The Exposome and Adductomics: Promises and Pitfalls

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Work supported by NIEHS

The exposome shifts the paradigm

[redacted]

Air

[redacted]

Water

[redacted]

Diet

[redacted]

Lifestyle

[redacted]

VOCs

[redacted]

RO&CS

If the exposome includes all chemicals - from all sources,

then how should we characterize exposures for health studies?

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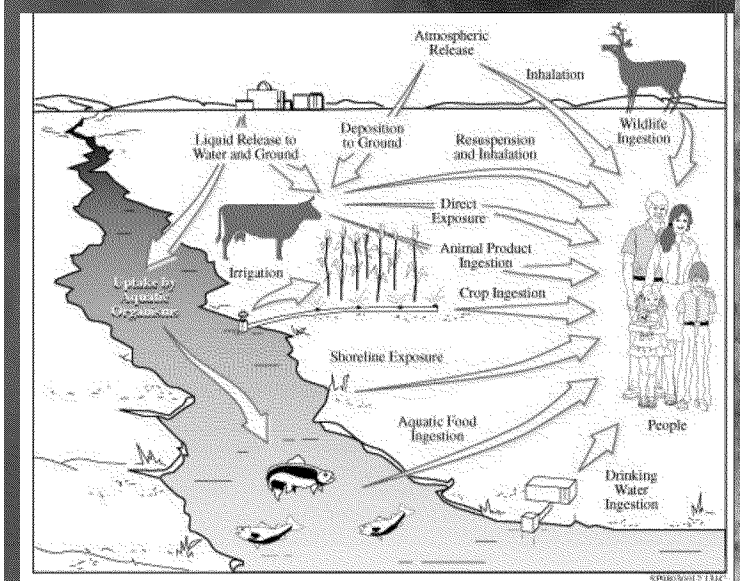
Bottom-up exposomics

- Large sampling effort
- Ignores endogenous sources of exposure



Courtesy United States Department of Energy, Hanford site

Evaluate uptake, metabolism, etc. of *important* agents (to estimate dose)

↑

Test for associations with case status

↑

Measure all analytes in air, water, food, etc. from cases and controls

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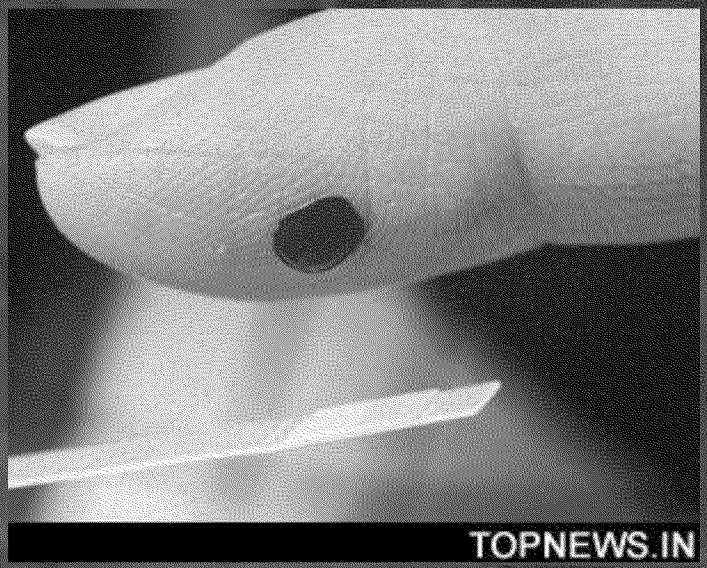
Top-down exposomics



- Relevant to internal dose
- Manageable sampling effort
- Includes all sources

Measure all analytes in blood from cases and controls

↓

Test for associations with case status

↓

Identify *important* agents and determine sources of exposure

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Reducing the universe of chemicals

"The studies in recent years … indicate to us that the formation … of alkylating or arylating structures in chemical carcinogenesis is emerging as a central, but not necessarily universal, theme".

EC Miller and JA Miller, 1966, *Pharmacol Rev* 18:806

"The liver … can convert stable organic compounds to potent alkylating agents … that … cause damage to liver, kidney, bone marrow, and other tissues… Similarly, allergic responses … may be mediated by the reaction of body proteins with trace amounts of chemically active … metabolites".

BB Brodie *et al*., 1971, *PNAS* 68:160

Alkylating agents are *electrophiles* (*'electron lovers'*) that react with *nucleophiles* (electron-rich substances), including DNA and proteins. The Millers and Brodie were among the fist to recognize that toxicants are generally electrophiles.

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Focus upon electrophiles

"Although there are important exceptions, the vast majority of effects attributed to reactive toxicity arise from the reaction between an electrophilic chemical and a biological nucleophile."

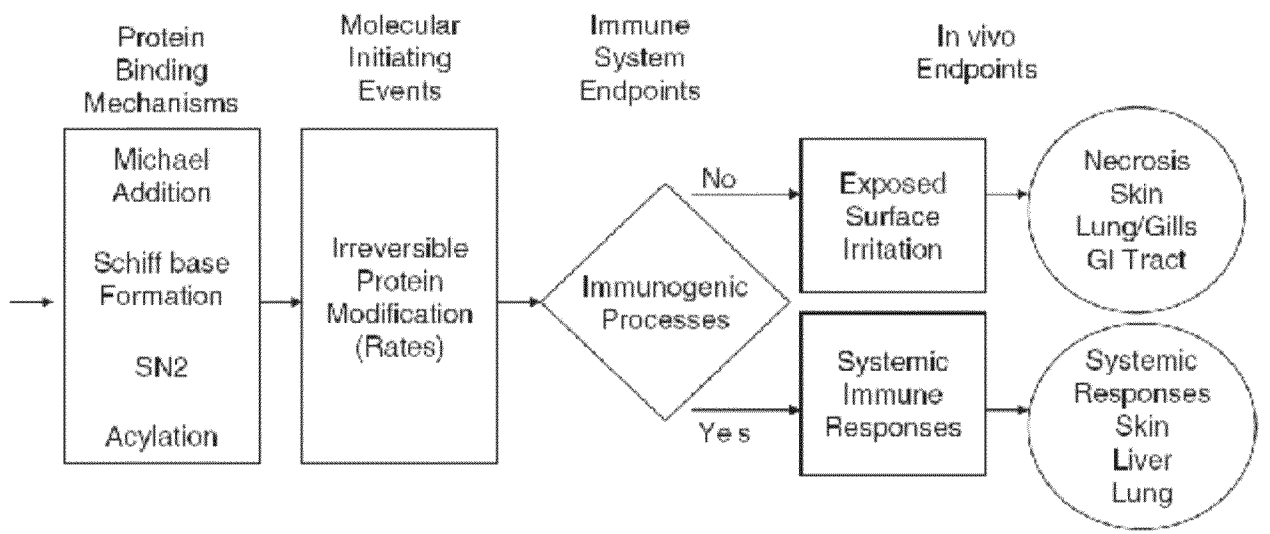

Figure 2. Schematic representation of the proposed pathways for reactive toxicity from soft electrophiles.

Schultz, TW, *et al., 2006, SAR and QSAR Environ Res*, 17(4): 413-428.

Adductomics: characterizing exposures to electrophiles

- Electrophiles have short life spans in vivo
  - E.g., alkylating agents, aldehydes, epoxides, quinones, reactive oxygen species, reactive carbonyl species
- But they form stable adducts (addition products) by reacting with biological nucleophiles (e.g., DNA, proteins, glutathione)
- By measuring all adducts of a given nucleophile we perform 'adductomics'
  - Triple quadrupole MS with selected reaction monitoring (SRM) has the necessary sensitivity and specificity

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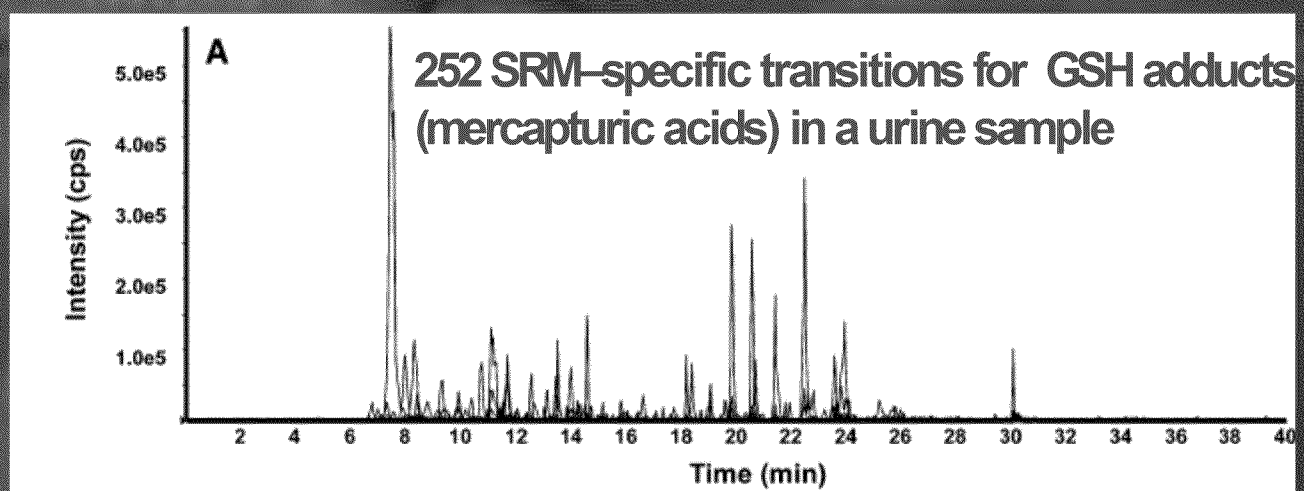
Glutathione adductomics: an extension of metabolomics

Anal. Chem. **2007**, *79*, 2918–2926

Tools in Metabonomics: An Integrated Validation Approach for LC-MS Metabolic Profiling of Mercapturic Acids in Human Urine

Silvia Wagner,† Karoline Scholz,† Maximilian Sieber,† Marco Kellert,† and Wolfgang Voelkel†,‡,*



GSH adducts are transient (vary greatly within days)

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DNA adductomics

ANTIOXIDANTS & REDOX SIGNALING
Volume 8, Numbers 5 & 6, 2006
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Requires 100 μg DNA (equivalent to 20 ml blood)

Forum Original Research Communication

Development of the Adductome Approach to Detect DNA Damage in Humans

ROBERT A. KAN
HIROKAZU T

374 SRM-specific transitions for DNA adducts in human lung tissue

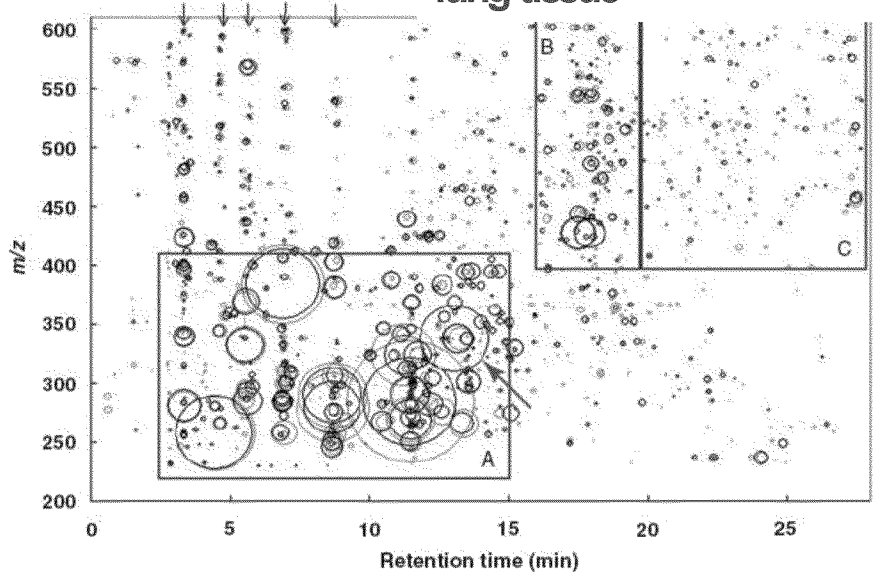

FIG. 2. Adductome map of putative DNA adducts detected in human lung tissue from a nonsmoker (*light circles*) and a smoker (*dark circles*). The neutral loss of 2′-deoxyribose from positively ionized 2′-deoxynucleoside putative adducts was analyzed by LC/ESI–MS/MS in MRM mode transmitting the $[M + H]^+ > [M + H - 116]^+$ transition over a total of 374 transitions in the mass range from *m/z* 228.8 to *m/z* 602.8. *Boxes A* through *C* define zones of activity that are discussed in the text. The *arrow* indicates the presence of a large putative adduct detected in the DNA of the smoker that was 4.8 times larger than in the DNA of the nonsmoker.

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Protein adductomics?

- Protein adducts have longer residence times than urinary adducts (28 d for HSA and 63 d for Hb)
- Proteins much more abundant than DNA in blood (1 ml contains 150 mg Hb, 30 mg of HSA, and 0.003-0.008 mg of DNA)
- HSA and/or Hb adducts have been related to some occupational or environmental exposures
  - Ethylene oxide, 1,3-butadiene, benzene, aflatoxin, PAHs, acrylamide

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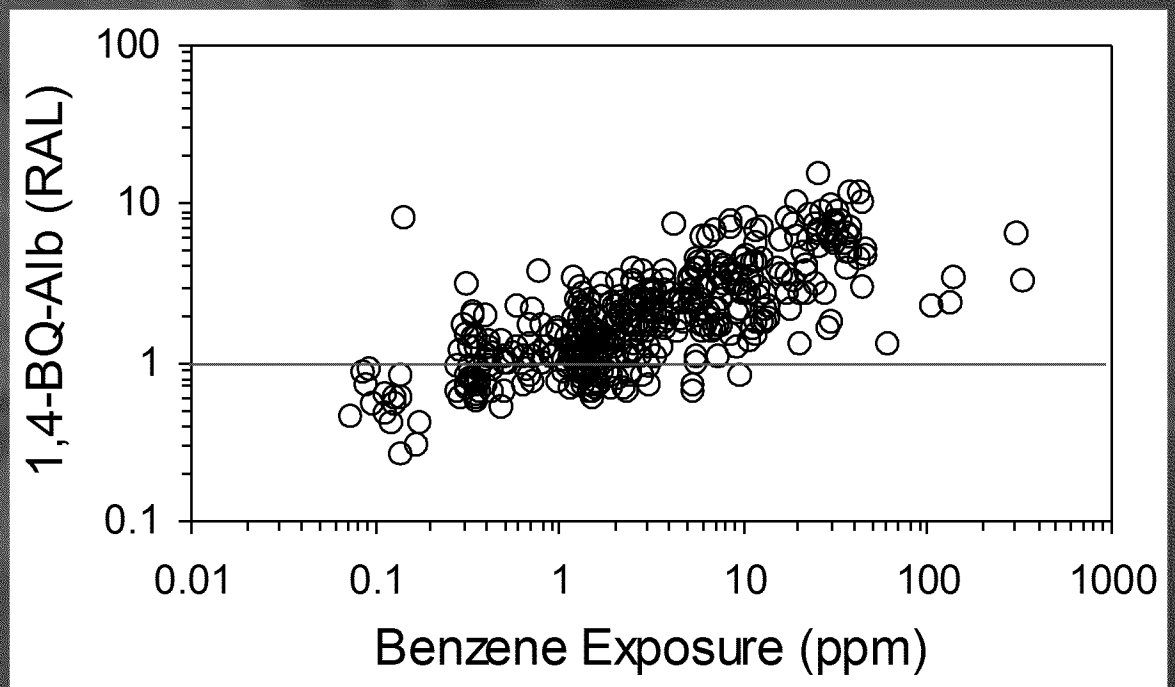
Benzoquinone adducts of HSA in benzene-exposed workers (*n* = 439)

Benzene → Phenol → Hydroquinone → Benzoquinone



Background adducts:

- Environmental benzene
- Hydroquinone in food and cigarette smoke
- Endogenous production of phenol

Data from: Yeowell O'Connell et al. *CEBP* (2001); Rappaport et al. *Cancer Res* (2002); Lin et al. *EHP* (2007)

Human Serum Albumin (HSA)

- Most abundant protein in serum
- 585 Amino acids
- 35 Cys (-SH) residues
  - 34 Used for disulfide bonds
- One free thiol (Cys^{34})
  - Represents 90% of free thiols in serum
  - Scavenges ROX and other electrophiles
- *Preferred site for adduction of electrophiles in serum (conserved in all mammalian species)*

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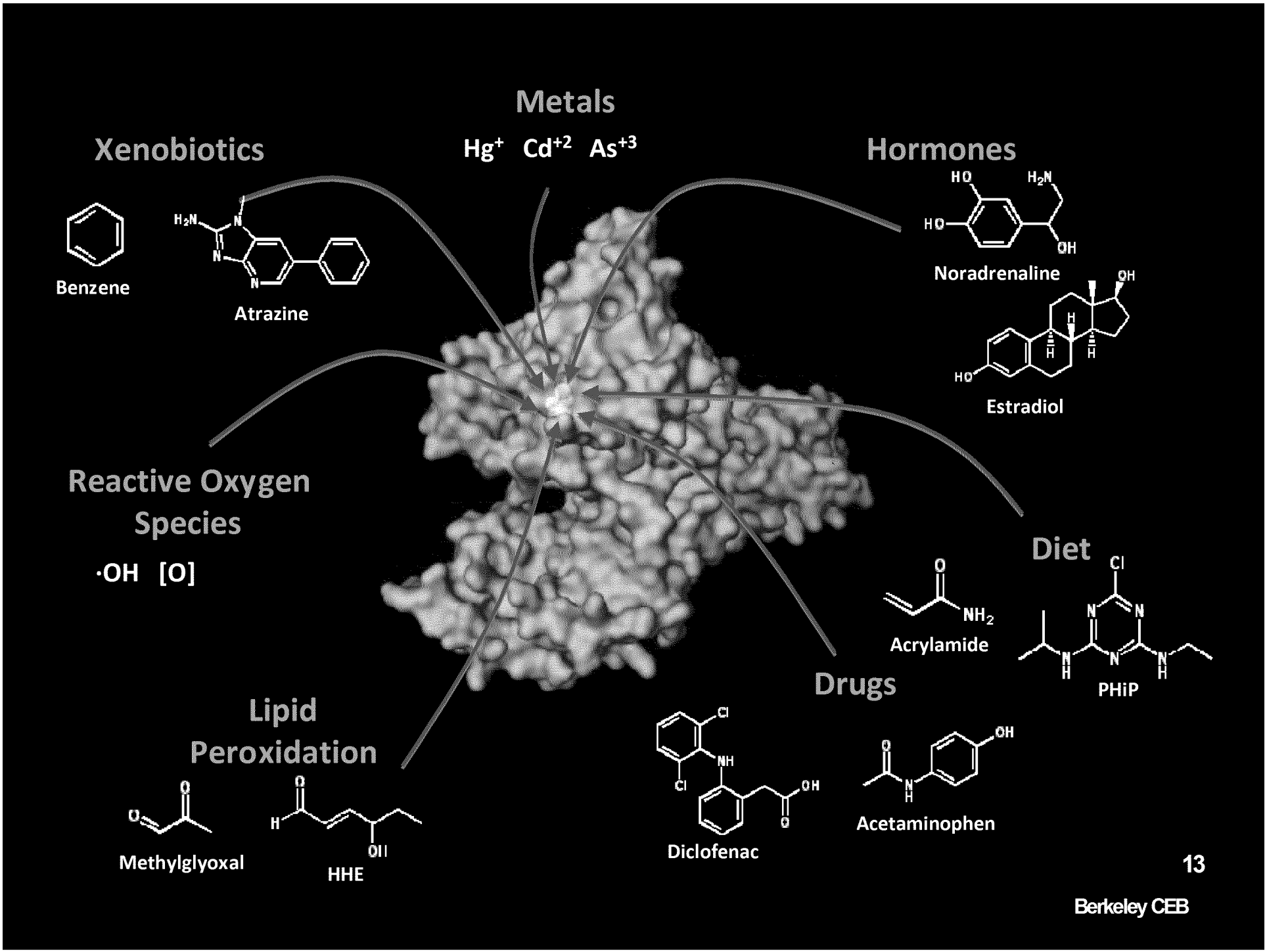
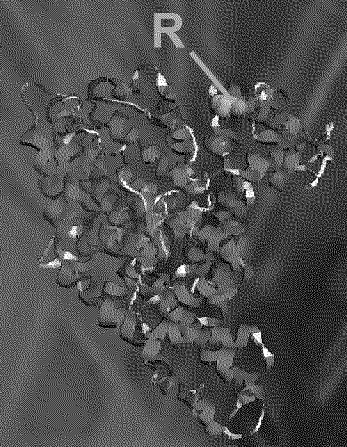

Metals
Hg+ Cd+2 As+3
Xenobiotics
Benzene
Atrazine
Hormones
Noradrenaline
Estradiol
Reactive Oxygen Species
·OH [O]
Diet
Acrylamide
PHiP
Drugs
Diclofenac
Acetaminophen
Lipid Peroxidation
Methylglyoxal
HHE
13
Berkeley CEB

Scheme for characterizing adducts of HSA-Cys^{34}

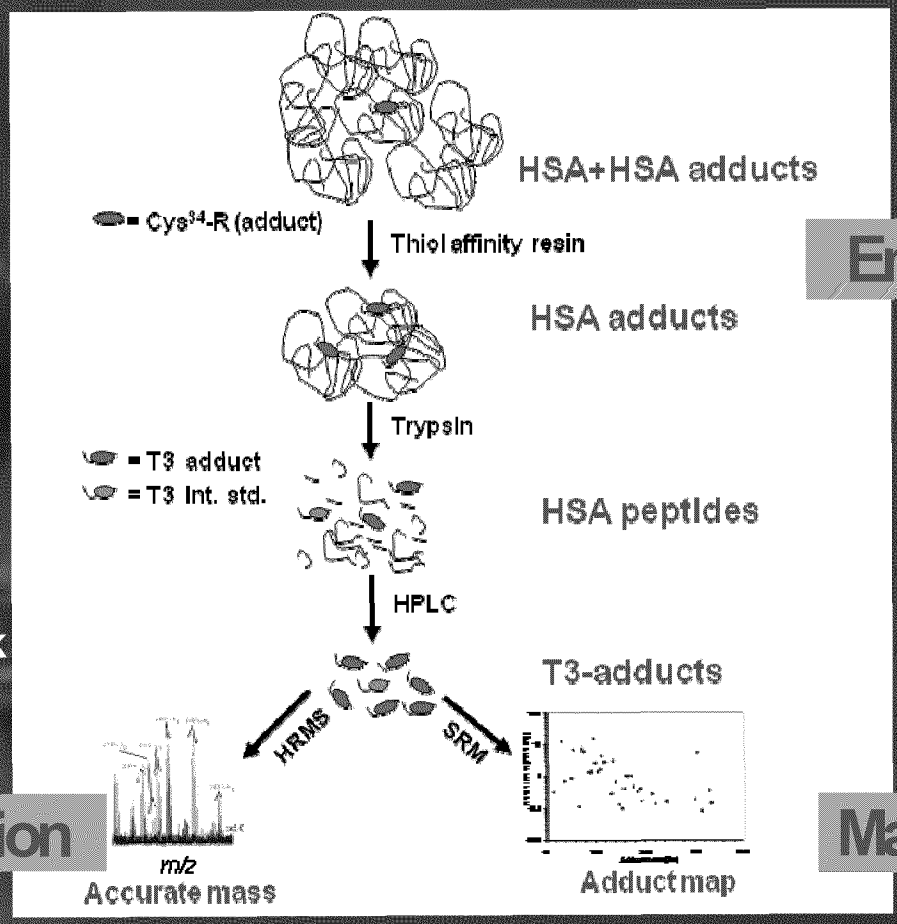


HSA Adduct

R

ALVLIAFAQYLQQCPVEDHVK

T3 Adduct



Enrichment

Identification

Mapping

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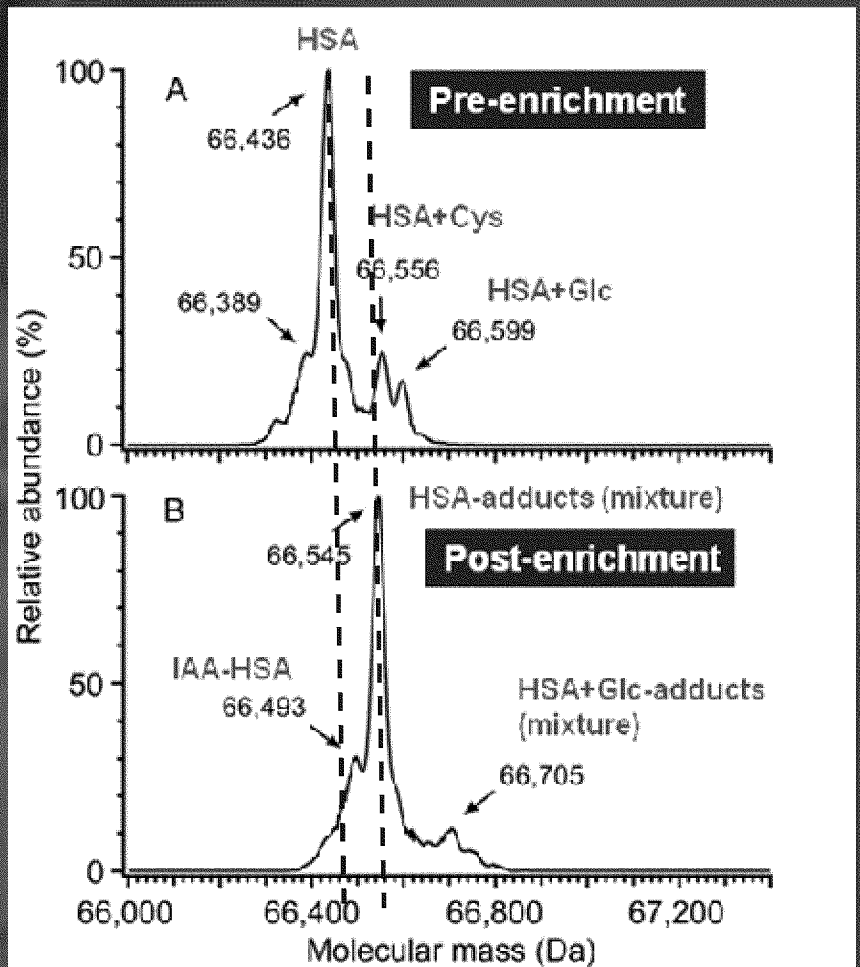
Adduct enrichment

HSA from a volunteer subject was analyzed by OrbiTrap MS (HSA-IAA was added as a positive control)

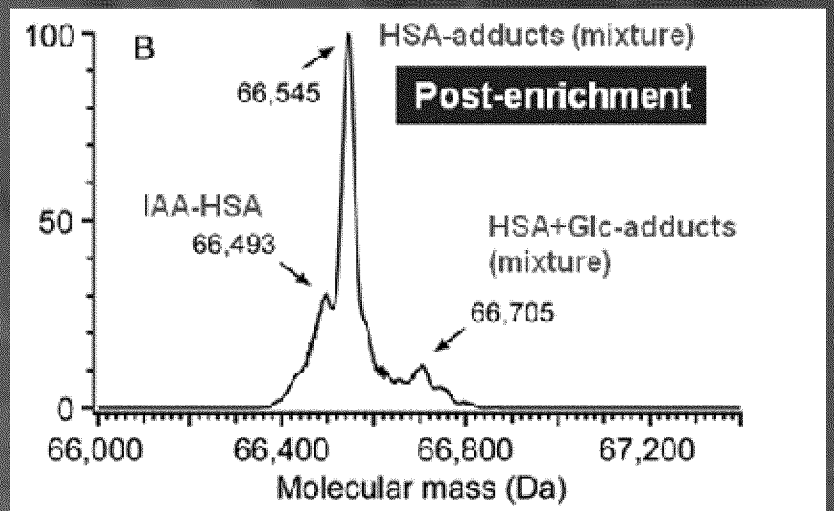


- Average adduct mass = 66,545-66,436 = 109 Da.
- About 3% of HSA is adducted

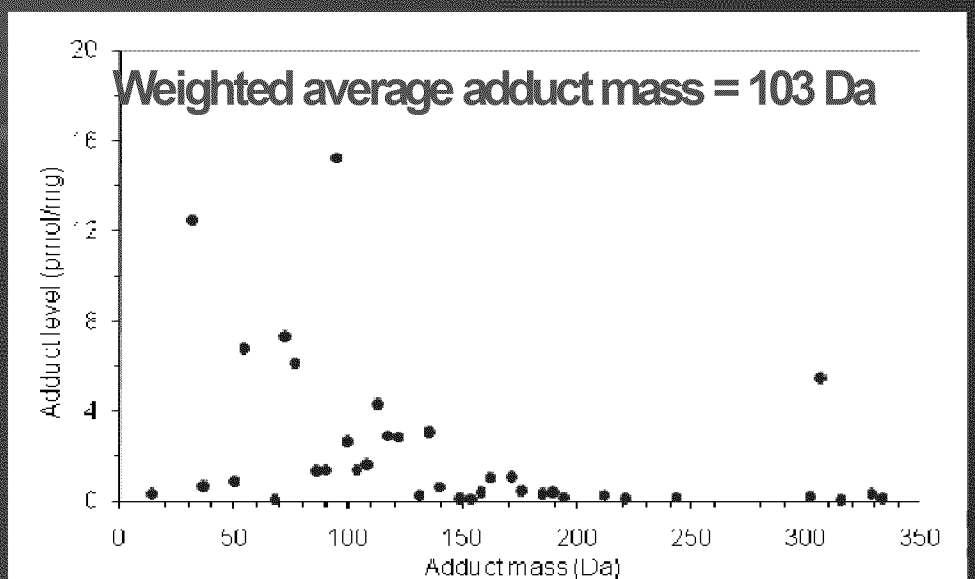
W. Funk *et al. Analyt Biochem,* in press.

Adduct mapping

Average adduct mass = 109 Da



Tryptic digest of 0.5 mg HSA from the same subject (312 SRM-specific transitions for T3 adducts)



- 37 Points contained one or more adducts
- Mass range: 12 - 335 Da
- Abundance range: 0.08 – 15 pmol/mg HSA
- Avg. abundance = 2 pmol/mg (0.015% of HSA) suggesting 200 expected adducts

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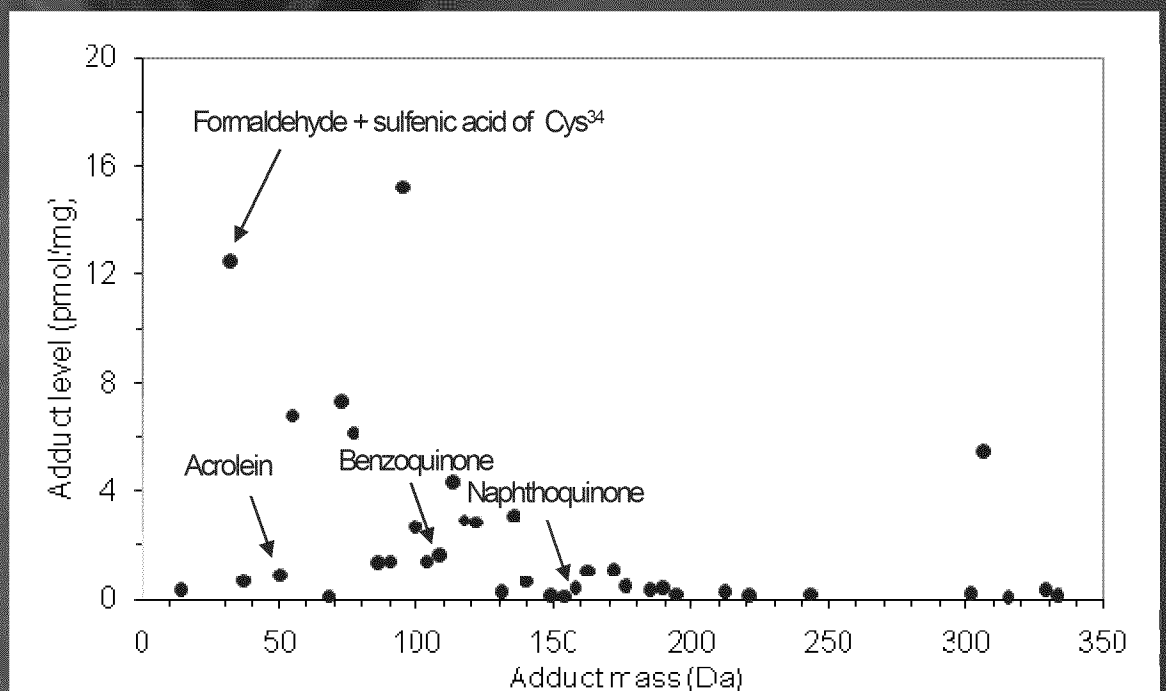
Adduct identification

Some adducts provided accurate masses via HRMS



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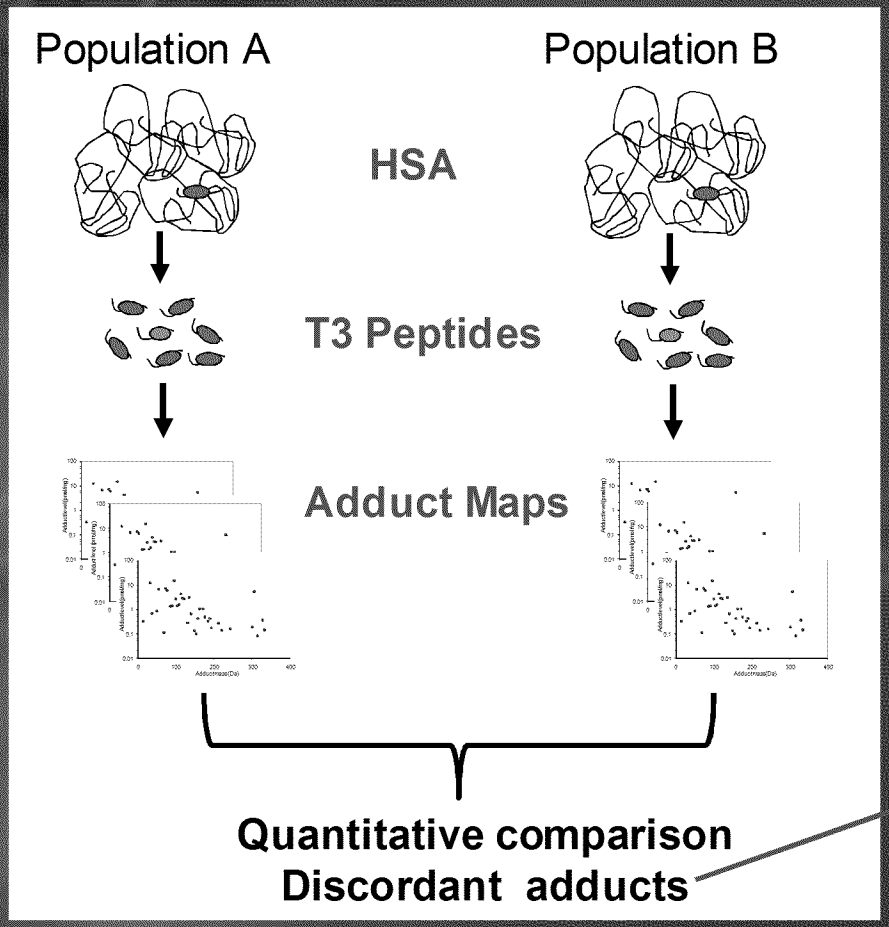
Adductomics and biomarker discovery



Currently comparing adduct maps between smokers & nonsmokers and between lymphoma cases & controls

Possible biomarkers of exposure or disease status

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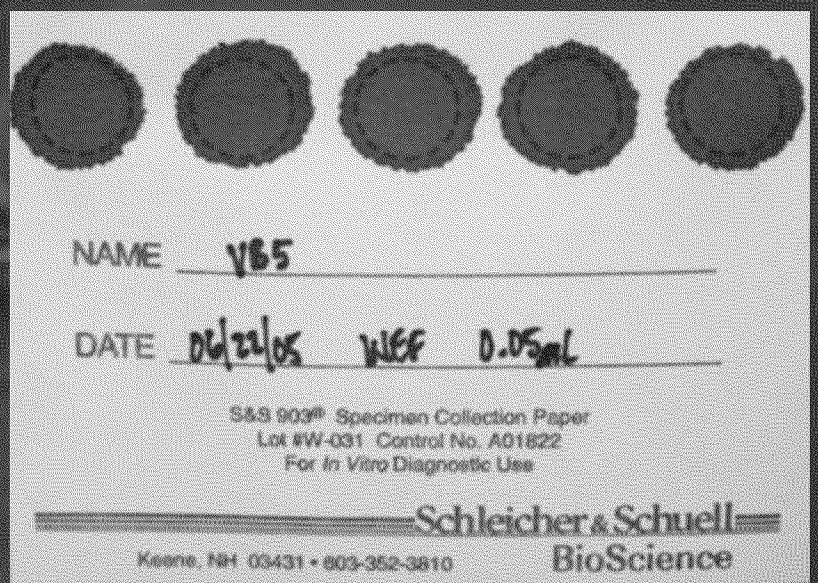
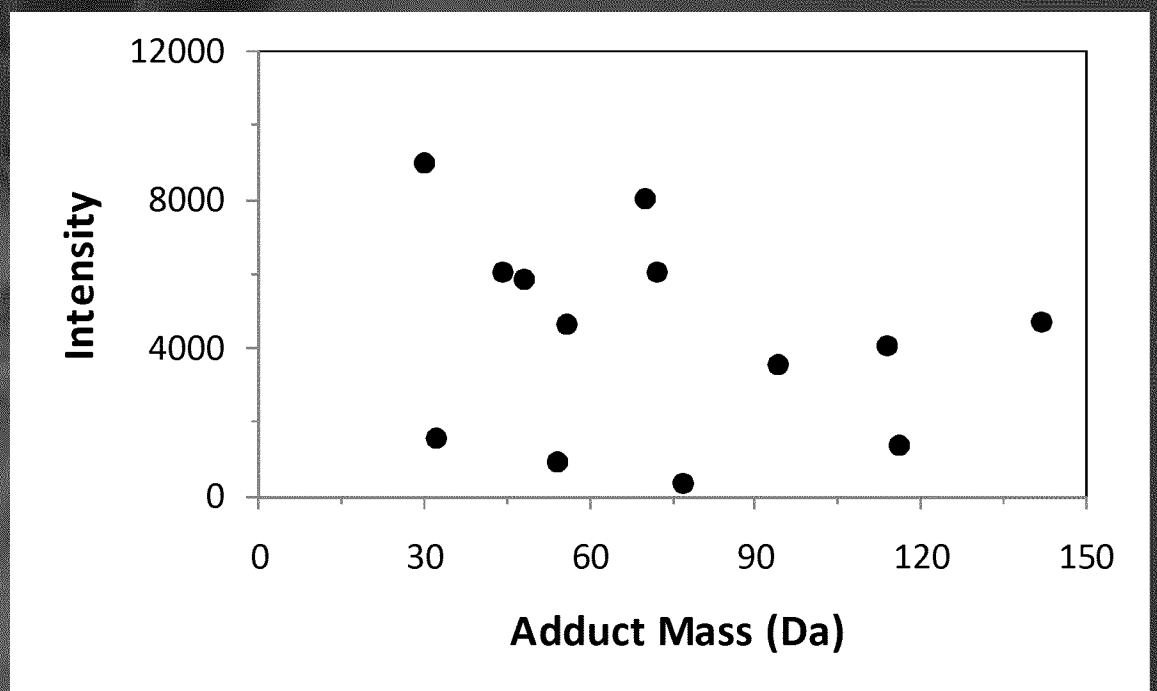
Adductomics with dried blood spots (DBS)

- One DBS contains 800 μg HSA (50 μl blood)
- SRM can detect HSA-Cys^{34} adducts in 1/8 – 1/4 of a DBS (3-mm or 6-mm punch)



HSA-Cys^{34} adducts in DBS

Fresh DBS from a volunteer subject (6-mm punch)



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Fetal dried blood spots and childhood leukemia

- HSA adducts reflect exposures during the last month of gestation
- The Northern California Childhood Leukemia Study has DBS from 1000 cases and controls (P. Buffler *et al.*)
  - *HSA adducts will be measured in a subset of these DBS*

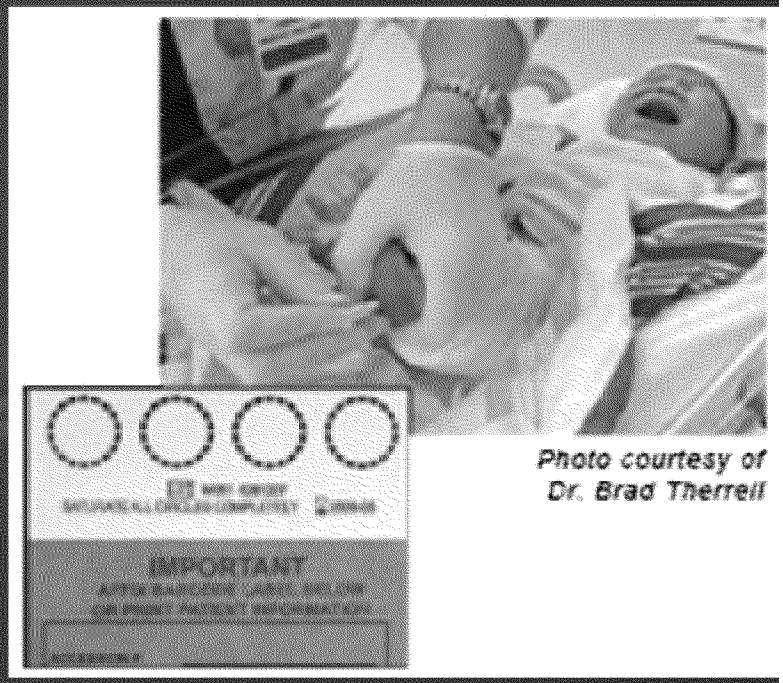

Photo courtesy of Dr. Brad Therrell

Measuring HSA adducts

Technology	Time frame	Volume of blood (ml)	Sensitivity (M)	Multiplex	Samples/d per analyst
Test tubes, handwritten labels, GC-MS	1980-1990	0.1 - 1	pM - nM	No	1 - 10
96-Well plates, bar codes, ELISA	1990-2000	0.01 – 0.1	fM - pM	No	10 - 100
Robotics, bar codes, SRM	2000-2010	0.01 – 0.1	aM - fM	Yes	10 - 100
Microfluidics, ELISA	2010 - 2020	0.001 – 0.01	aM - fM	??	100 - 1000

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Adductomics and P4 medicine

"P4 medicine is what I think is going to happen over the next 20 years. Medicine will move from its current largely reactive state to one that is *predictive*, that is *personalized*, that is eventually *preventive* and *participatory*… The idea would be that nanotech and microfluidic measurements will make it possible to have a device in the home that could prick the thumb, take a fraction of a droplet of blood, make 2500 measurements, … and send the information via wireless to a server."

Adductomics could provide some of these measurements!

A Conversation with Dr. Leroy Hood: Visionary Biologist and Biotechnologist
Paul S. Weiss, ACS Nano, 2007, 1 (4), pp 242–247.

With thanks to:

Evan Williams
Harry Li
Maria Demireva
Hasmik Grigoryan
Tony Iavarone
Patricia Buffler
Catherine Metayer
Mark van der Laan
Sherri Rose
Martyn Smith
Luoping Zhang

William Funk (UNC)
Suramya Waidyanatha (UNC)
Karen Yeowell-O'Connell (UNC)
Yu-Sheng Lin (UNC)
Nathaniel Rothman (NCI)
Qing Lan (NCI)
Roel Vermeulen (Utrecht U.)
Qingshan Qu (NYU)

Work supported by NIEHS