SUMMARY

Organized by Paul R. West, PhD (Stemina Biomarker Discovery, Madison, WI) and Gary Patti, PhD (Washington University, St. Louis, MO).

The Workshop was held in Room 101 of the Minneapolis Convention Center on Wednesday, June 12 from 5:45 PM to 7:00 PM.

- Attendance was approximately 120 individuals

- Following a brief introduction, our distinguished expert panelists were introduced:
  - Lloyd Sumner, PhD; Noble Foundation, OK
  - Sunia Trauger, PhD; Harvard University, MA
  - Steffen Neumann, PhD; Leibniz Institute of Biochemistry IPB Halle, Germany

- The following slides (#2-#30) were presented (about 40 minutes) followed by an open audience question and answer period where a wide range of topics were discussed including:
  - Metabolite databases
  - Data analysis
  - Chemical standards
  - Chemical structure confirmation procedures and techniques
  - Normalization of data
  - A location to list useful information for the metabolomics community

ASMS Workshop Metabolomics

Organizers

Paul West (Stemina)

Gary Patti (Washington University)

Panelists

Sunia Trauger (Harvard University)

Lloyd Sumner (Noble Foundation)

Steffen Neumann (IPB Halle)

ASMS Workshop Metabolomics

Progress, Updates, and *State of Innovation*
Gary Patti

“Metabolomic Gotchas” Paul West

Discussion Points Panelists

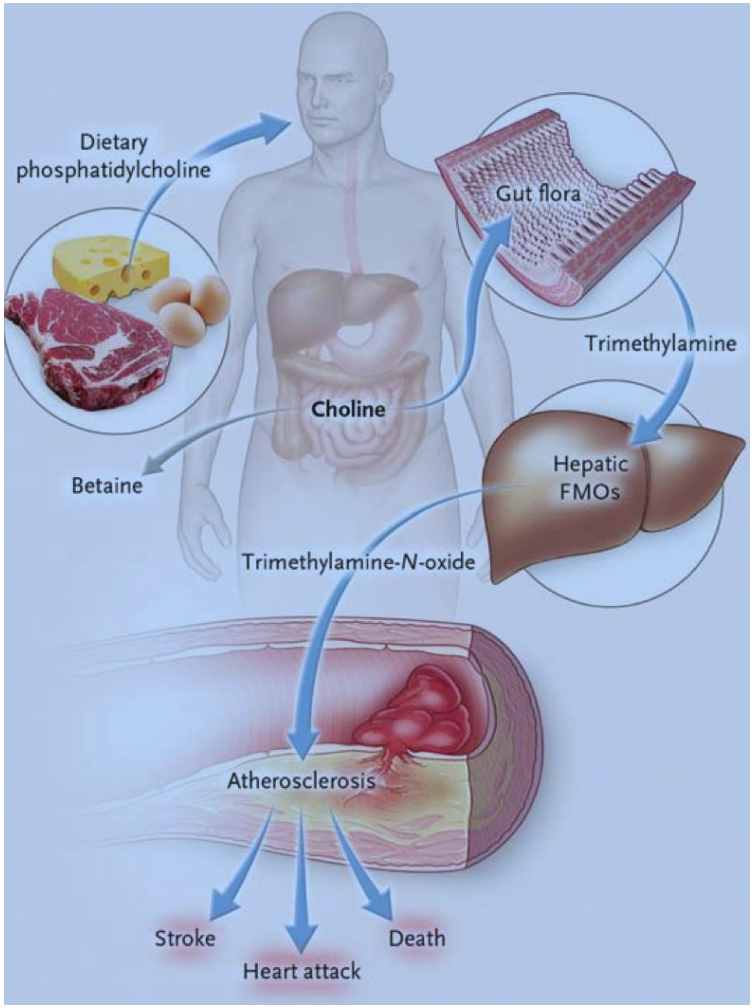
ASMS Workshop Metabolomics

Progress, Updates, and State of Innovation

Gary Patti

1. Translating metabolomic research into the clinic.

1. Translating metabolomic research into the clinic.



Gut flora metabolism of phosphatidylcholine promotes cardiovascular disease. Hazen et al., Nature 2011.

Increased trimethylamine N-oxide (TMAO) associated with increased risk of cardiovascular disease

1. Translating metabolomic research into the clinic.

The NEW ENGLAND JOURNAL of MEDICINE

ESTABLISHED IN 1812 APRIL 25, 2013 VOL. 368 NO. 17

Intestinal Microbial Metabolism of Phosphatidylcholine and Cardiovascular Risk

W.H. Wilson Tang, M.D., Zeneng Wang, Ph.D., Bruce S. Levison, Ph.D., Robert A. Koeth, B.S., Earl B. Britt, M.D., Xiaoming Fu, M.S., Yuping Wu, Ph.D., and Stanley L. Hazen, M.D., Ph.D.

1. Translating metabolomic research into the clinic.

The NEW ENGLAND JOURNAL of MEDICINE

ESTABLISHED IN 1812 APRIL 25, 2013 VOL. 368 NO. 17

Intestinal Microbial Metabolism of Phosphatidylcholine and Cardiovascular Risk

W.H. Wilson Tang, M.D., Zeneng Wang, Ph.D., Bruce S. Levison, Ph.D., Robert A. Koeth, B.S., Earl B. Britt, M.D., Xiaoming Fu, M.S., Yuping Wu, Ph.D., and Stanley L. Hazen, M.D., Ph.D.

4007 patients examined at multiple time points

1. Translating metabolomic research into the clinic.

The NEW ENGLAND JOURNAL of MEDICINE

ESTABLISHED IN 1812 APRIL 25, 2013 VOL. 368 NO. 17

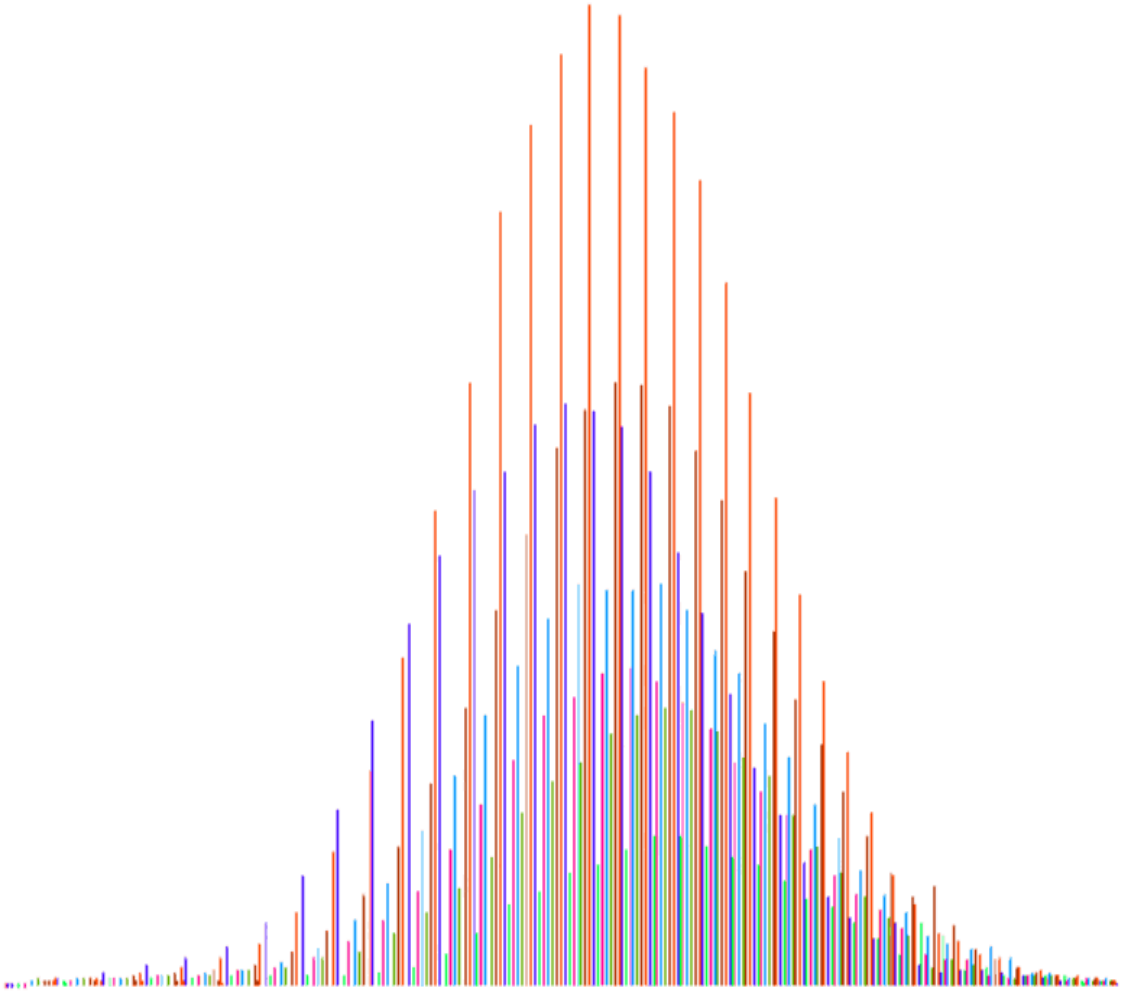
Intestinal Microbial Metabolism of Phosphatidylcholine and Cardiovascular Risk

W.H. Wilson Tang, M.D., Zeneng Wang, Ph.D., Bruce S. Levison, Ph.D., Robert A. Koeth, B.S., Earl B. Britt, M.D., Xiaoming Fu, M.S., Yuping Wu, Ph.D., and Stanley L. Hazen, M.D., Ph.D.

4007 patients examined at multiple time points

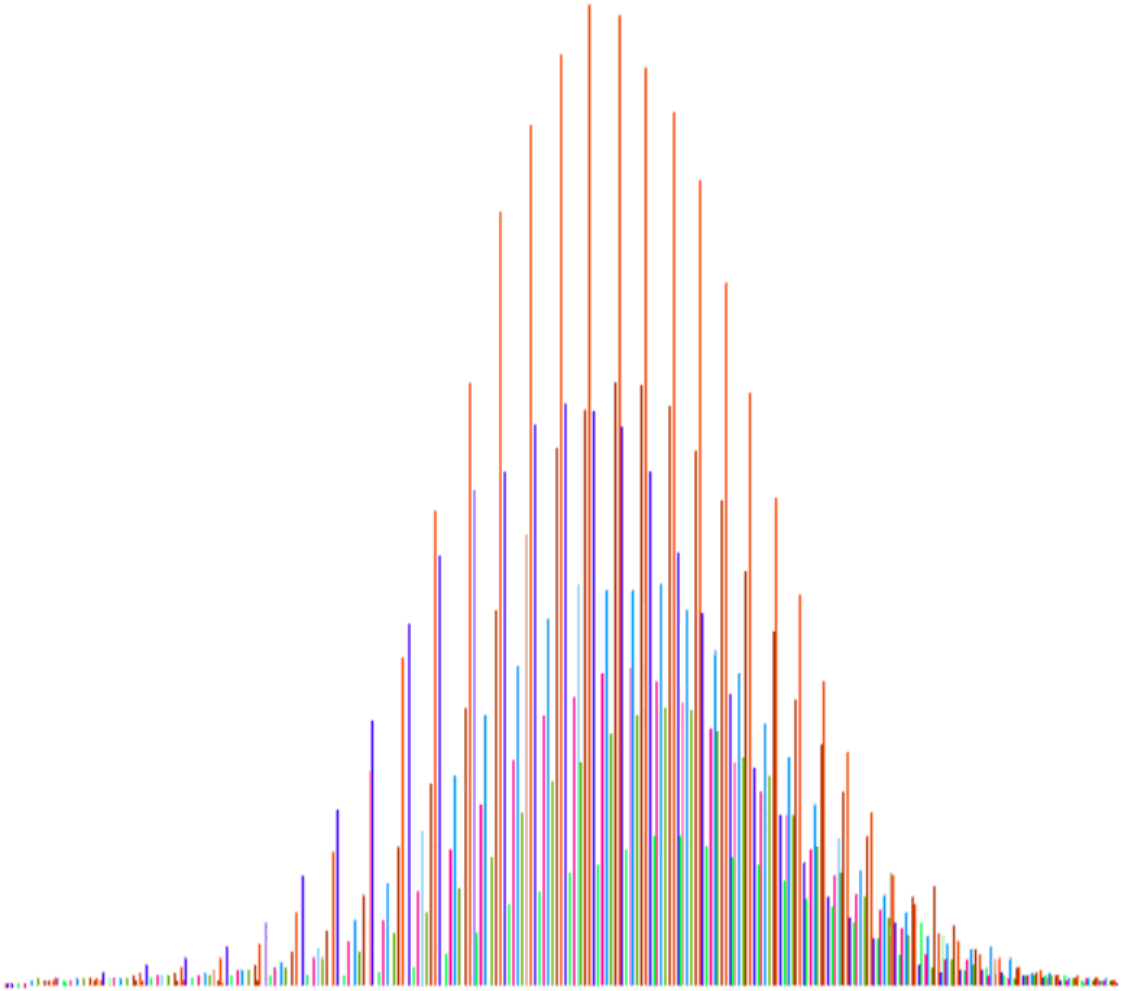
>10,000 analyses

2. Improvements in MS technologies



2. Improvements in MS technologies

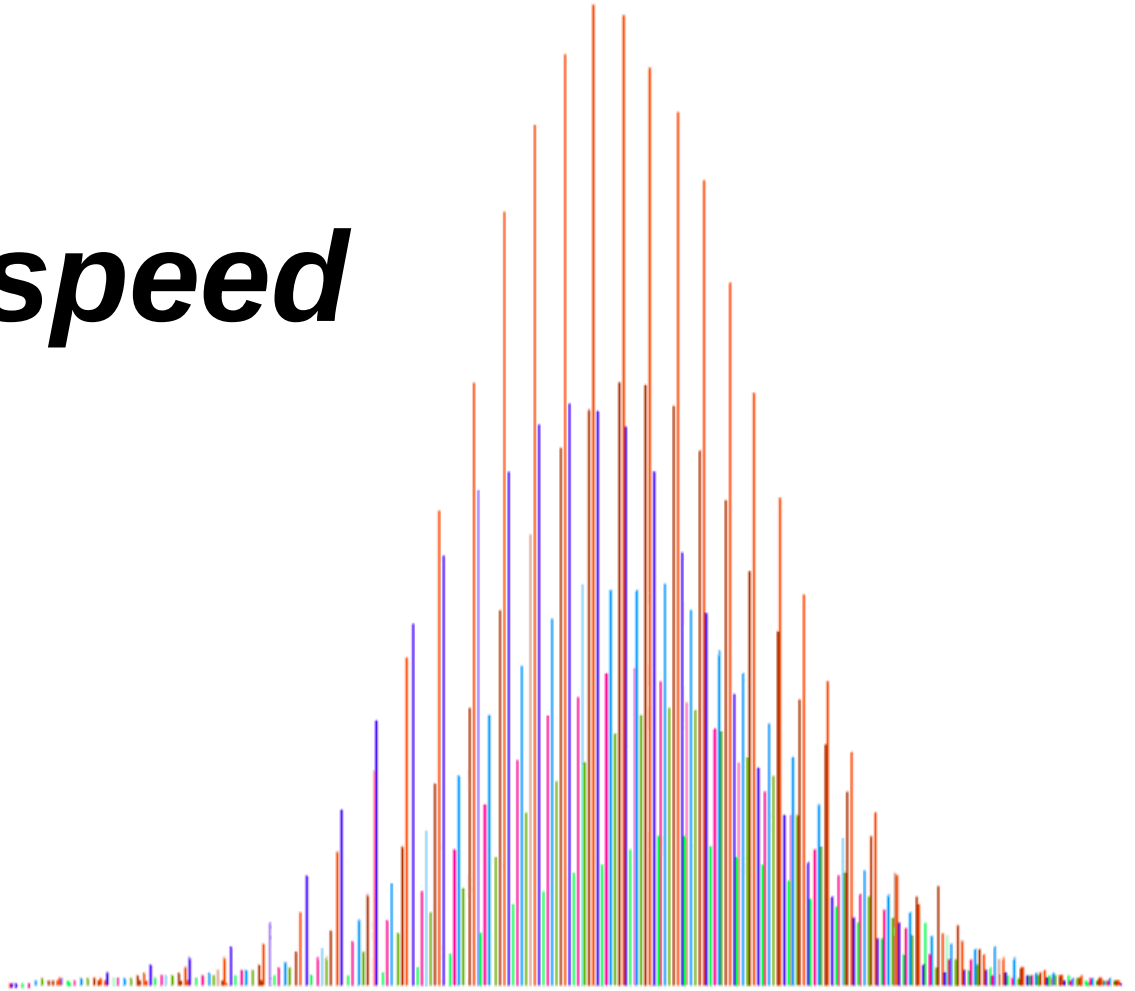
ion funnel



2. Improvements in MS technologies

ion funnel

MS^2 acq. speed

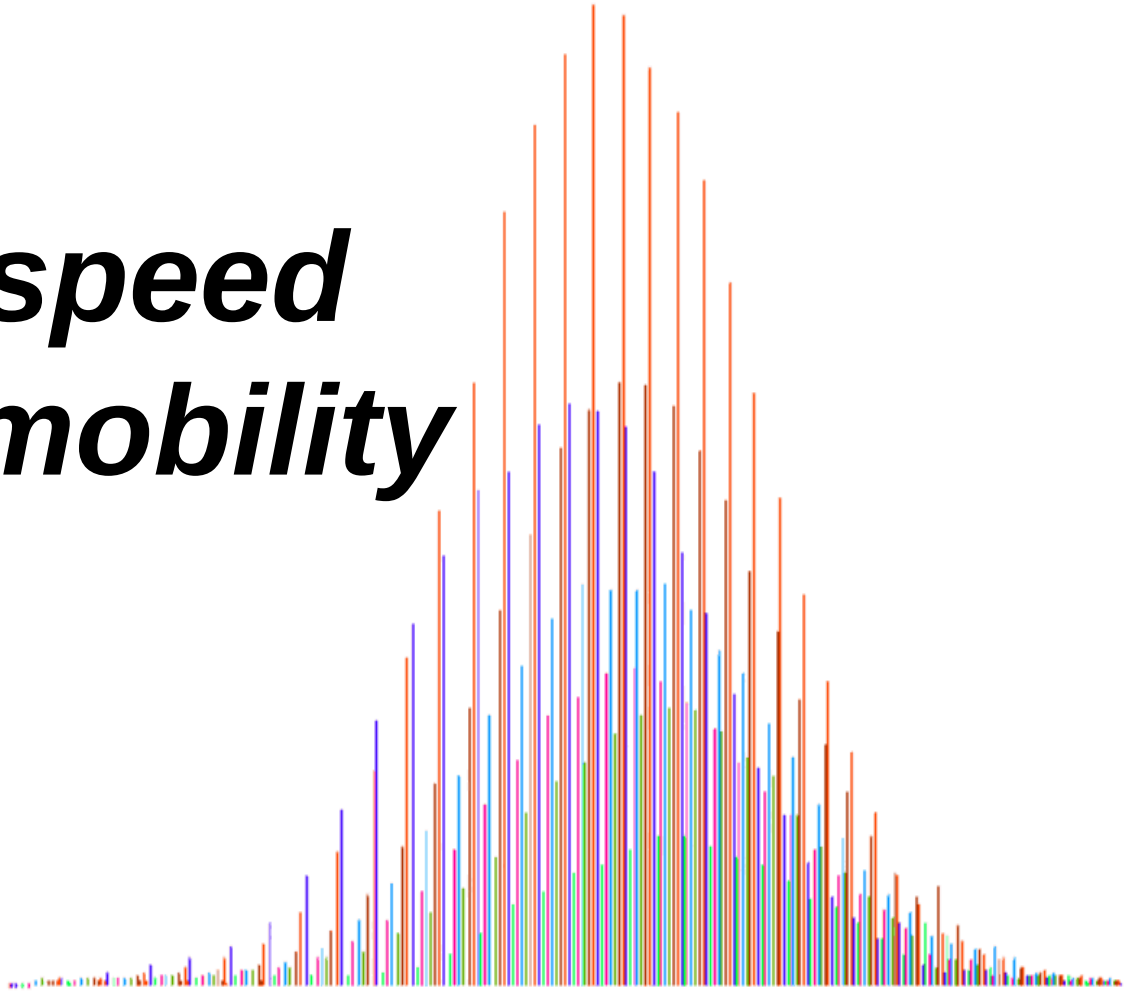


2. Improvements in MS technologies

ion funnel

MS^2 acq. speed

ion mobility



3. Impacting our basic understanding of cancer metabolism.

3. Impacting our basic understanding of cancer metabolism.

The Warburg effect

3. Impacting our basic understanding of cancer metabolism.

LETTER

nature

Reductive glutamine metabolism by IDH1 mediates lipogenesis under hypoxia

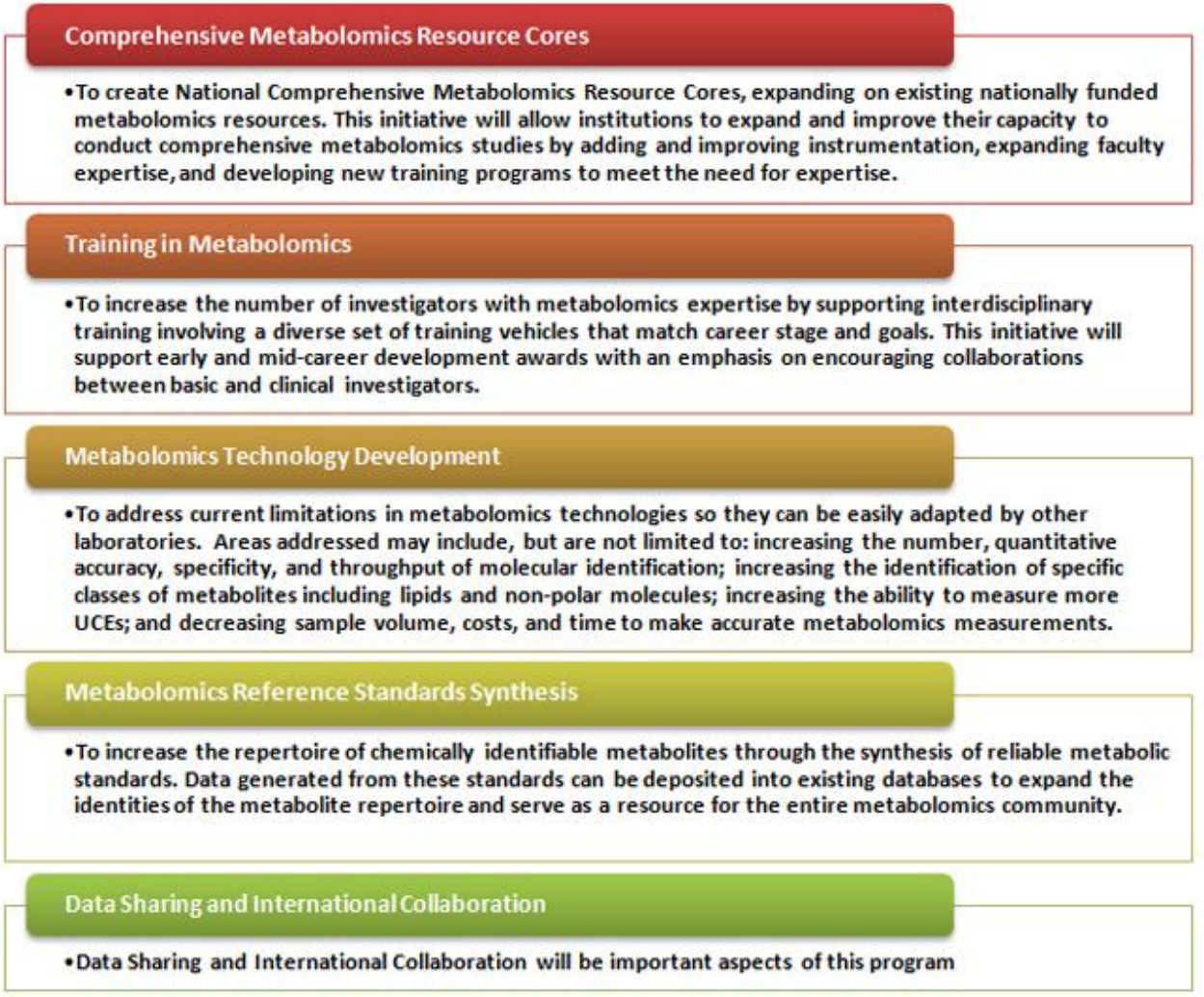
Christian M. Metallo[1]†, Paulo A. Gameiro[1,2,3,4], Eric L. Bell[5], Katherine R. Mattaini[5,6], Juanjuan Yang[3,4], Karsten Hiller[1]†, Christopher M. Jewell[6], Zachary R. Johnson[6], Darrell J. Irvine[6,7], Leonard Guarente[5], Joanne K. Kelleher[1], Matthew G. Vander Heiden[5,6,8], Othon Iliopoulos[3,4] & Gregory Stephanopoulos[1]

380 | NATURE | VOL 481 | 19 JANUARY 2012

19 January 2012

4. Increases in metabolomic funding/resources.

4. Increases in metabolomic funding/resources.



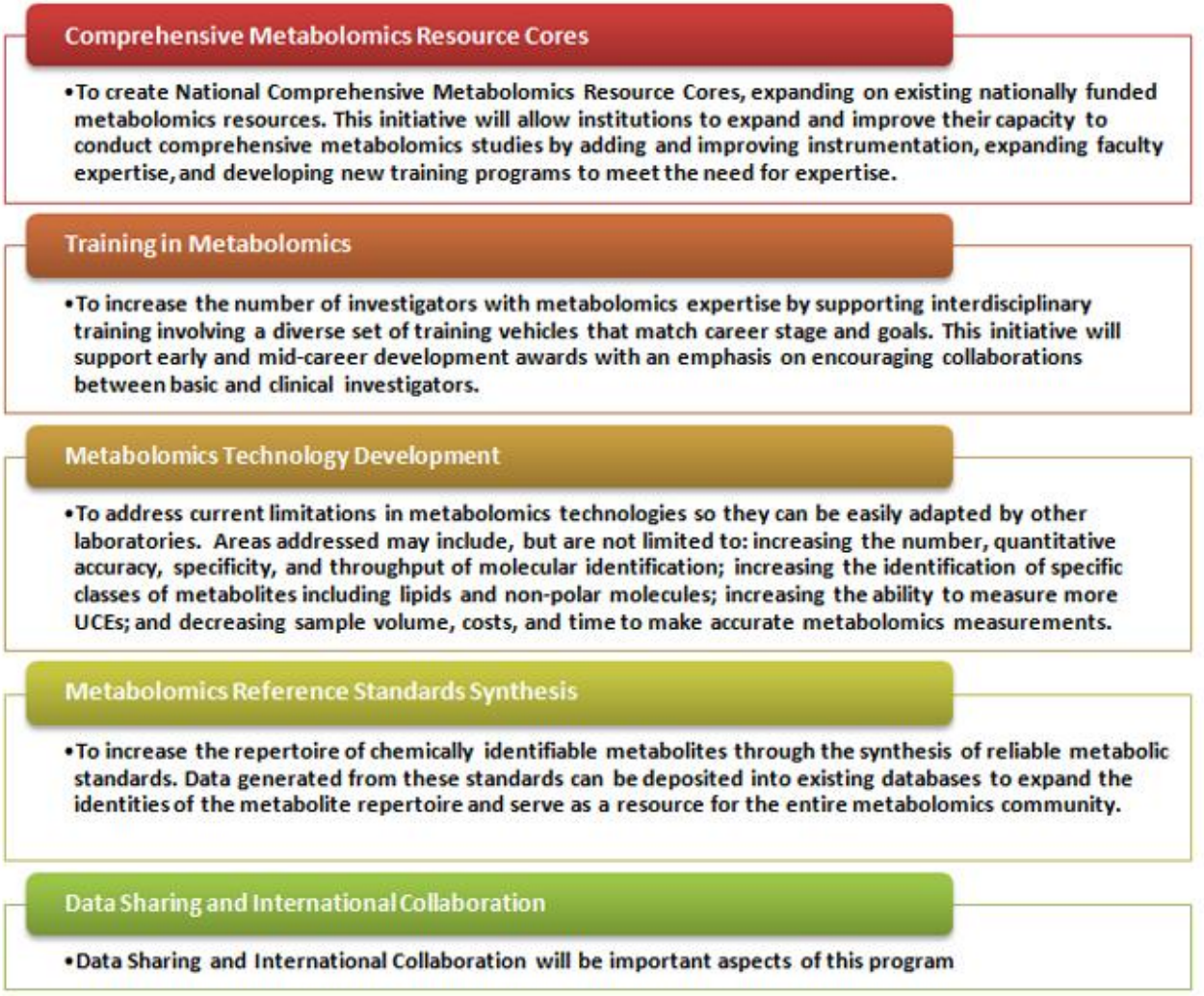
4. Increases in metabolomic funding/resources.

3 U24's

2 R25's

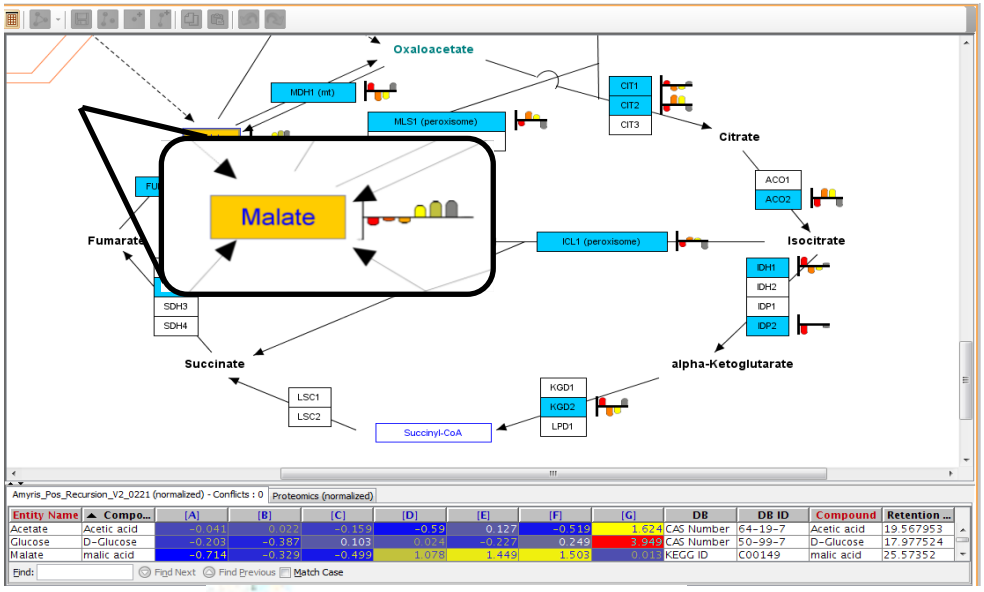
6 R01's

1 U01



5. Improving quality of metabolomic software and databases.

5. Improving quality of metabolomic software and databases.



Agilent Technologies

XCMS Online

Register ›

Current Users:

E-mail:

(e.g. researcher@scripps.edu)

Password:

Sign In

5. Improving quality of metabolomic software and databases.

More standard MS²:

2012: 7,533

2013: 11,209

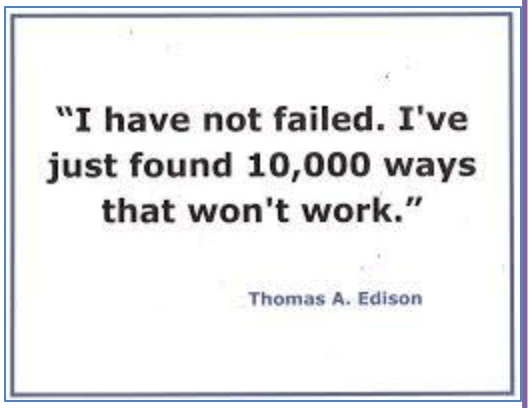
“Gotchas” in Metabolomics

Non-targeted metabolomics

Don’t overlook these important things…

COMMUNICATION!!

- Many metabolomics projects are collaborative efforts.
  - It is critical that the principle investigator promote continual quality communications.
  - For example:
    - Lab A cultures or cultivates an organism
    - Lab B person 1 harvests the samples
    - Lab B person 2 performs the sample prep
    - Lab C performs the MS analyses
    - Informatics group performs data analysis.
  - Each area must communicate its science and techniques in great detail to the others and try not to leave out anything or make assumptions. <u>Sometimes these little details can be project killers.</u>



Experiment Design

Take the time to carefully design metabolomics experiments

What are the questions/hypotheses and can they realistically be answered?

- **Number of samples?**
  - More is usually better but how to optimize the number of samples without going too far for practicality?
  - *Power analysis can* address the optimization problem by quantifying the relationship between the number of experiments and the statistical significance of the effect. Unfortunately, often the number of metabolites greatly exceeds the number of samples.
  - THERE MUST BE A STATISTICALLY VALID SAMPLE NUMBER.
- **Analytical Instrument and Method Limitations**
  - Dynamic range
    - Metabolite concentration range in human blood is 10^{12}
    - The best MS systems are capable of about 10^{5}.
  - Chromatography
    - To obtain the best nontargeted coverage of the metabolome MUST use orthogonal techniques such as GC-MS & HILIC-MS & C18-MS.

Sampling and Prep

"OK, so check this out….they're doing another #$%^ *#&#^*$ metabolomics experiment.
So I didn't eat for 3 days, then I didn't sleep for two days, then I ate part of the cage, then you wanna know the best part?"

<Sigh> "What?"

"I peed in Ralph's special food and then you ate it."
"That should REALLY screw up their data!"

Sampling and Prep

For a set of samples, sampling technique must be as consistent as possible. Small variations can cause big variations in the data, particularly when significant metabolite fold changes can be as low as 1.5.

- **Diurnal (time of day sample is taken)**
  The time of day can have significant impact on the level of some metabolites, particularly mammals and sometimes plants.

- **Sampling techniques are CRITICAL**
  Many projects have been torpedoed by "discovering" and spending significant resources on an interesting metabolite, only to find out later that it was a sample contaminant (for example a rubber glove component).

  Pipetting techniques that differ between lab personnel can introduce variability

Sampling and Prep

Sample prep (consistency is key)

- **Solubility variability**
  - *Important to ensure consistency in procedures and results.*

- **Filtering**
  - Particularly when removing proteins and other large MW components – must be consistent regarding vacuum application, centrifugation G force, time

  - BE CAREFUL! Filtering products offered by vendors to remove components such as phospholipids are often NON SPECIFIC. They will remove many other molecular species that may be important metabolites!

Internal Standards

Spiking internal standards into sample solutions

- You can introduce different standards at different steps in the prep process
  - Can be used for QC to figure out where sampling error was introduced

- ALWAYS use ^{13}C standards instead of ^{2}H if possible.
  - Stable
  - Solid isotope patterns
  - No exchangeable protons
  - More consistent chromatography
  - More expensive but worth it

- Internal standard data is very useful for QC of each data file to look for misinjections, retention time drifting, column problems, hiccups in acquisition and mass accuracy etc.
  - Using a sufficient number of test samples, set CV pass/fail criteria for retention time, area and abundance.

- To Normalize or NOT
  - Sometimes or not, maybe – be decisive!

Data Analysis

Multivariate analysis – can be more useful for metabolite discovery than for modeling. Too many variables (mass features, treatment variables etc.) and not enough samples often causes…

"overfitting" of the data.

"If it's overfit…
you must quit…"

In statistics and machine learning, **overfitting** occurs when *a statistical model describes random error or noise instead of the underlying relationship*. Overfitting generally occurs when a model is excessively complex, such as having too many parameters relative to the number of observations. A model which has been overfit will generally have poor predictive performance, as it can exaggerate minor fluctuations in the data.

(The Wikipedia definition is excellent)