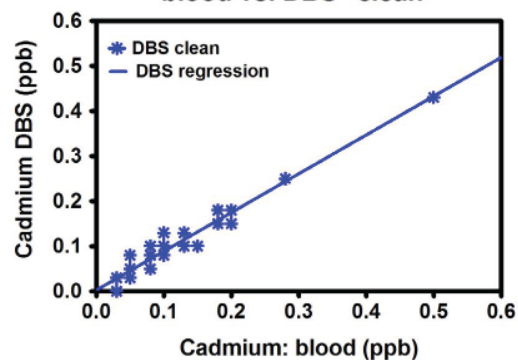
**Arsenic scatterplot:
blood vs. DBS "clean"**



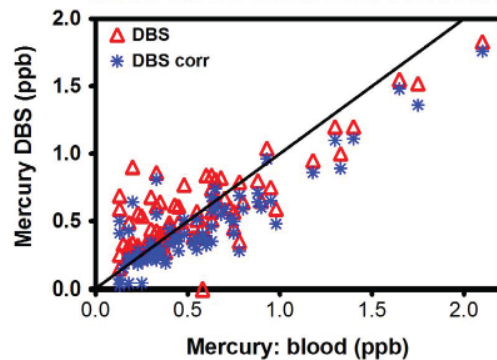
**Cadmium scatterplot:
blood vs. DBS and DBS corrected**



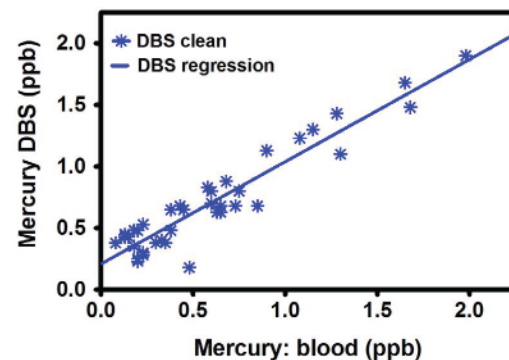
**Cadmium scatterplot:
blood vs. DBS "clean"**



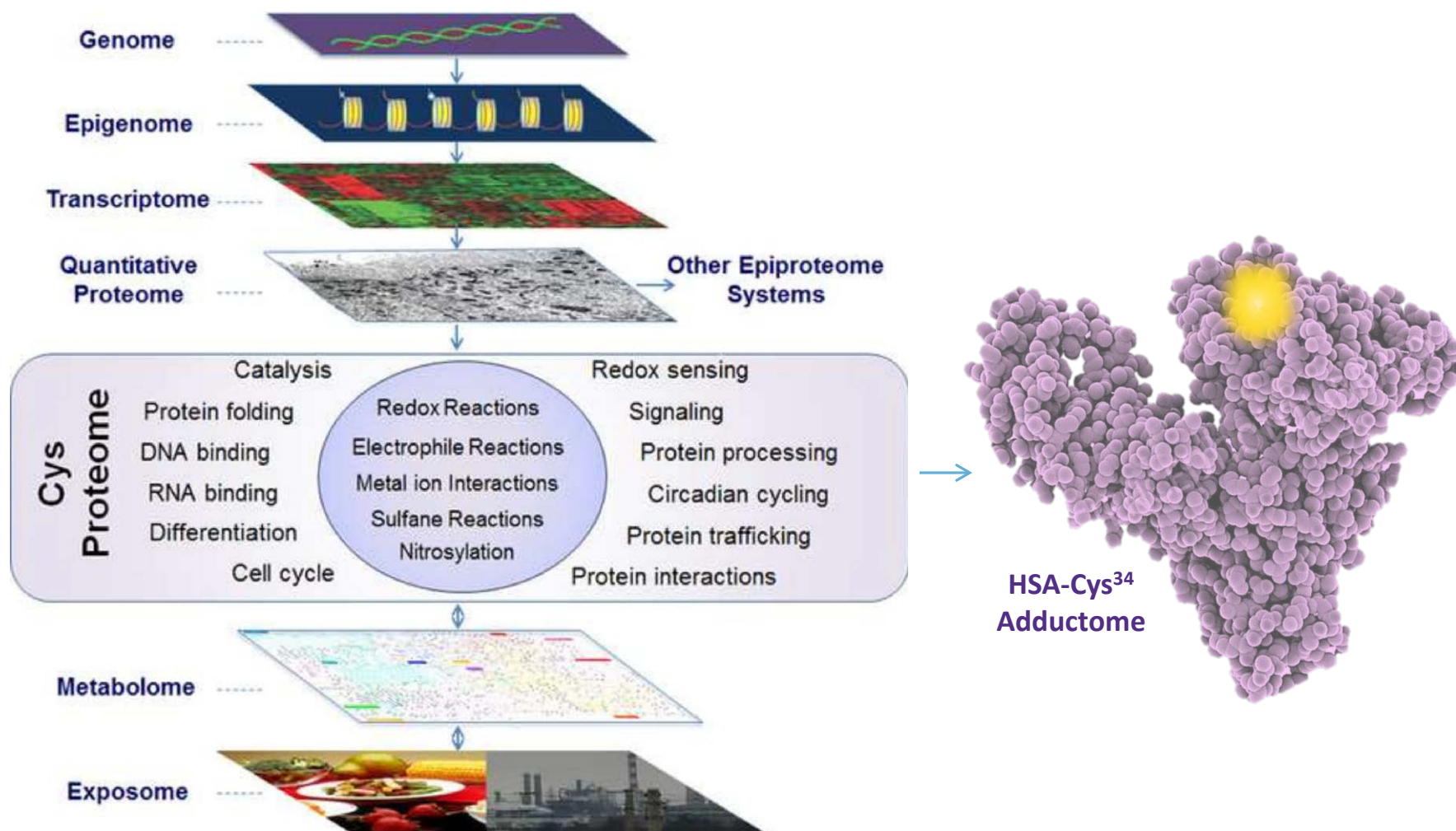
**Mercury scatterplot:
blood vs. DBS and DBS corrected**



**Mercury scatterplot:
blood vs. DBS "clean"**

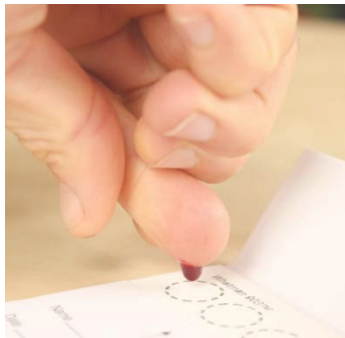


Integrating Omics data

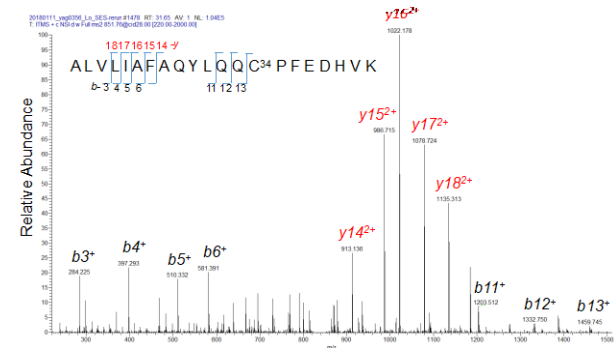
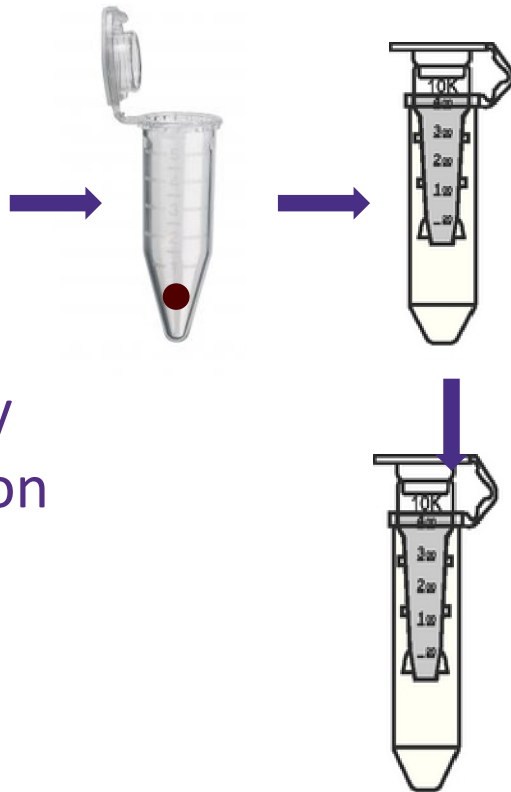


Multiple Omics in DBS Samples

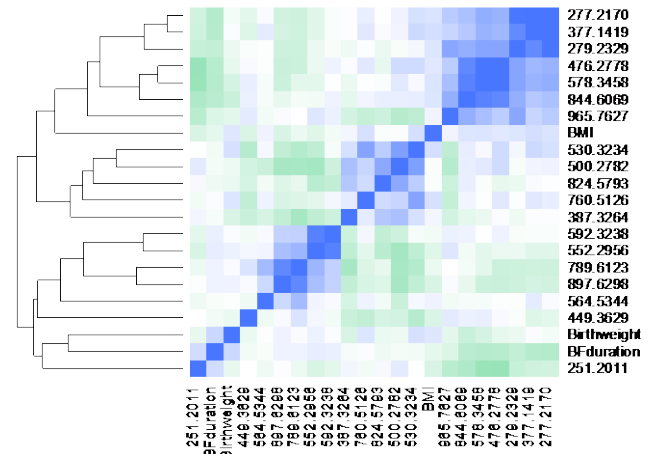
Untargeted & targeted analyses



Field-friendly
blood collection



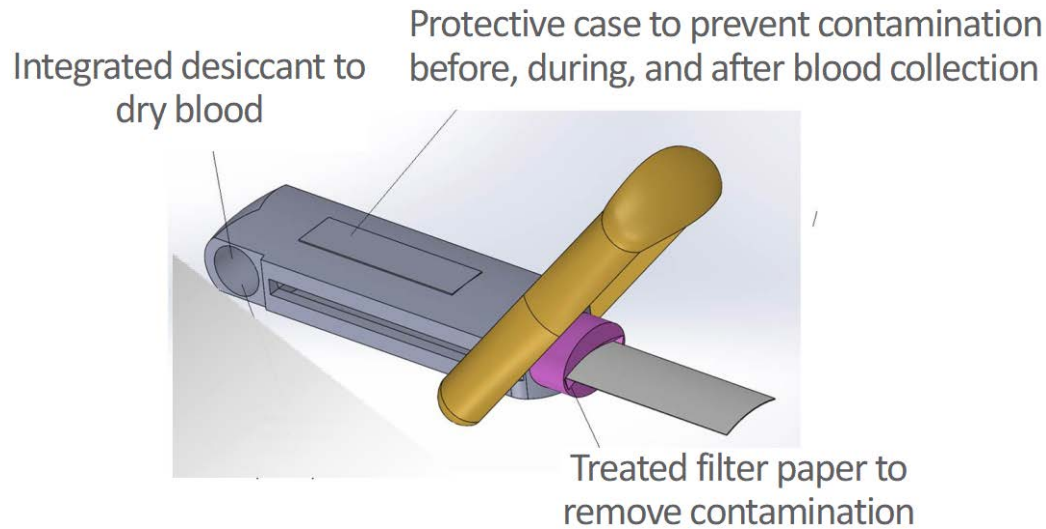
Adductomics



Metabolomics

Specialized DBS Collection Devices

Toxic metal screening



Non-invasive blood collection

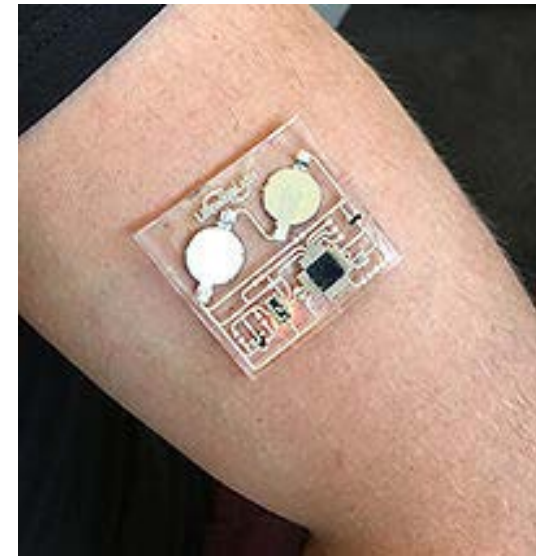


Photo curtesy of Lifeware Labs

Questions?