

APPLICATIONS OF METABOLOMICS IN SYSTEMS LEVEL BIOCHEMISTRY



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TAKE HOME MESSAGE

Metabolomic technologies can be used as an analytical lens into

- the *in situ* biochemical state of a given cell and/or organism protein
- the *in situ* or physiologic function/activity of its component proteins



Biochemistry: Chemical foundation of biology (ie, life)

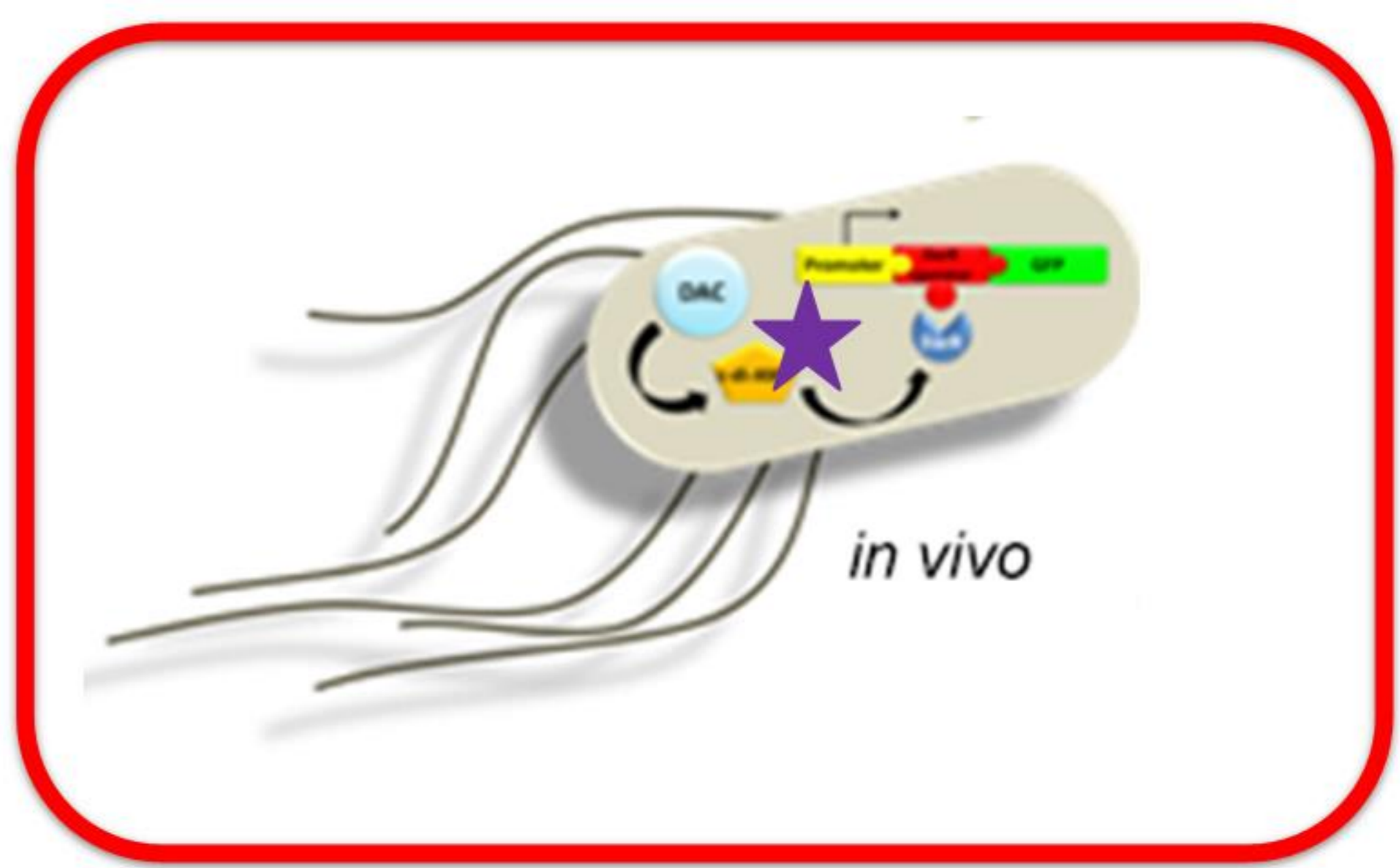
Most direct mediator of physiologic processes

Traditional methods limited by complexity and diversity of biochemical activities within cells and/or organisms

Components historically studied in isolation

Focused on intrinsic properties and potential capabilities, rather than actual level of activity

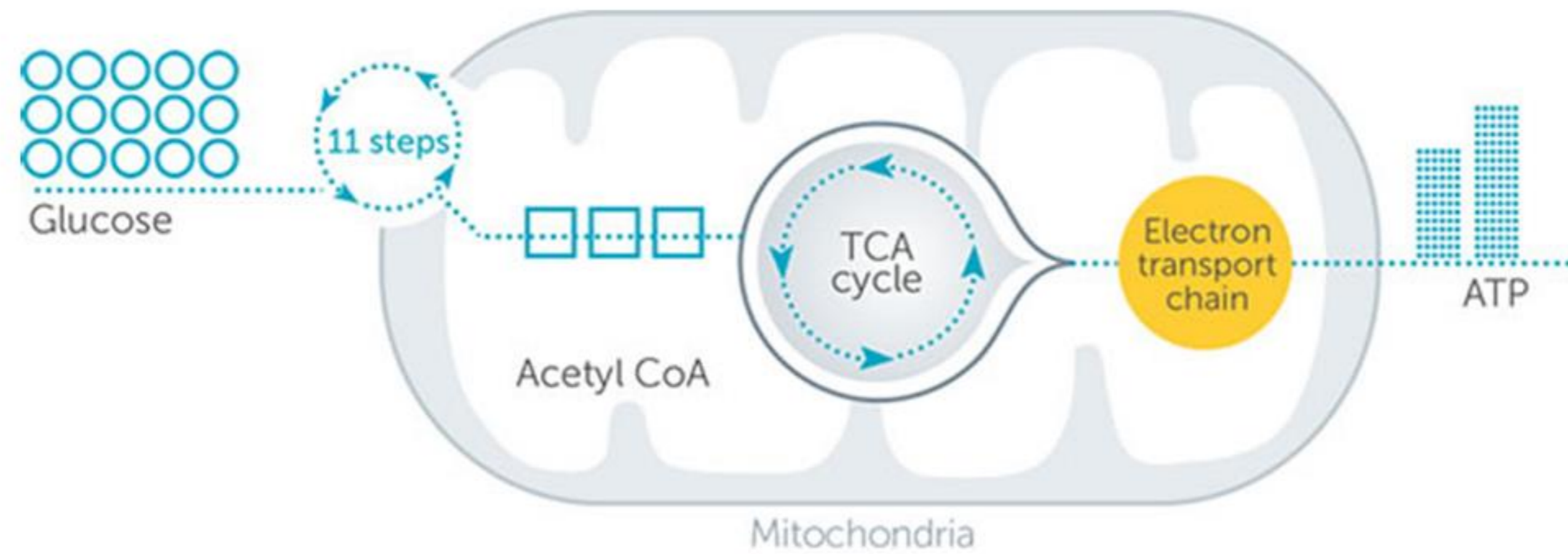
Neglected native milieu of viable cell



WHAT IS METABOLOMICS?

Conceptually unbiased study of the small molecule complement of a given cell or organism

METABOLISM: THE FUEL OF BIOCHEMISTRY



HOW DOES IT RELATE TO BIOCHEMISTRY?

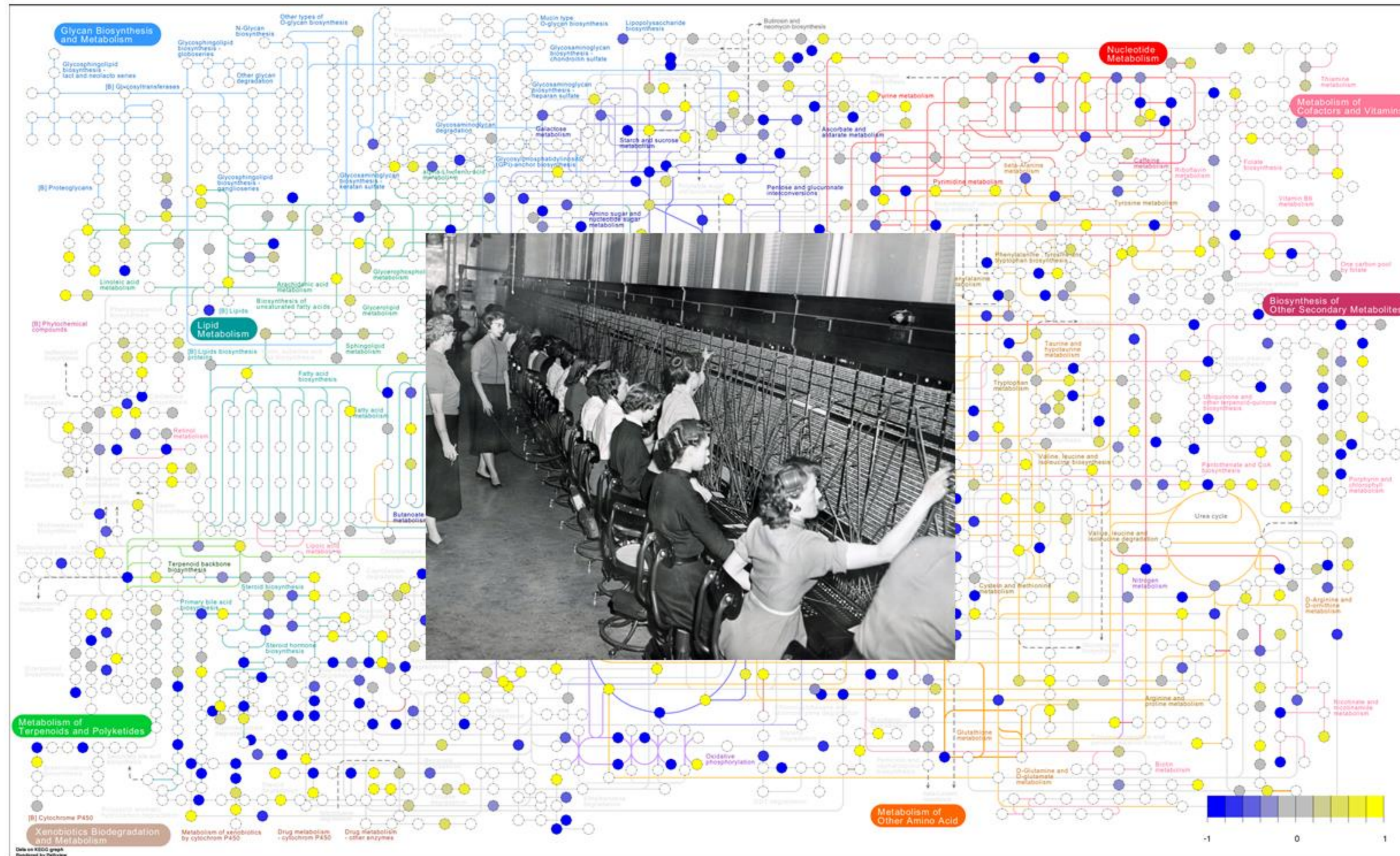
Metabolites: Chemical foundation of biochemistry

- Source of energy and precursors required for synthesis of all macromolecules
- Substrates, products and effectors of enzymes

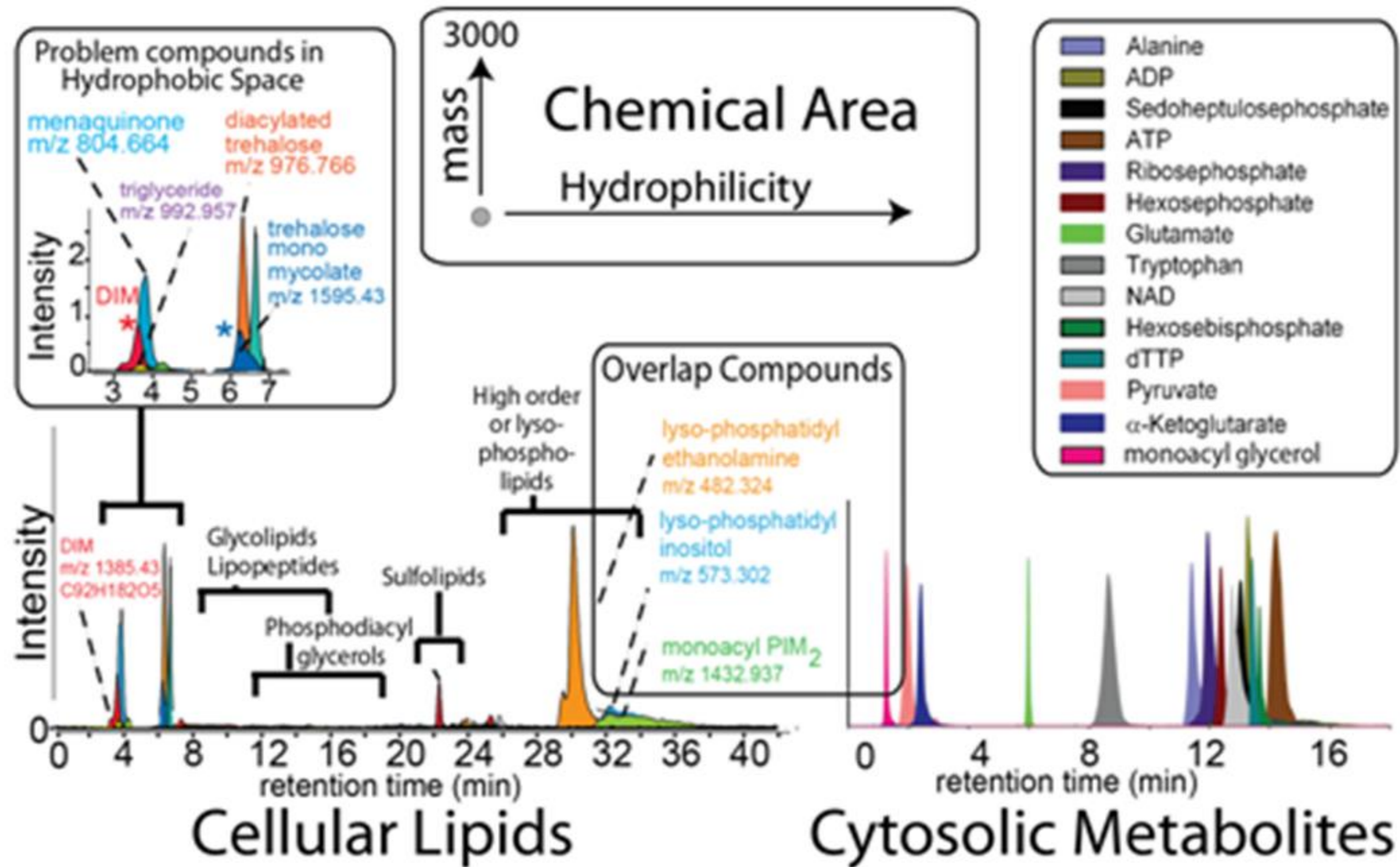
METABOLISM: MORE THAN FUEL?



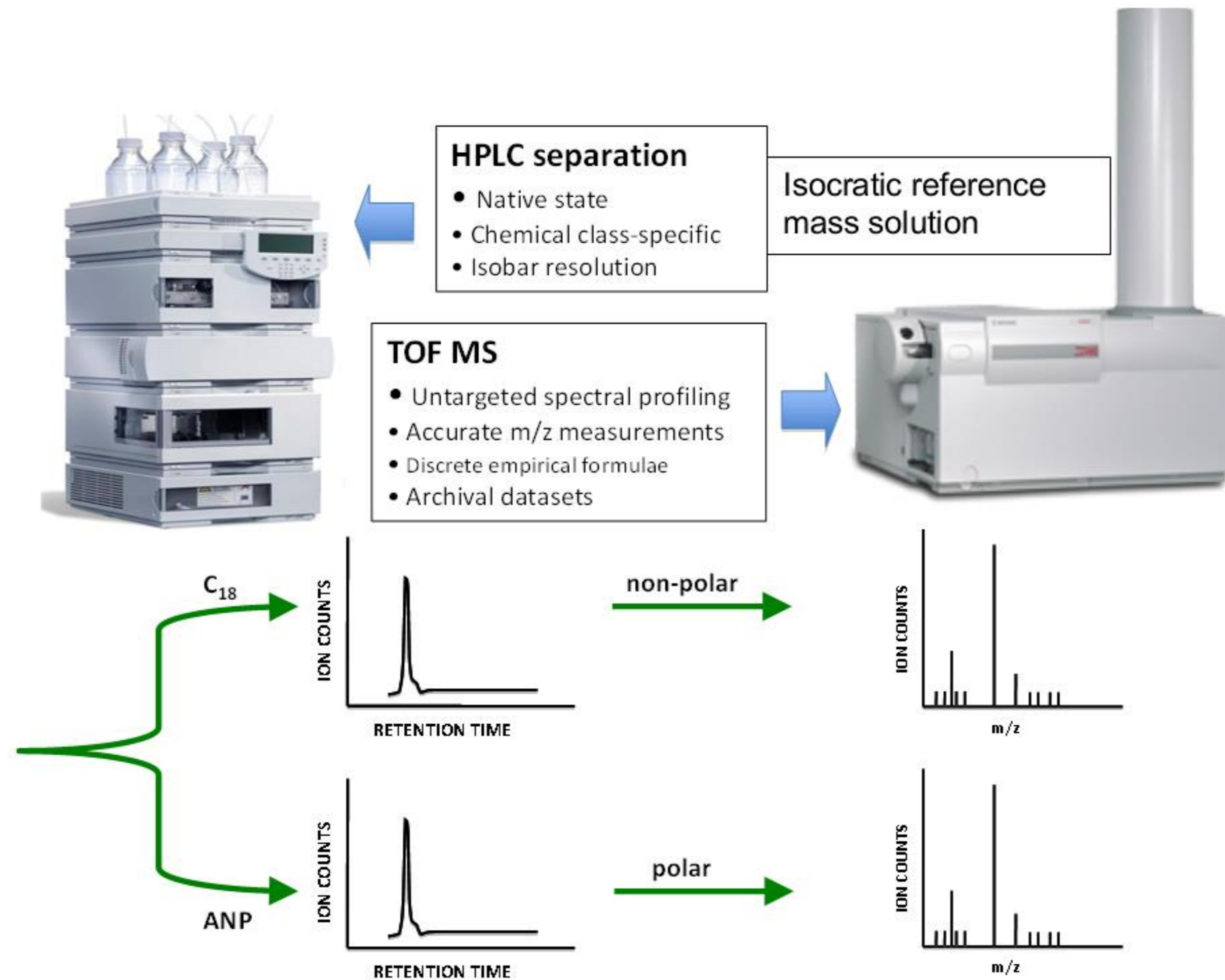
METABOLISM: MORE THAN FUEL?



HOW WE STUDY METABOLITES



HOW WE STUDY METABOLITES



WORKFLOW

Targeted: biologically agnostic (new relationships among known molecules)

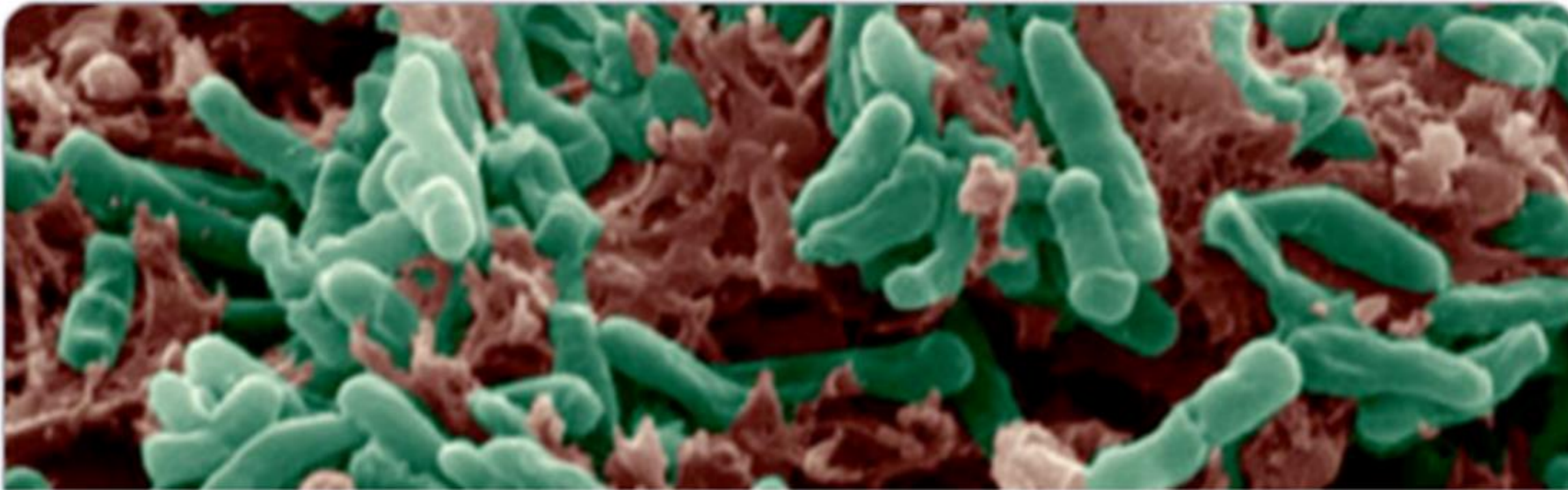
- Pre-establish 'reference' library of metabolites of interest; Agilent PCDL manager
- Compare batch pool sizes of library metabolites for individual metabolites and/or metabolite profiles; Agilent ProFinder and MPP
- Consider targeted flux experiments using batch pool size comparisons as flag

Untargeted: chemically agnostic (discovery of new or unanticipated molecules)

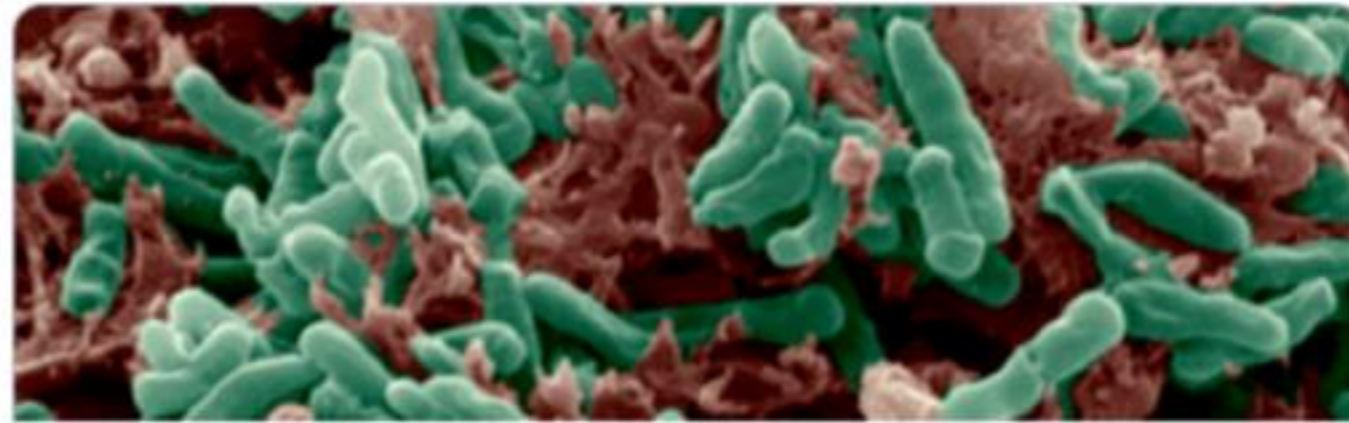
- Index metabolites (defined as chromatographically resolved ion families) based on accurate mass-retention time identifiers; Agilent MFE
- Identify statistically relevant molecules by comparative analysis
- Focus on molecular identification by empirical formula generation, comparison to candidate molecules (external addition, MS/MS) and de novo structural ID (NMR)

MYCOBACTERIUM TUBERCULOSIS

ONE PATHOGEN, 2 PUBLIC HEALTH CRISES

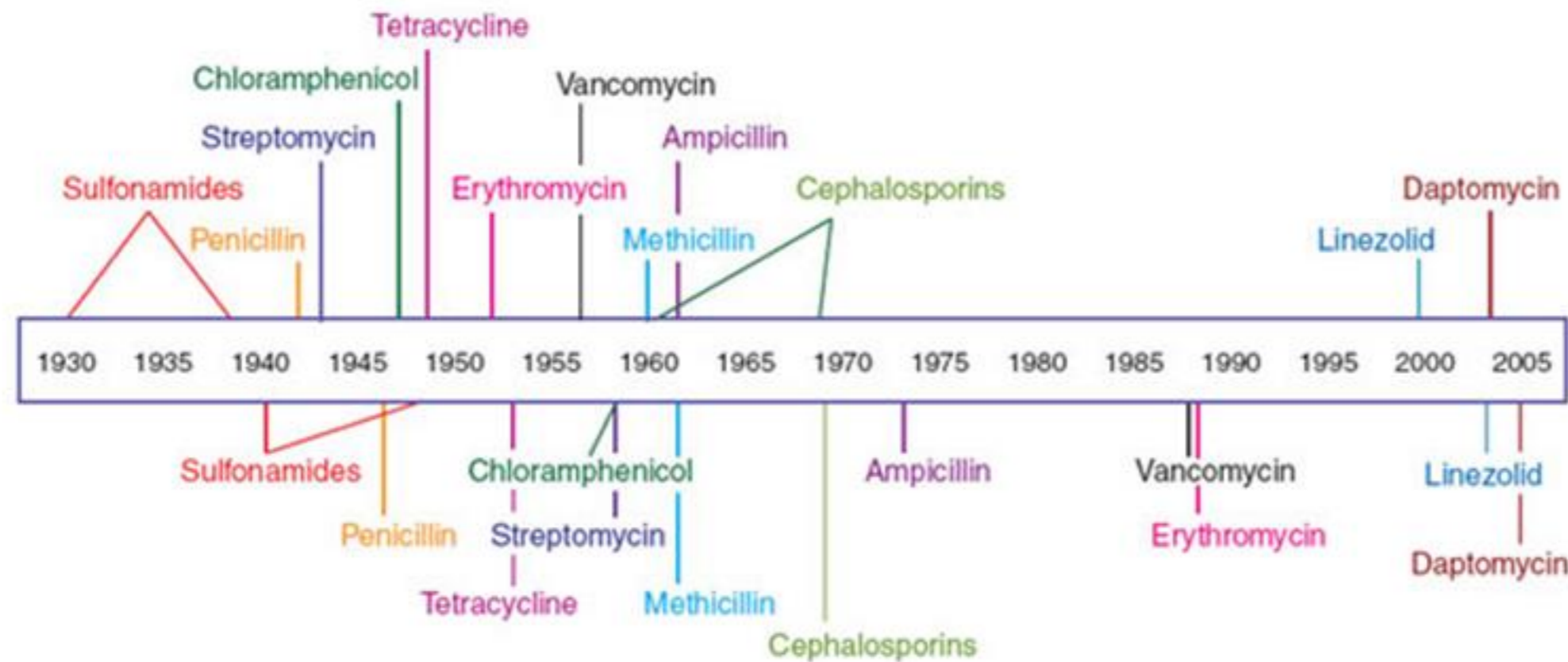


A GLOBAL HEALTH CRISIS...



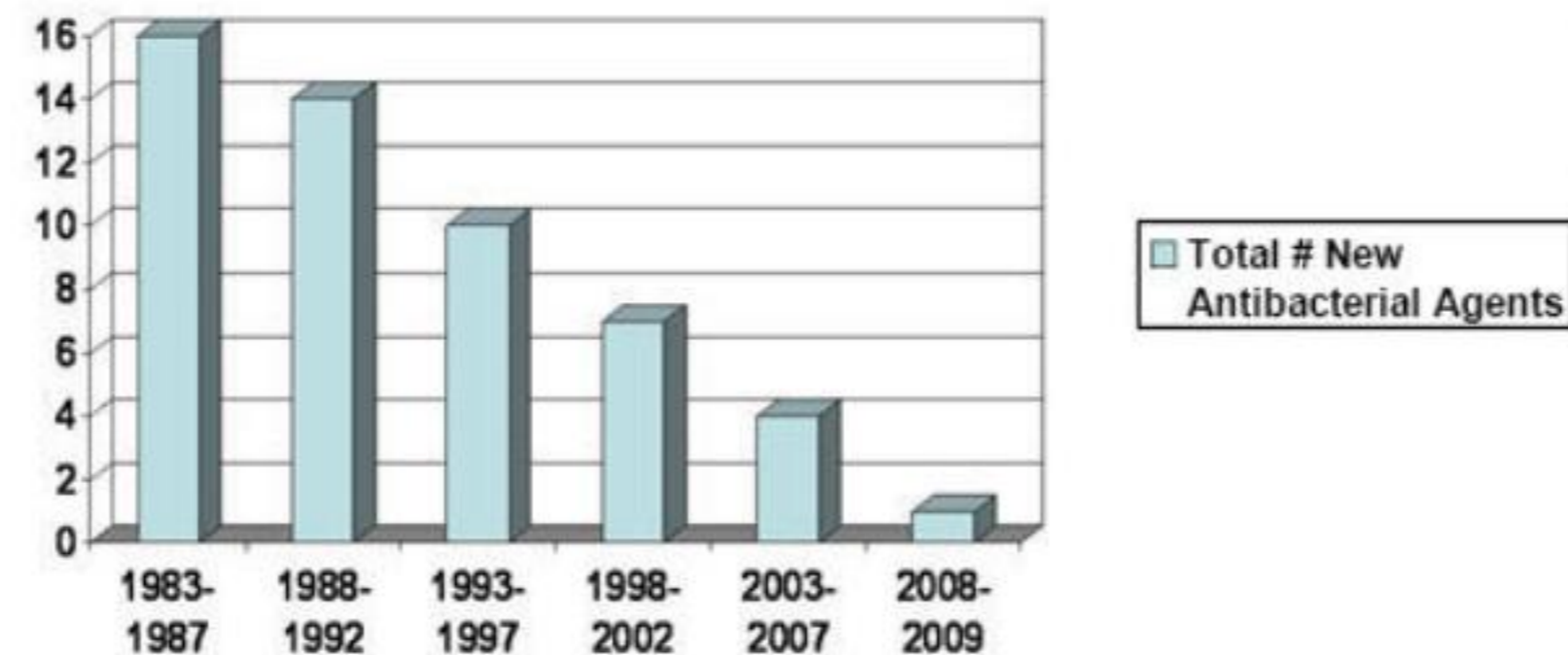
A CRISIS OF ANTI-INFECTIVES CHEMOTHERAPY

Antibiotic deployment

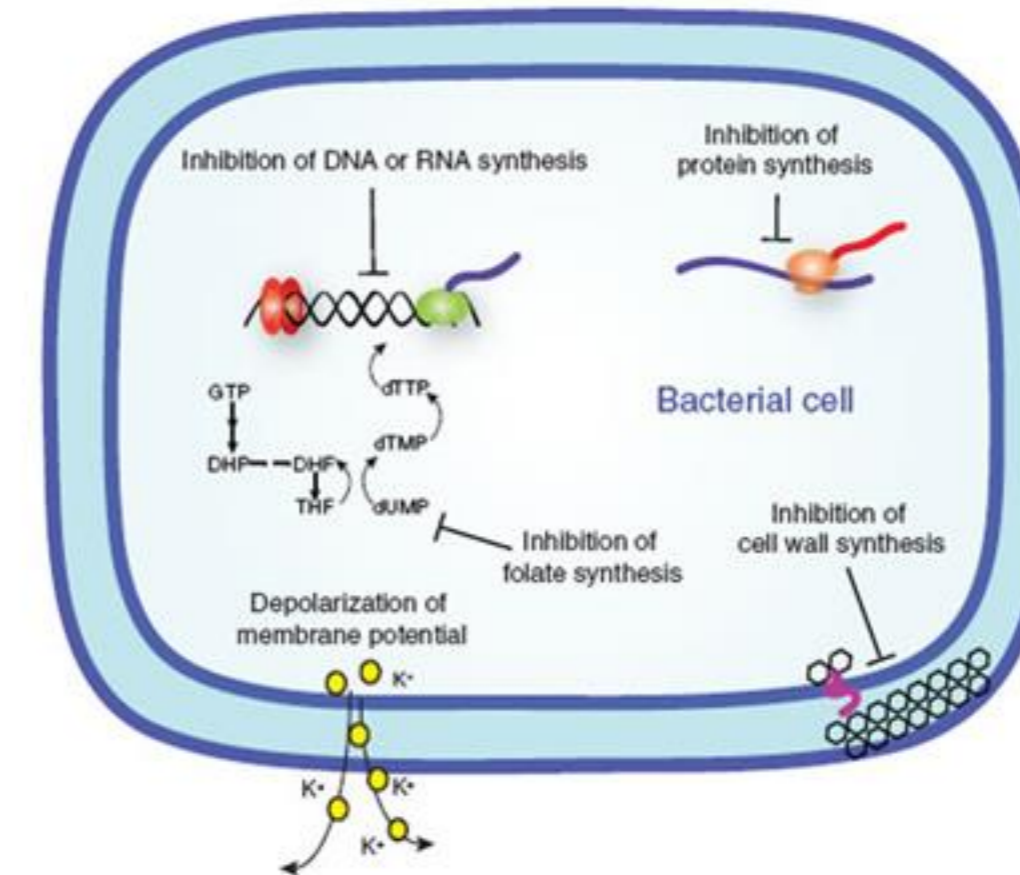


Antibiotic resistance observed

DECLINING ANTIBACTERIAL APPROVALS (PAST 25 YEARS)

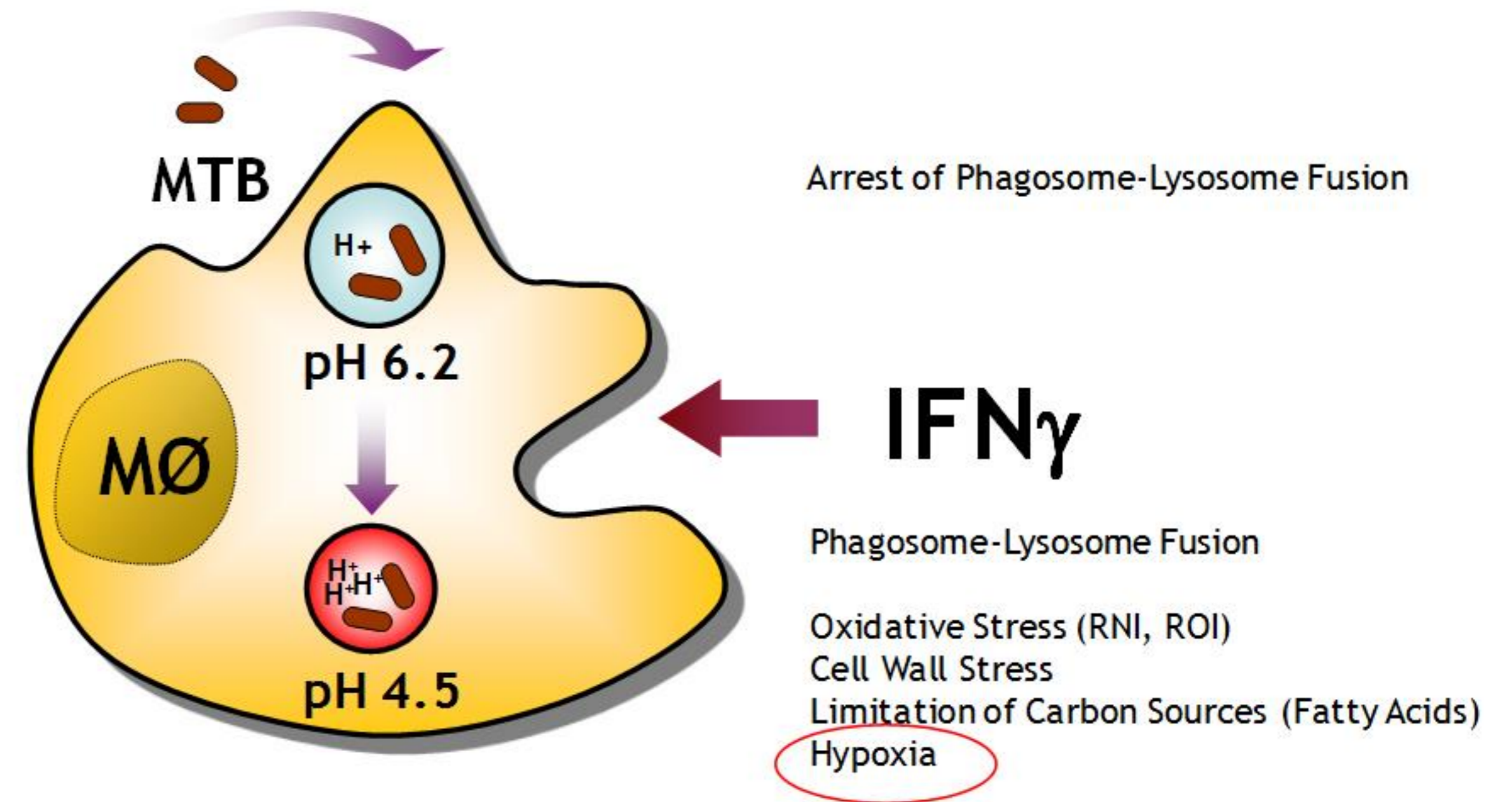
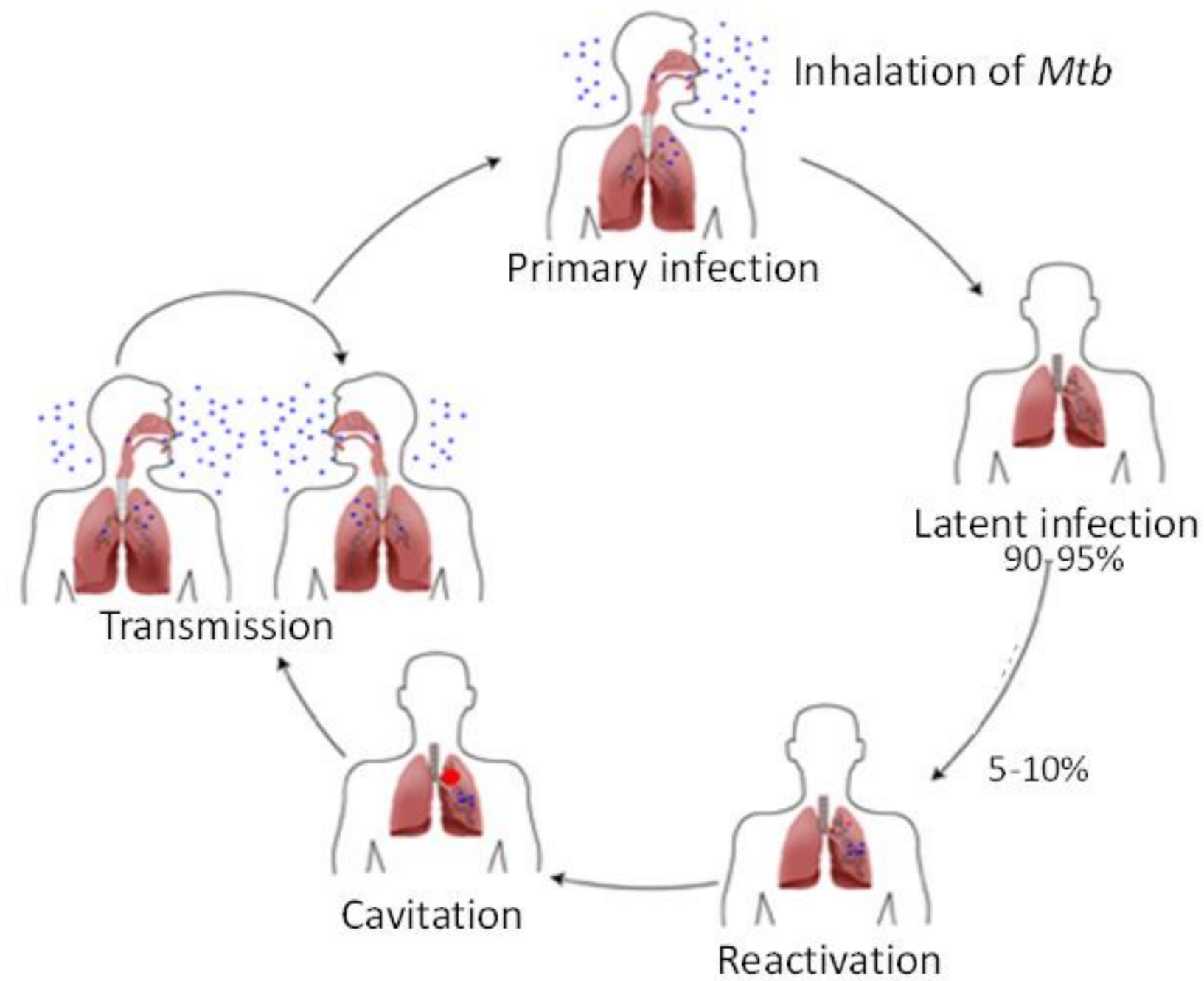


Spellberg, CID 2004, Modified



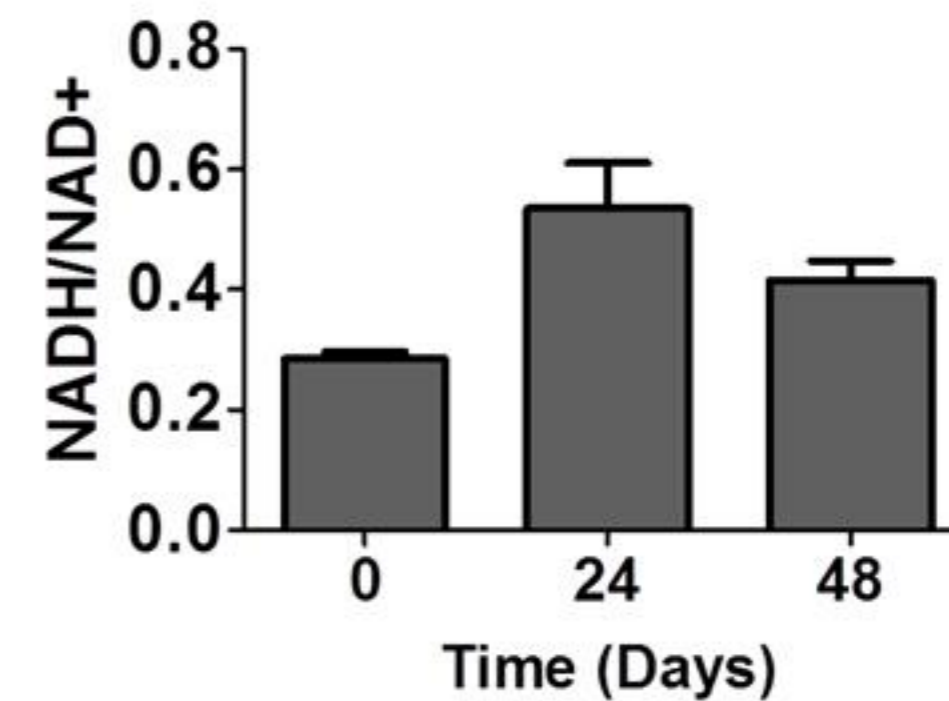
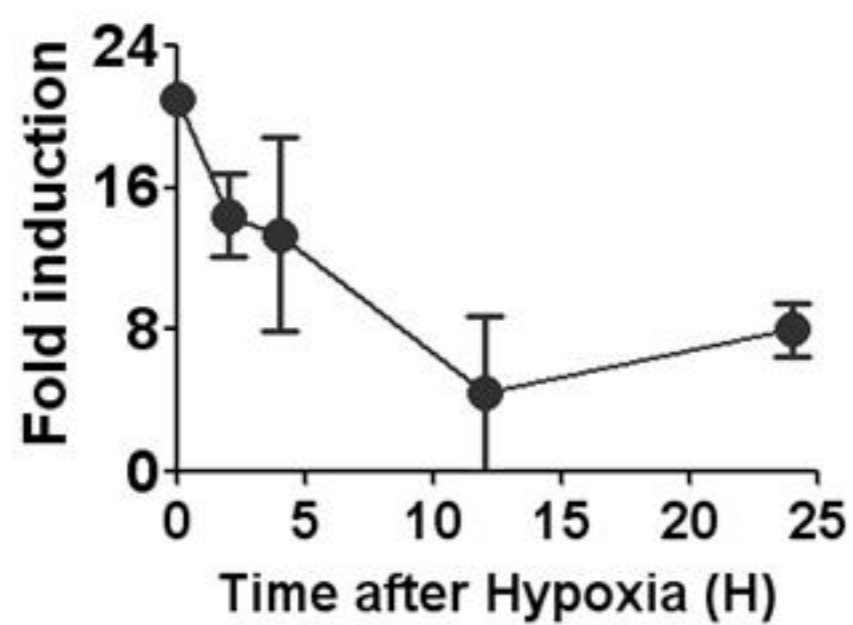
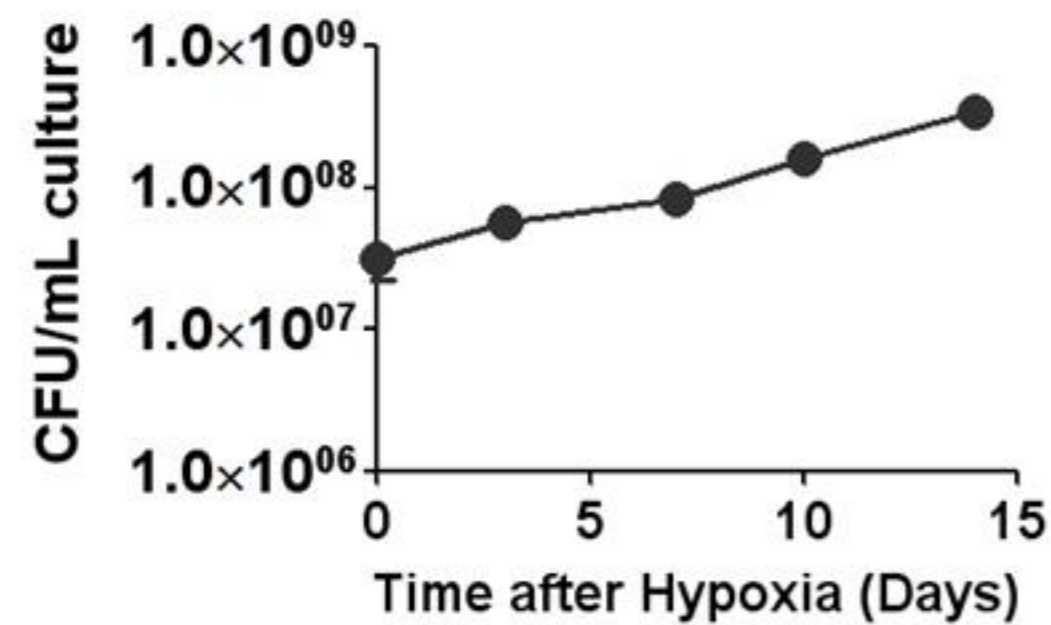
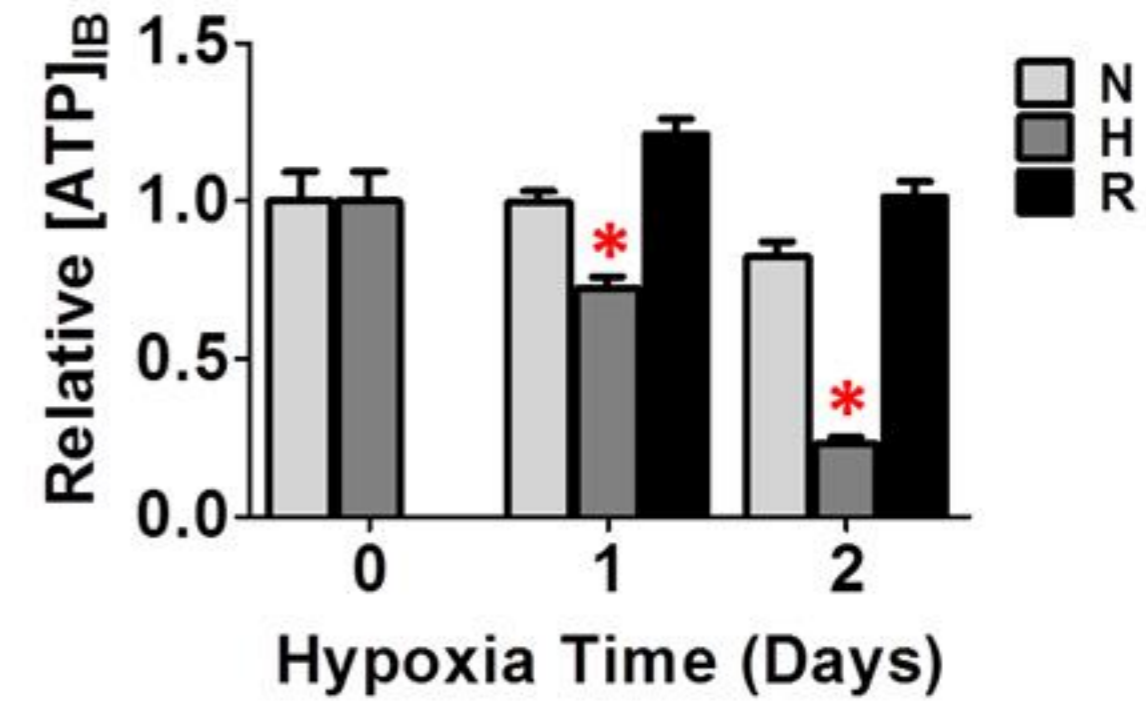
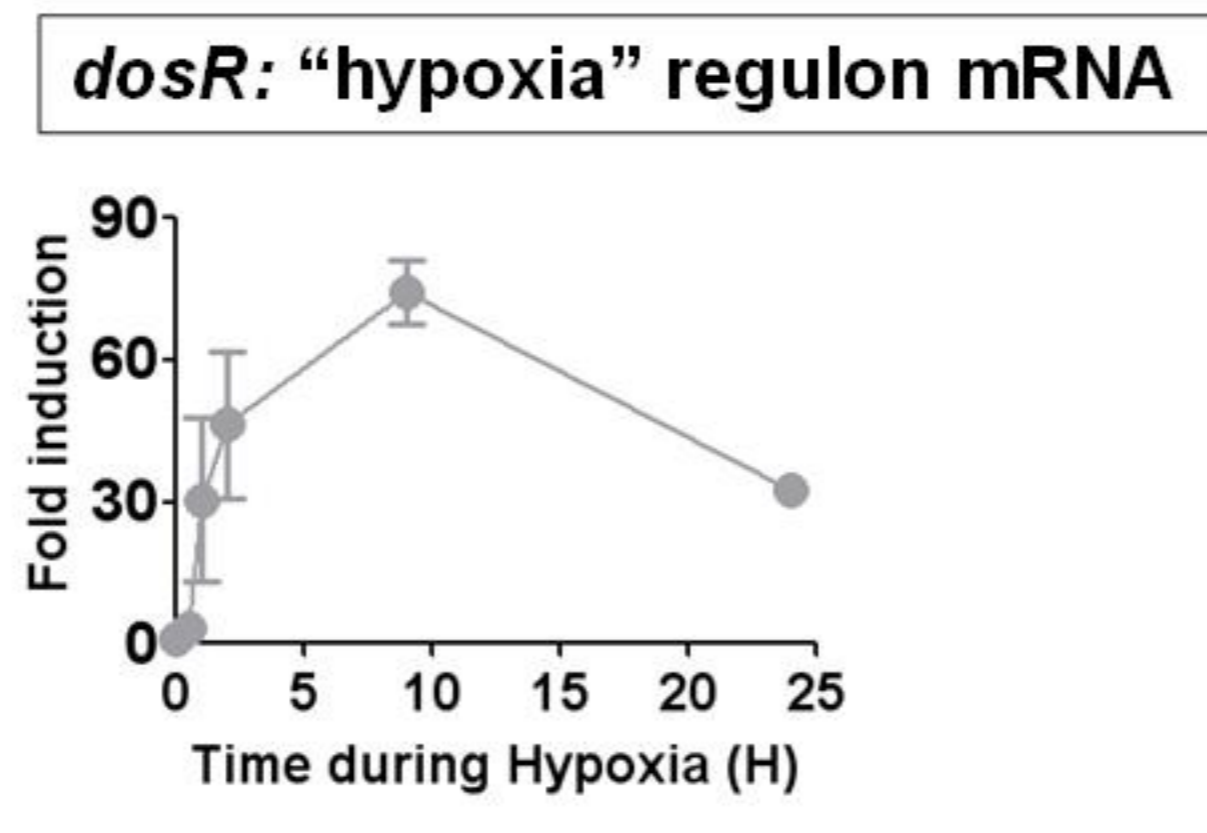
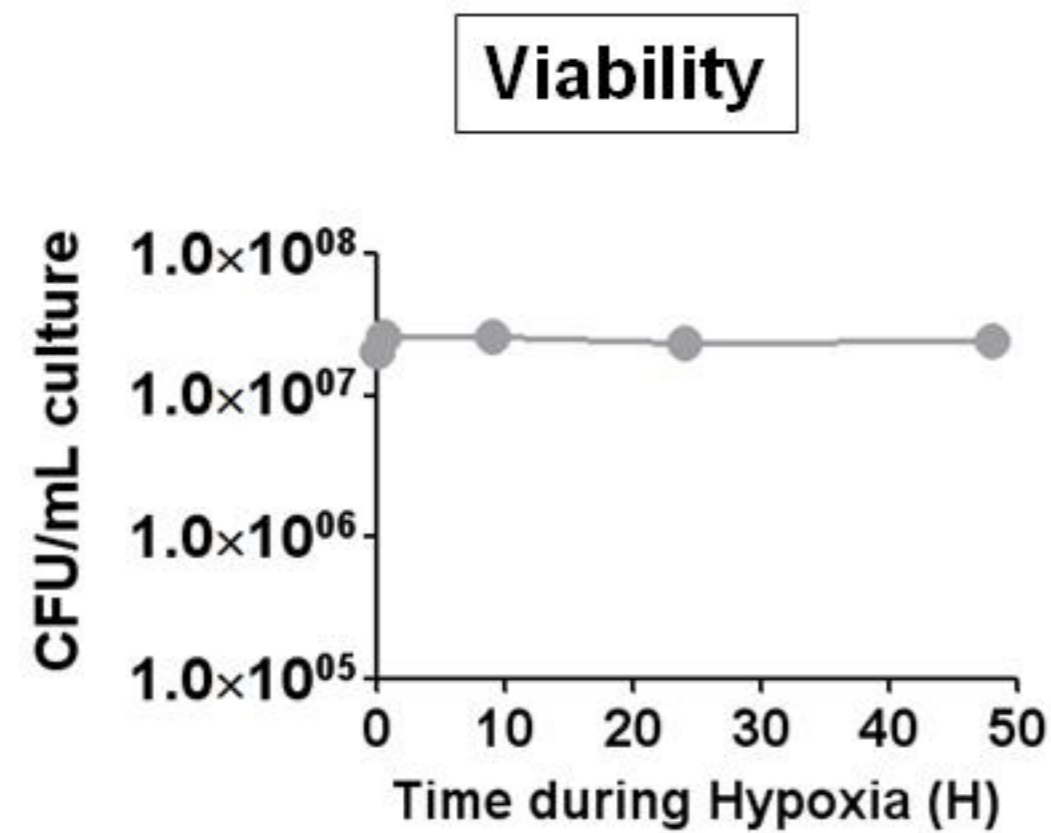
Top: Chatworthy & Hung. Nat Chem Biol. 2007 Sep;3(9):541-8.
 Bottom: Bad Bugs, No Drugs, IDSA. 2004.

A BIOCHEMICAL WINDOW INTO MTB PATHOGENICITY

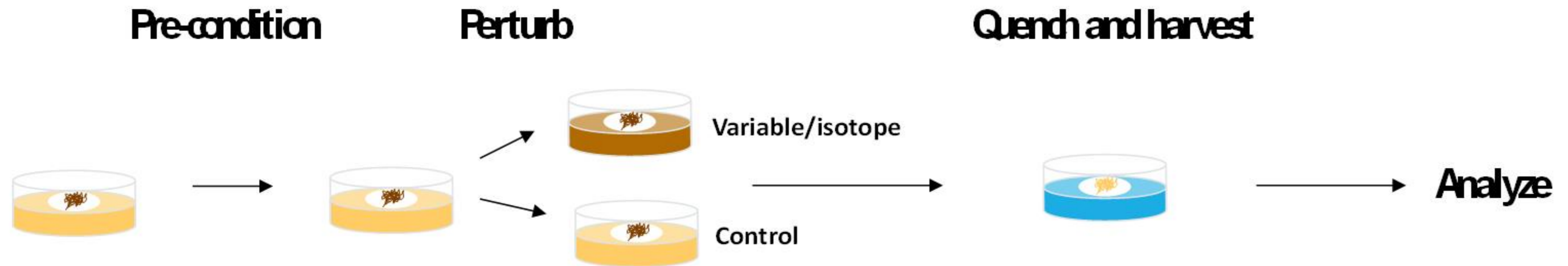


Adapted from:
Ramakrishnan Nat Rev Immunol 12 :352, 2012
Russell et al. Science 328:852, 2010
Kumar et al. Exp Rev Mol Med 13:39, 2011

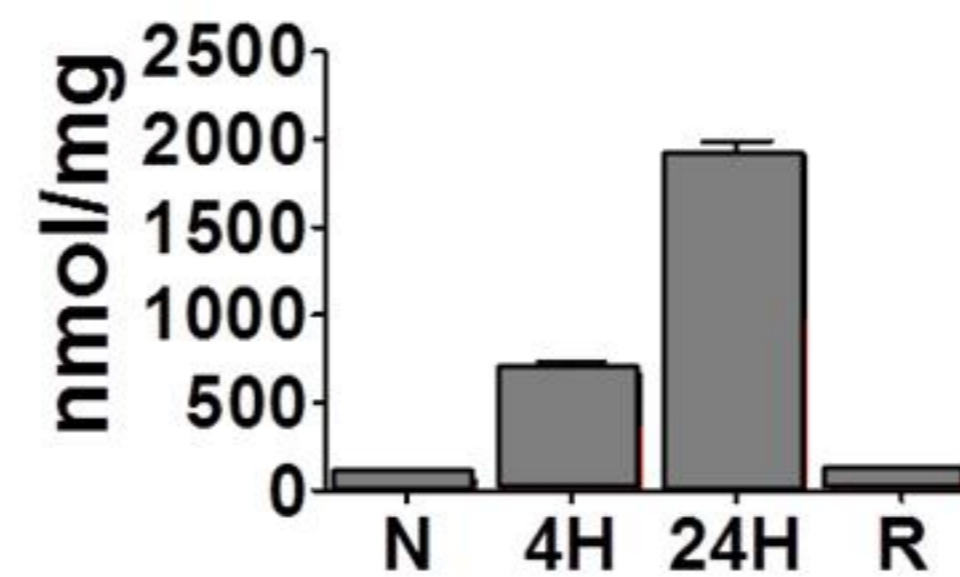
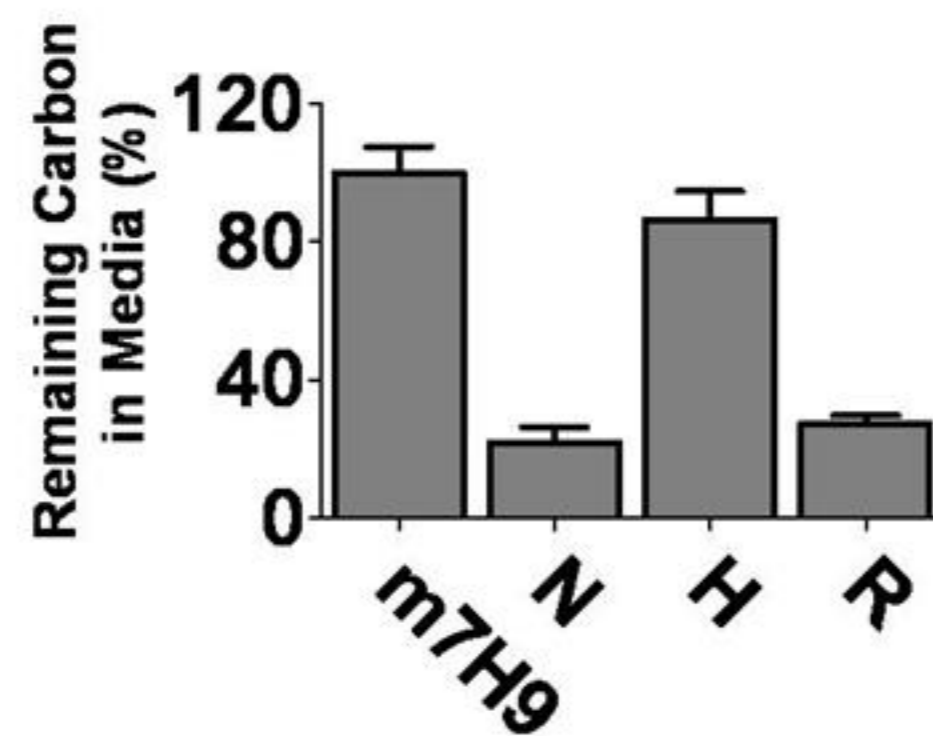
MODEL VALIDATION



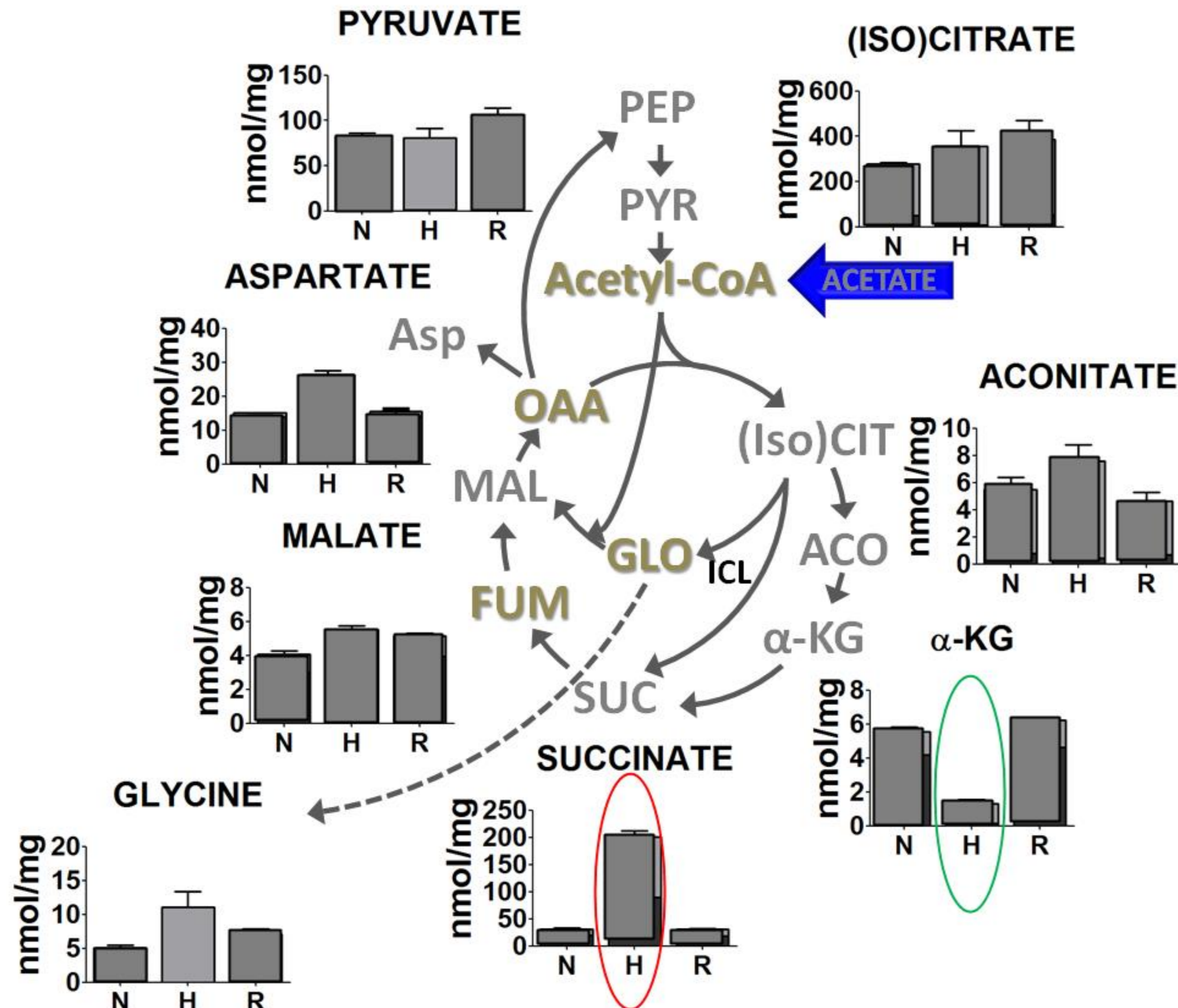
SURVEY TOOLS



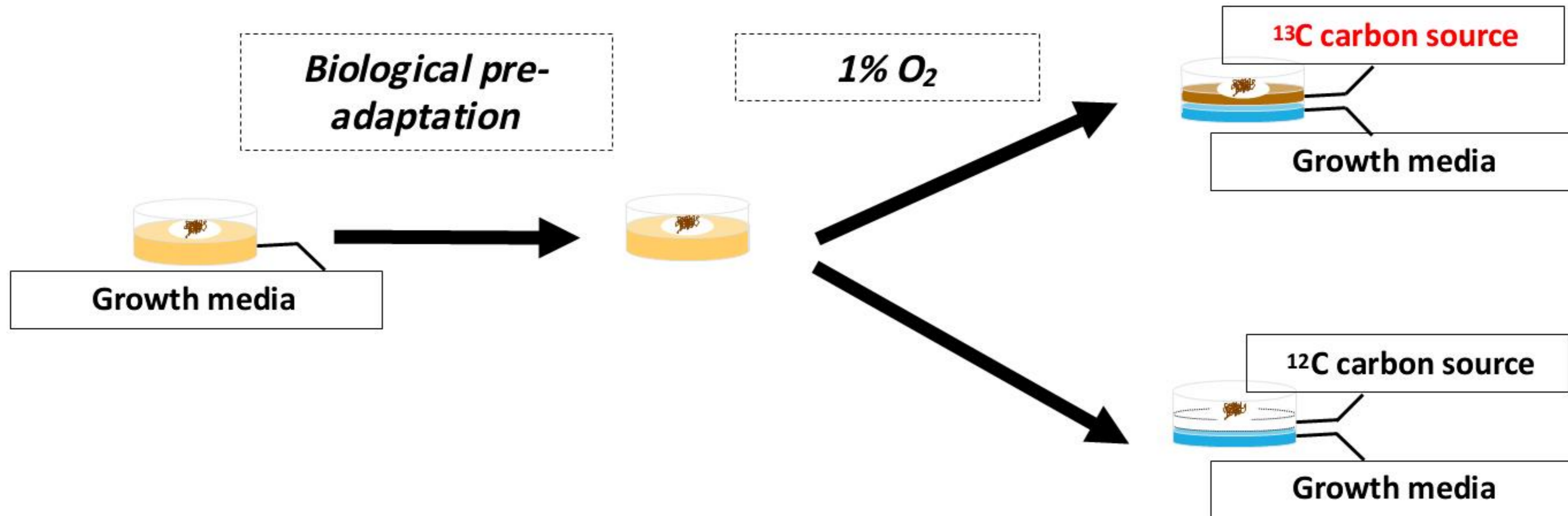
EXOMETABOLOME CHARACTERIZATION



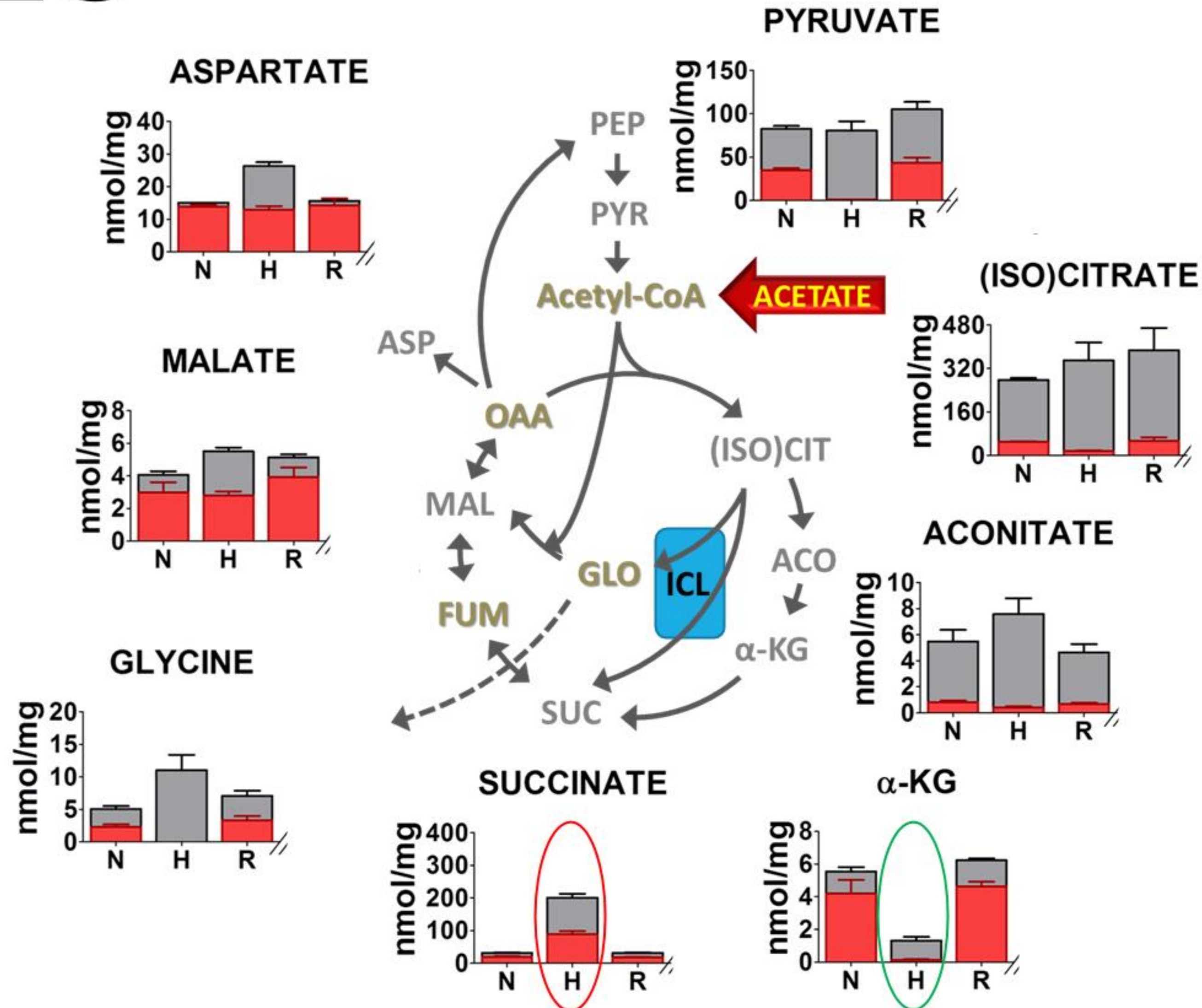
TARGETED PROFILING



TARGETED FLUX ANALYSIS

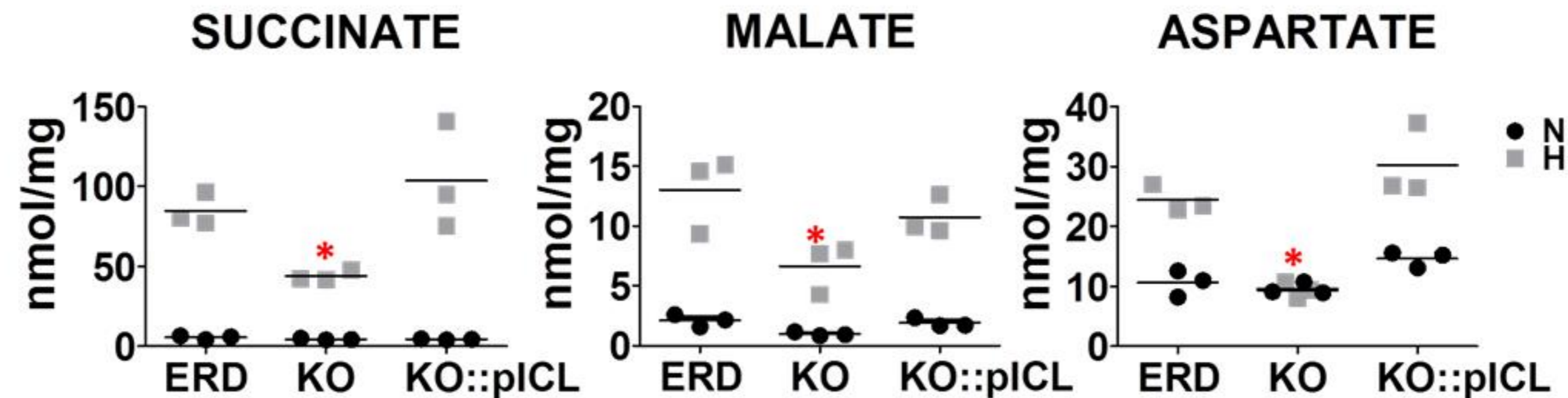
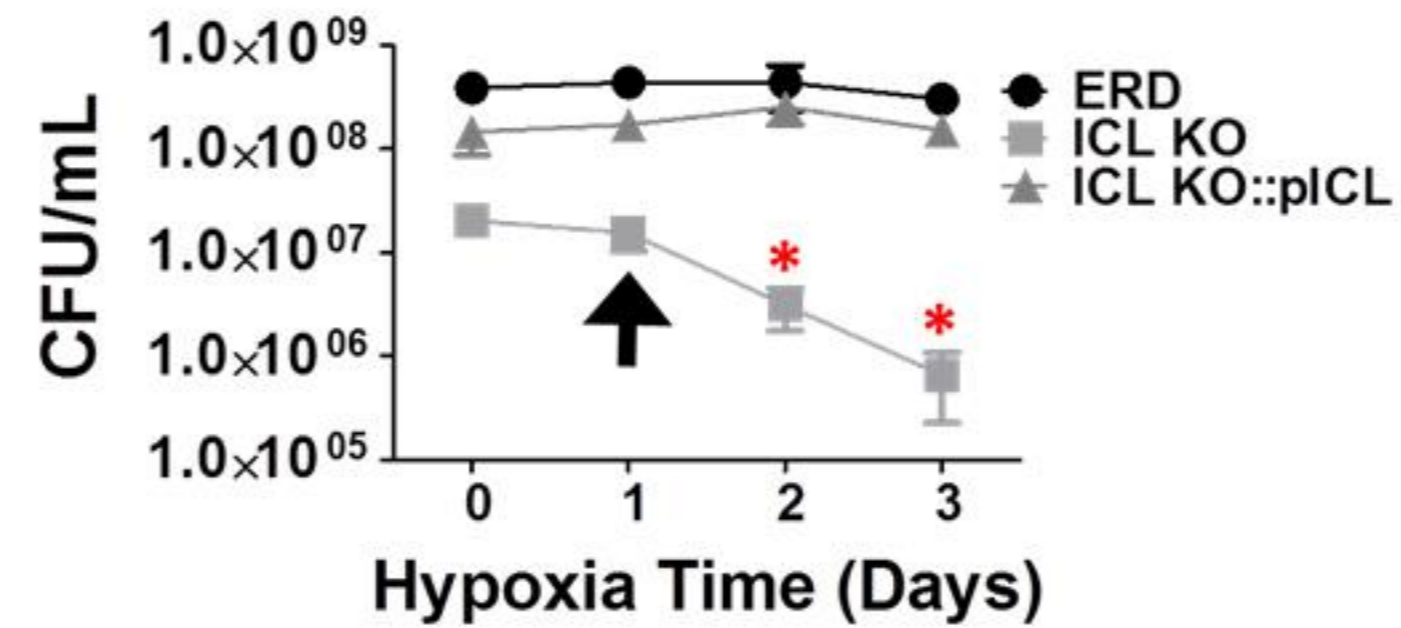
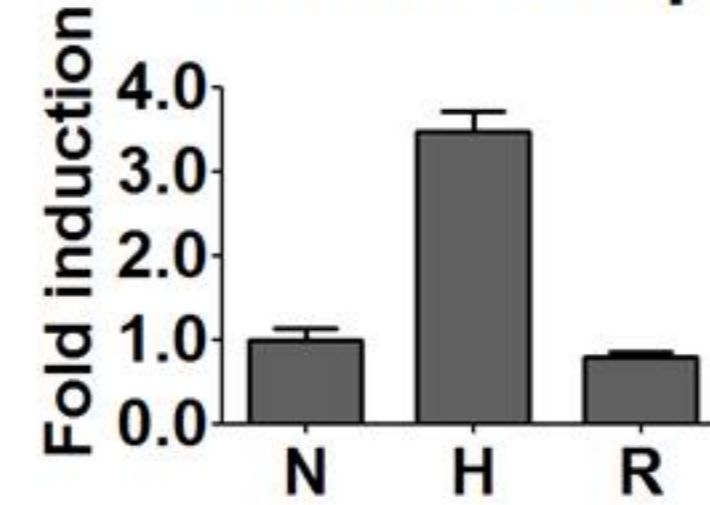


FROM METABOLITES TO GENES



OLD ENZYME, NEW FUNCTION

ICL transcript





AUTOBOT IRONHIDE IS A GMC TOPKICK

AUTOBOT BUMBLEBEE IS A CHEVY CAMARO

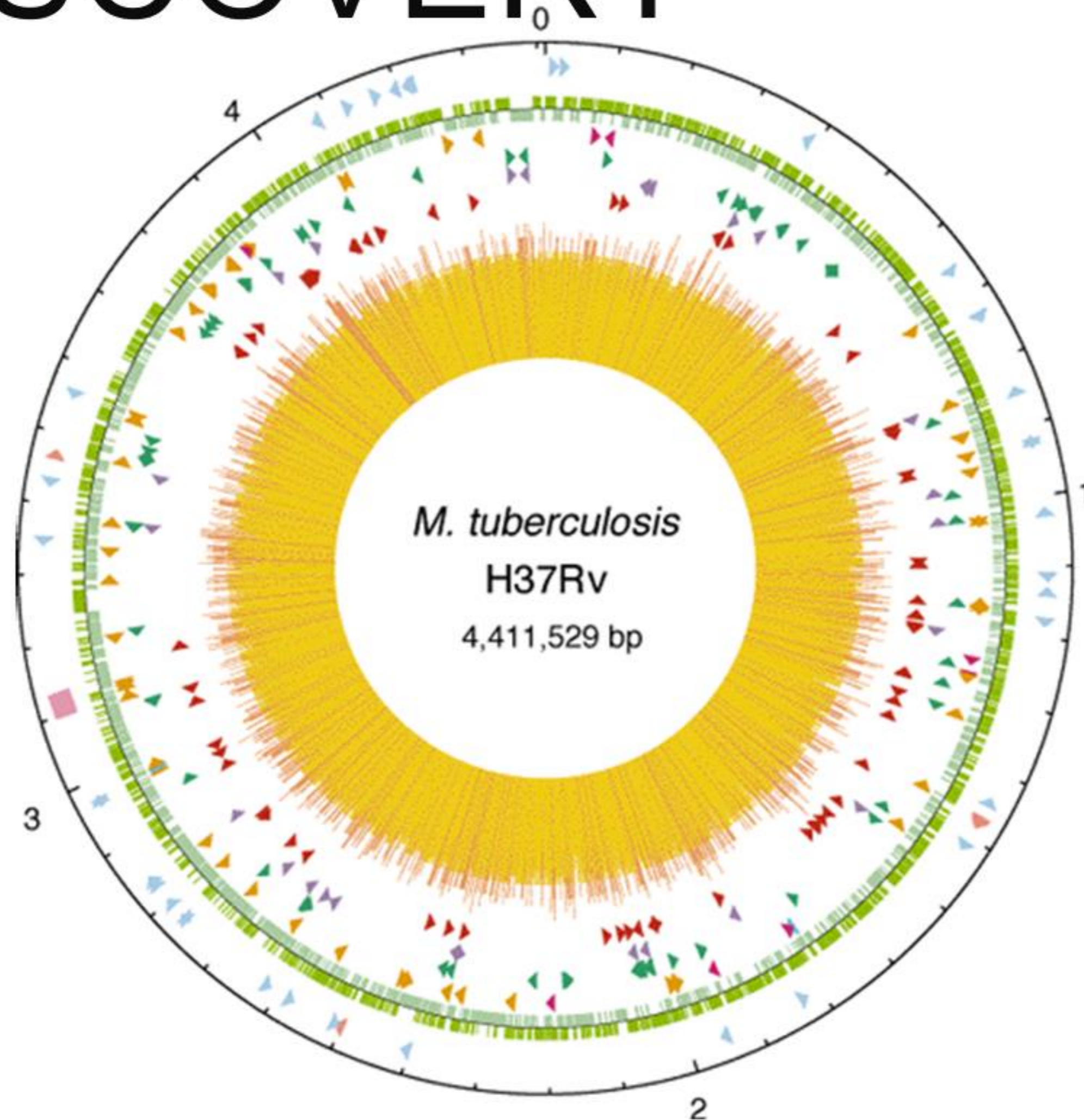
AUTOBOT JAZZ IS A PONTIAC SOLSTICE

AUTOBOT RATCHET IS A HUMMER H2



TRANSFORMERS

METABOLOMICS IN DRUG DISCOVERY



~ 40% functionally assigned*
(1,600)
~ 44% are conserved hypothetical proteins
(1,760)
~ 16% have no similarity to anything ever seen
(640)

Only 1 of 8 genes annotated to encode subunits of Mtb's PDH, KDH cxs fulfilled its prediction (aceE; E1 PDH)

METABOLOMICS IN DRUG DISCOVERY

Genes required for mycobacterial growth defined by high density mutagenesis

Christopher M. Sassetti,¹ Dana H. Boyd² and Eric J. Rubin^{1*}

¹Department of Immunology and Infectious Diseases, Harvard School of Public Health, Harvard Medical School, Boston, MA 02115, USA.

²Department of Microbiology and Molecular Genetics, Harvard Medical School, Boston, MA 02115, USA.

A postgenomic method for predicting essential genes at subsaturation levels of mutagenesis: Application to *Mycobacterium tuberculosis*

Gyanu Lamichhane*, Matteo Zignol*, Natalie J. Blades¹, Deborah E. Gelman*, Annette Dougherty*, Jacques Grosset*, Karl W. Broman*, and William R. Bishai**

*Center for Tuberculosis Research, Department of Medicine, The Johns Hopkins University School of Medicine, 424 North Bond Street, Baltimore, MD 21231-1001; and **Department of Biostatistics, The Johns Hopkins Bloomberg School of Public Health, 615 North Wolfe Street, Baltimore, MD 21205

Edited by John J. Mekalanos, Harvard Medical School, Boston, MA, and approved April 16, 2003 (received for review March 5, 2003)

Genetic requirements for mycobacterial survival during infection

Christopher M. Sassetti and Eric J. Rubin*

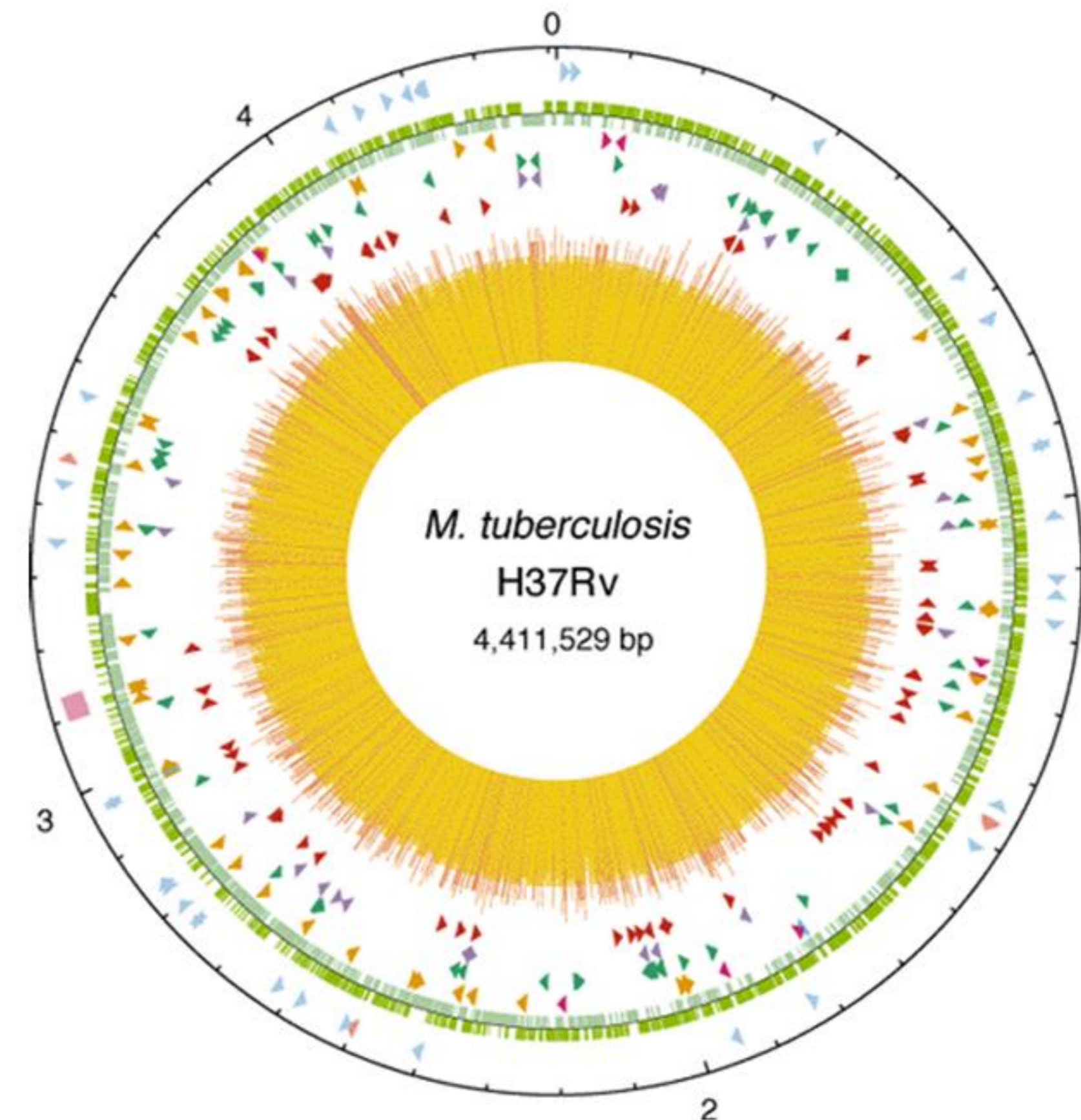
Department of Immunology and Infectious Diseases, Harvard School of Public Health, Building 1, Room 904, 665 Huntington Avenue, Boston, MA 02115

Edited by John J. Mekalanos, Harvard Medical School, Boston, MA, and approved August 19, 2003 (received for review July 8, 2003)

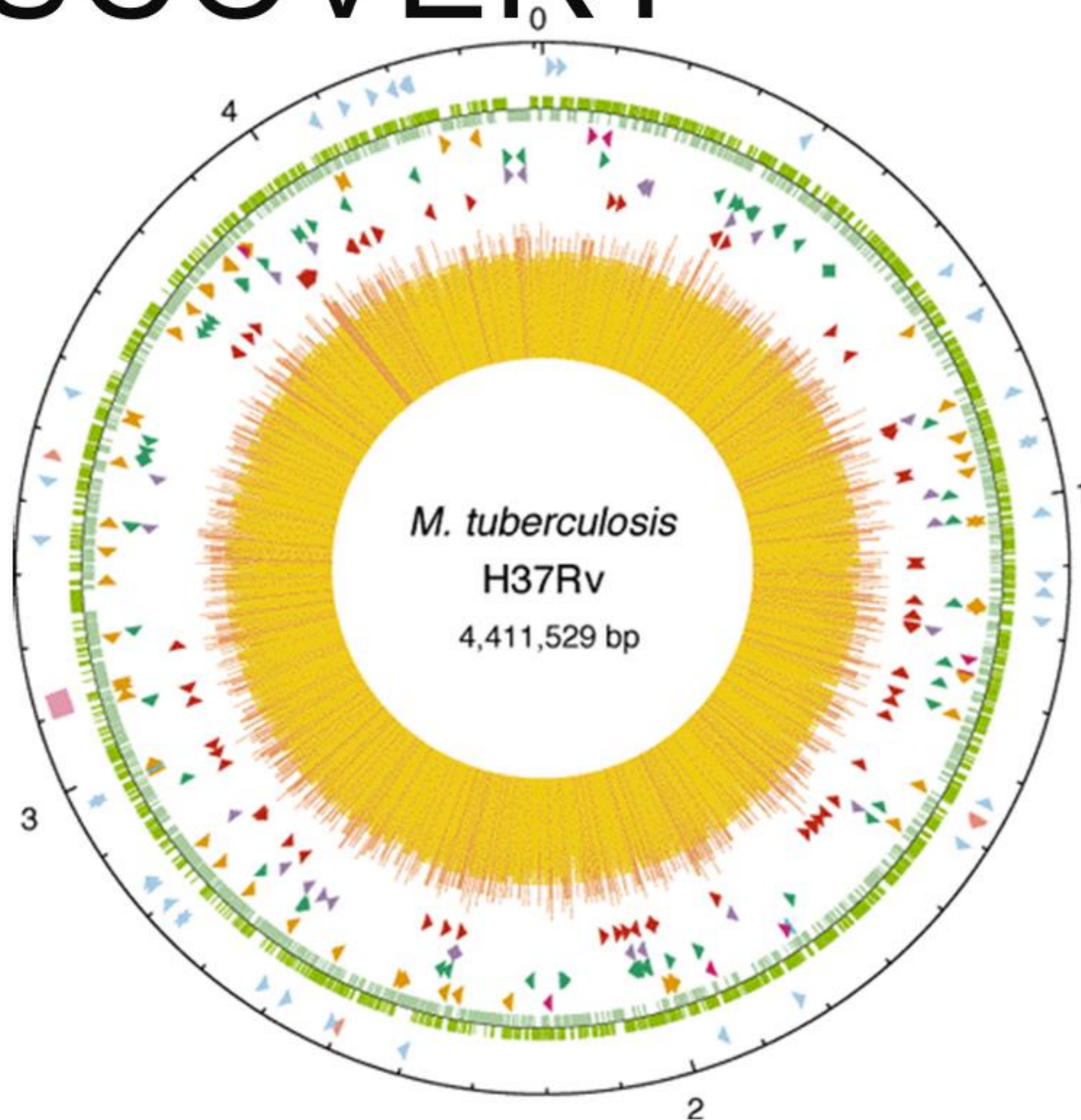
High-Resolution Phenotypic Profiling Defines Genes Essential for Mycobacterial Growth and Cholesterol Catabolism

Jennifer E. Griffin¹, Jeffrey D. Gawronski¹, Michael A. DeJesus², Thomas R. Ioerger², Brian J. Akerley¹, Christopher M. Sassetti^{1,3*}

¹ Department of Microbiology and Physiological Systems, University of Massachusetts Medical School, Worcester, Massachusetts, United States of America, ² Department of Computer Science, Texas A&M University, College Station, Texas, United States of America, ³ Howard Hughes Medical Institute, Chevy Chase, Maryland, United States of America



METABOLOMICS IN DRUG DISCOVERY



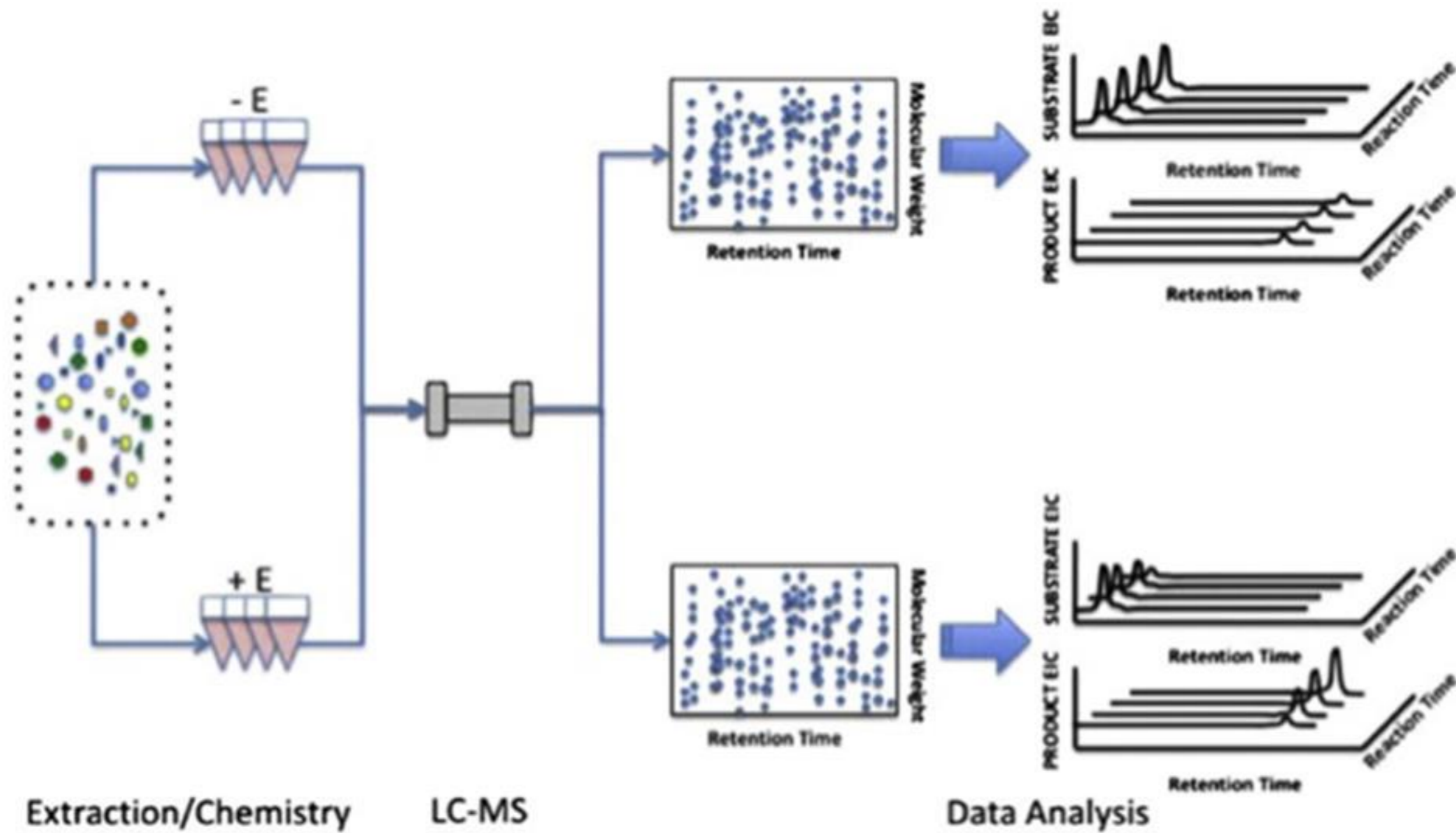
~ 40% functionally assigned*
(1,600)

~ 44% are conserved hypothetical proteins
(1,760)

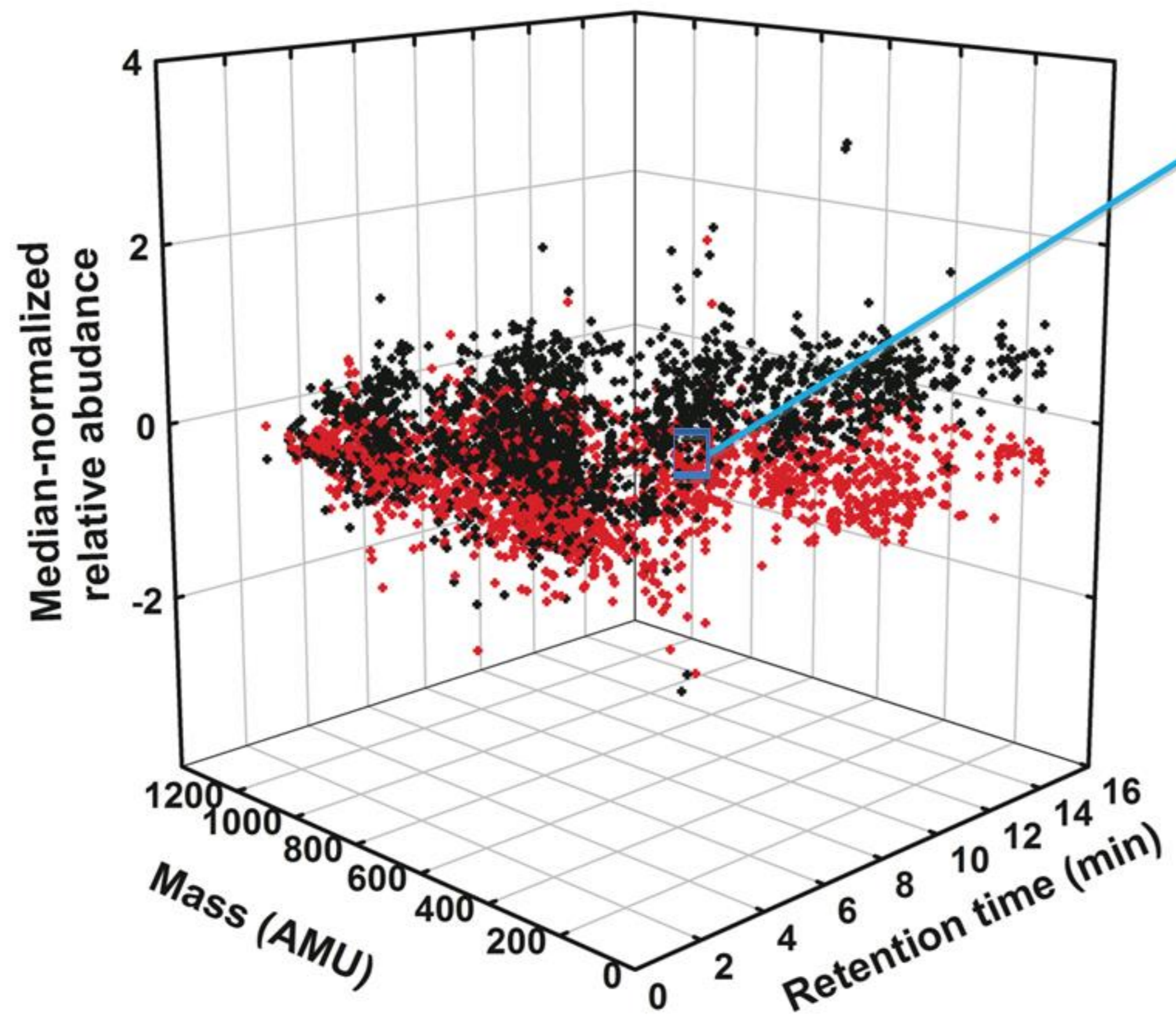
~ 16% have no similarity to anything ever seen
(640)

Only 1 of 8 genes annotated to encode subunits of Mtb's PDH, KDH cxs fulfilled its prediction (aceE; E1 PDH)

ACTIVITY-BASED METABOLOMIC PROFILING



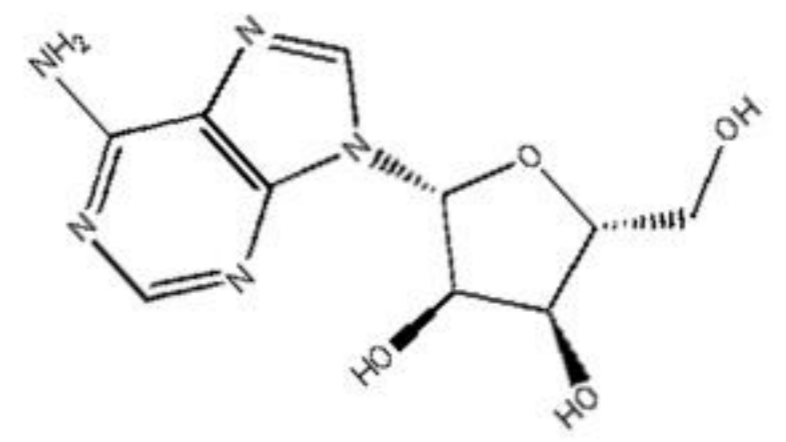
METABOLOMIC LIBRARY



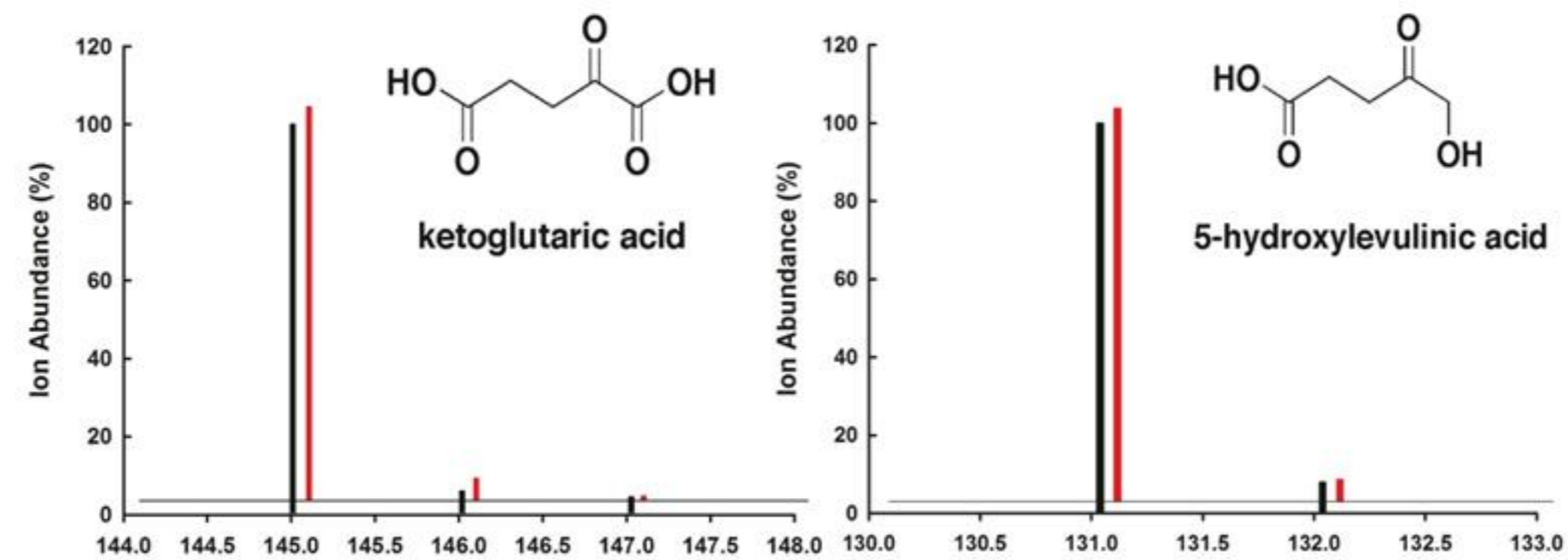
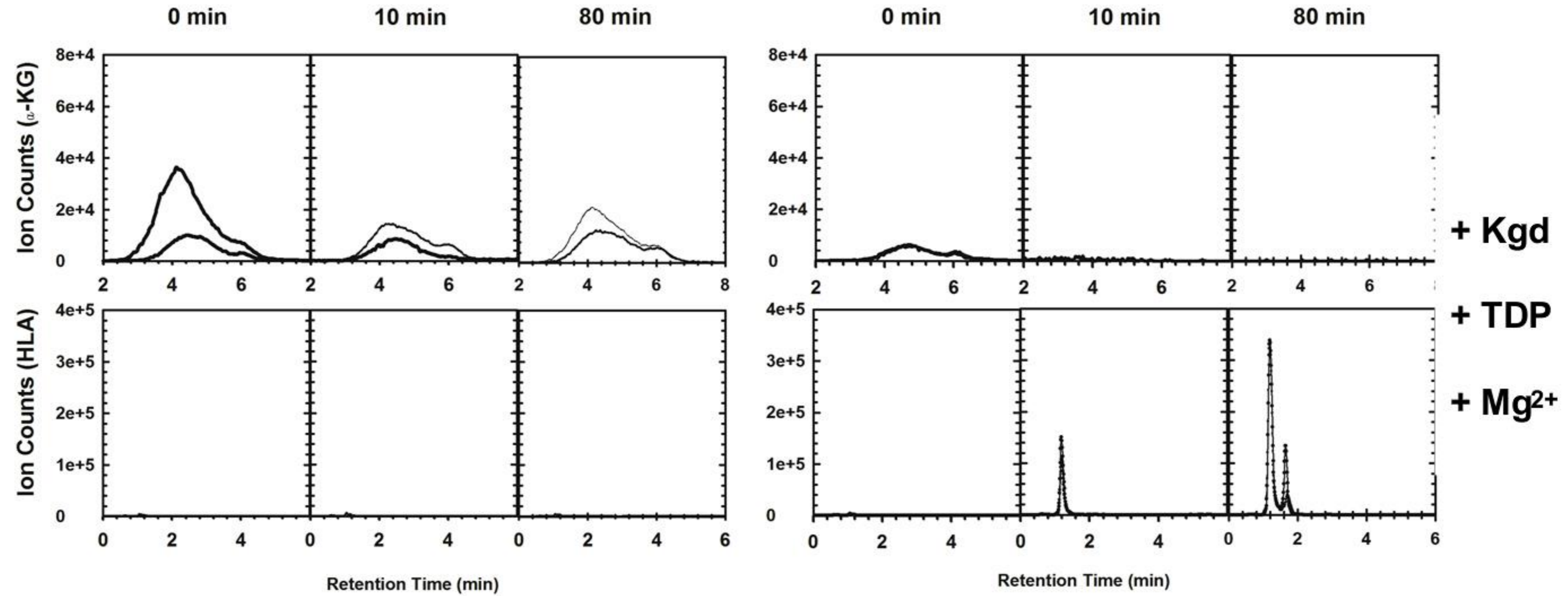
$m/z = 268.1040 [M+H]^+$
RT = 4.55 min
Isotopic envelope match (98%)

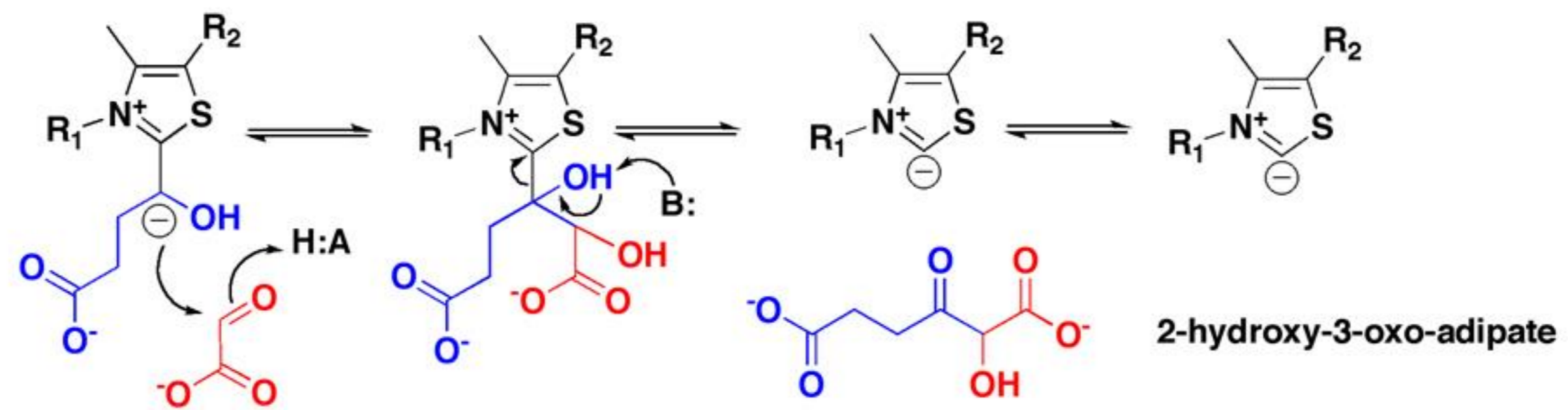
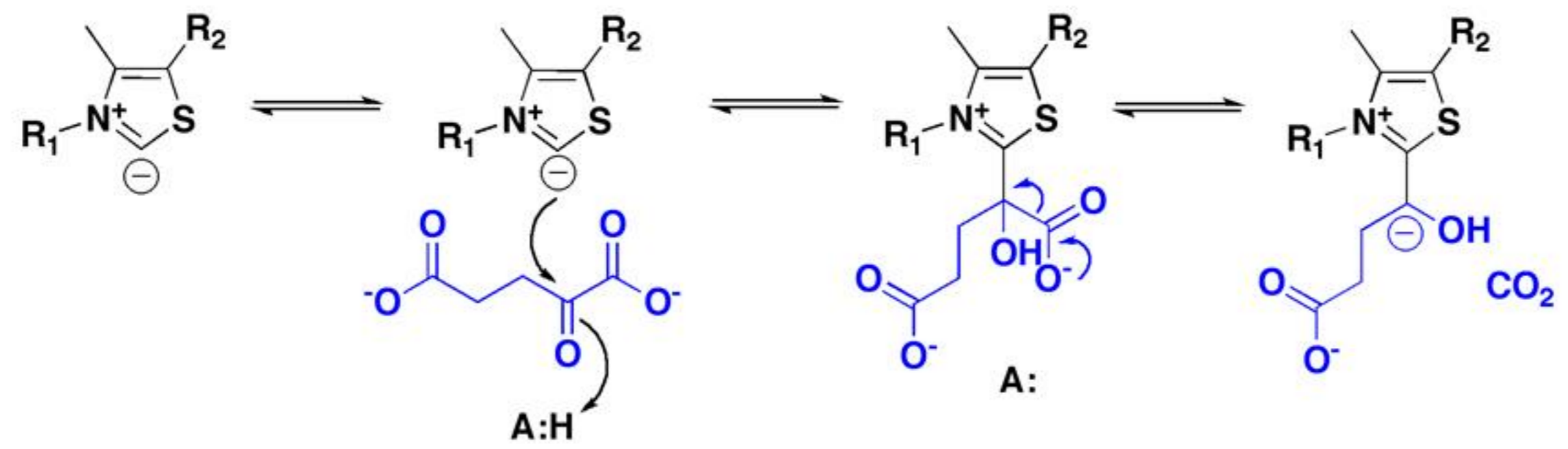


Adenosine



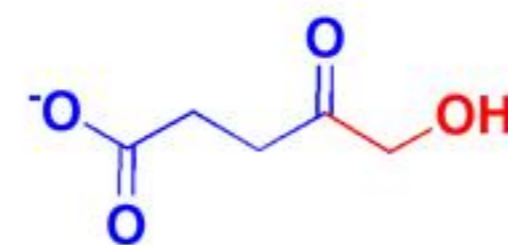
RV1248C



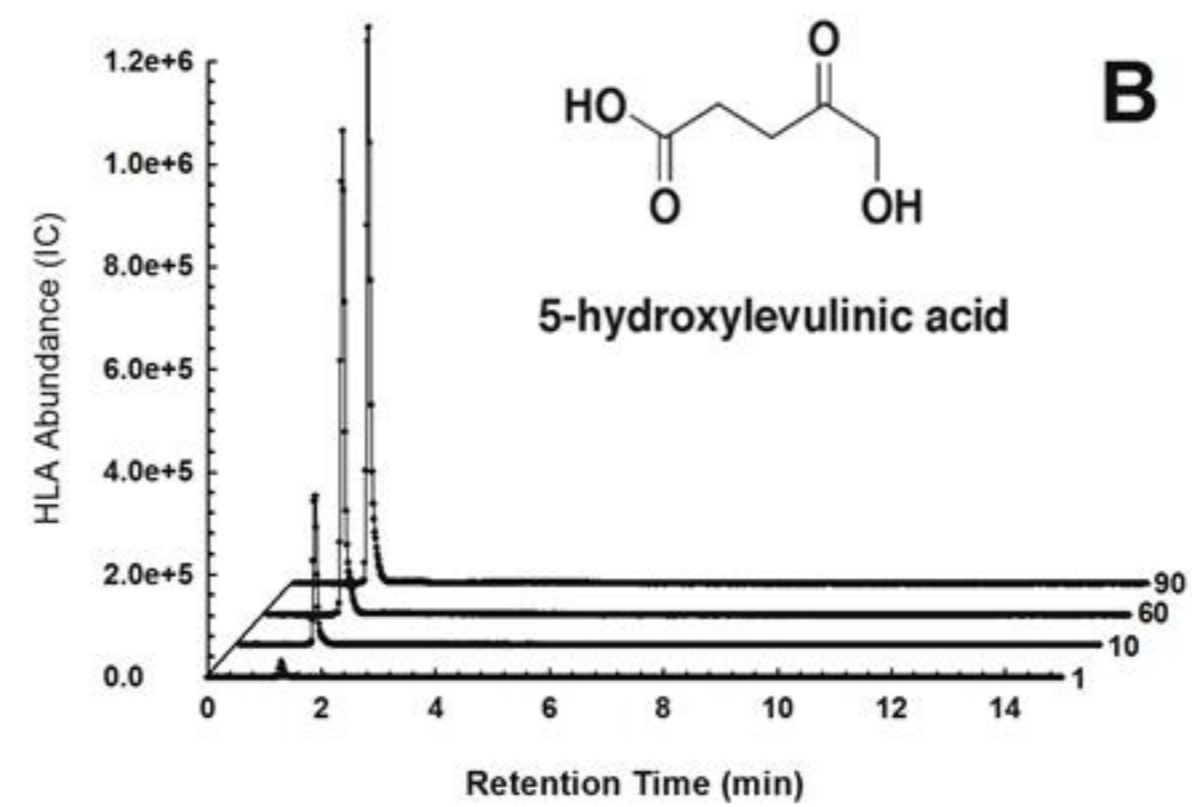
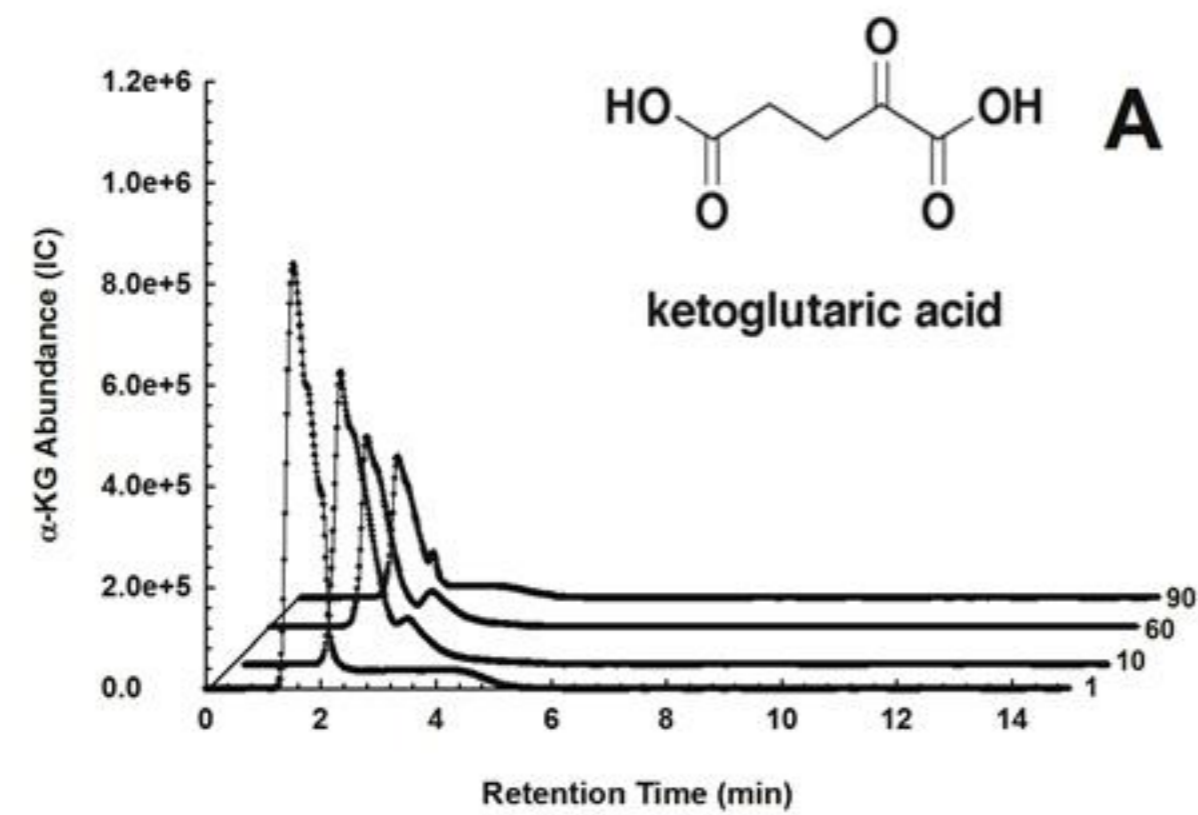


nonenzymatic decomposition
 CO_2

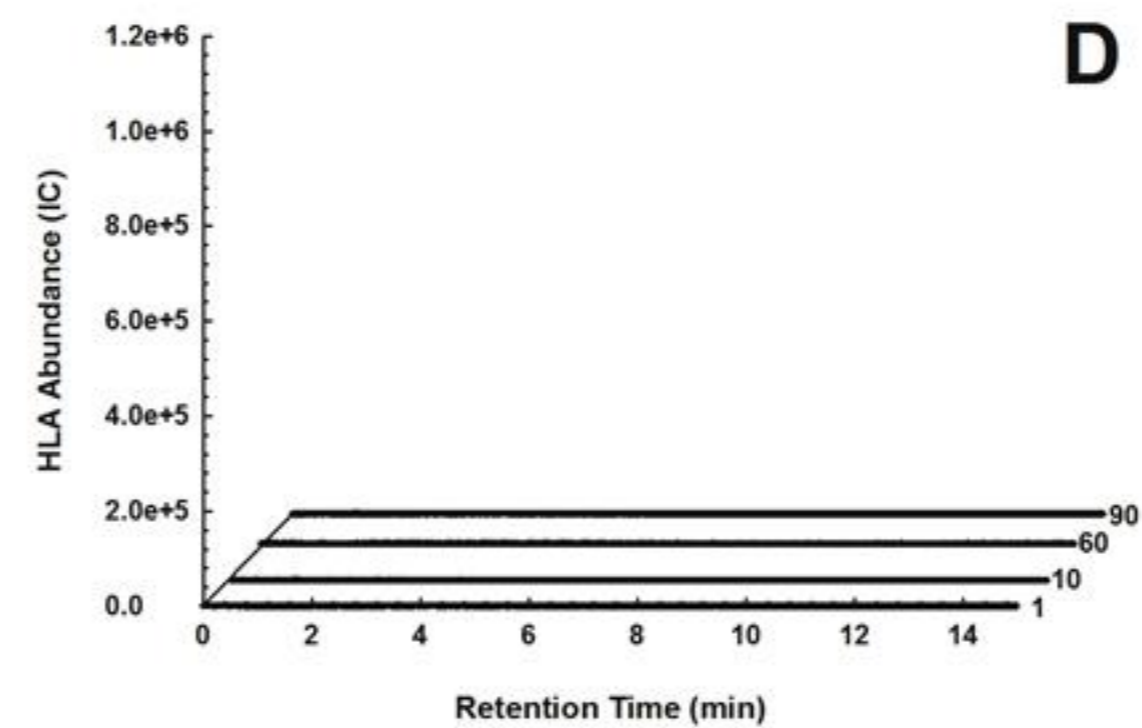
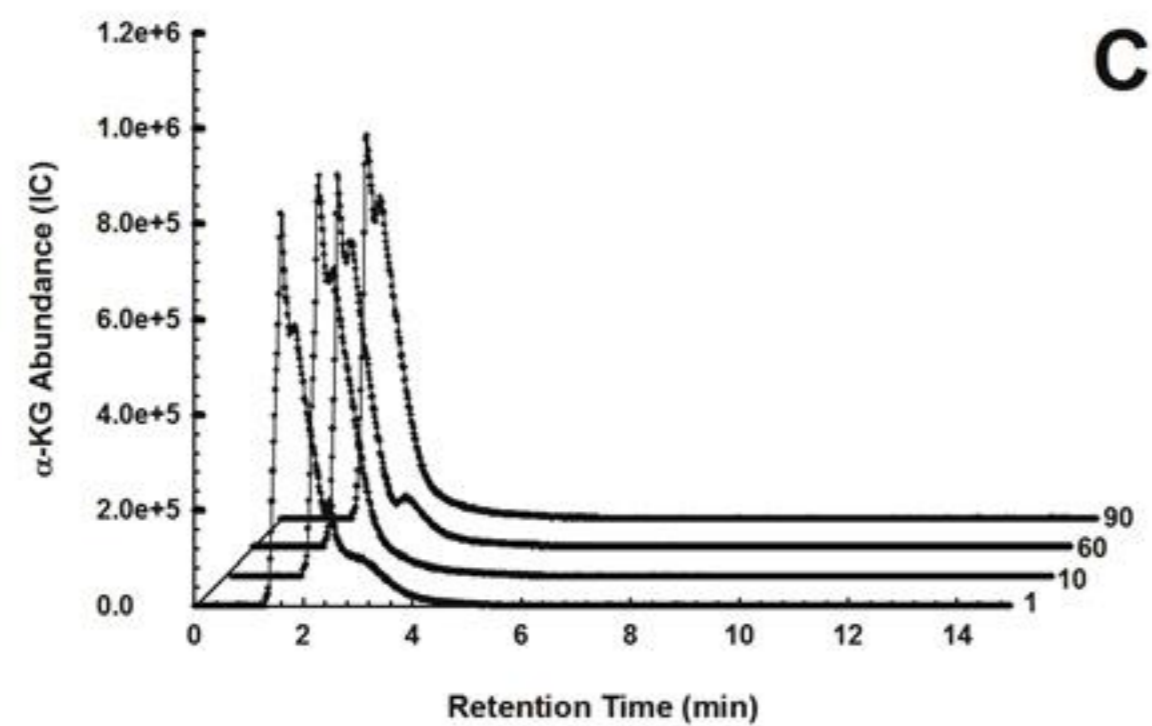
5-hydroxy-levulinate
 $m/z = 131.0350$ (M-H)-



IN VITRO CONFIRMATION

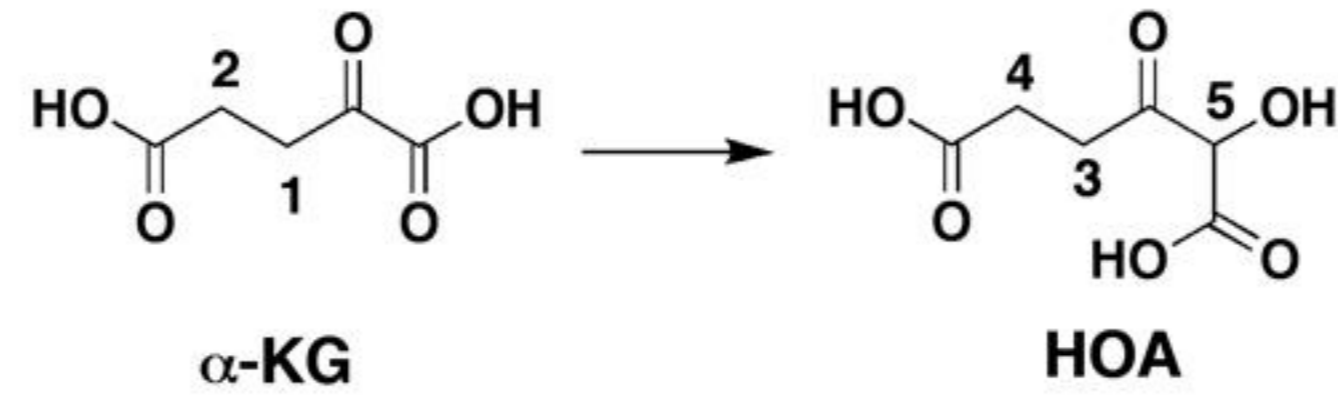


+Rv1248c



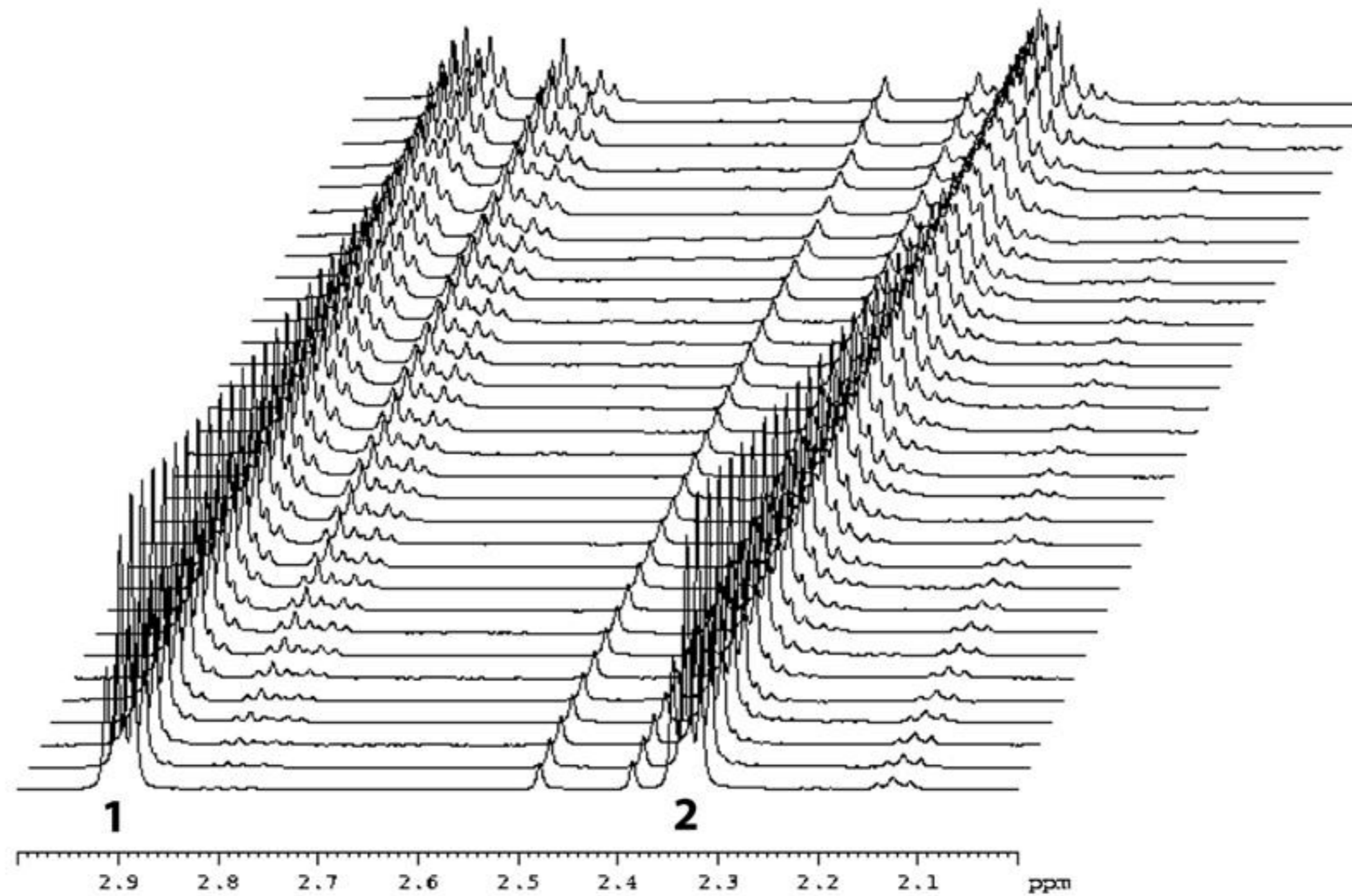
-Rv1248c

^1H NMR

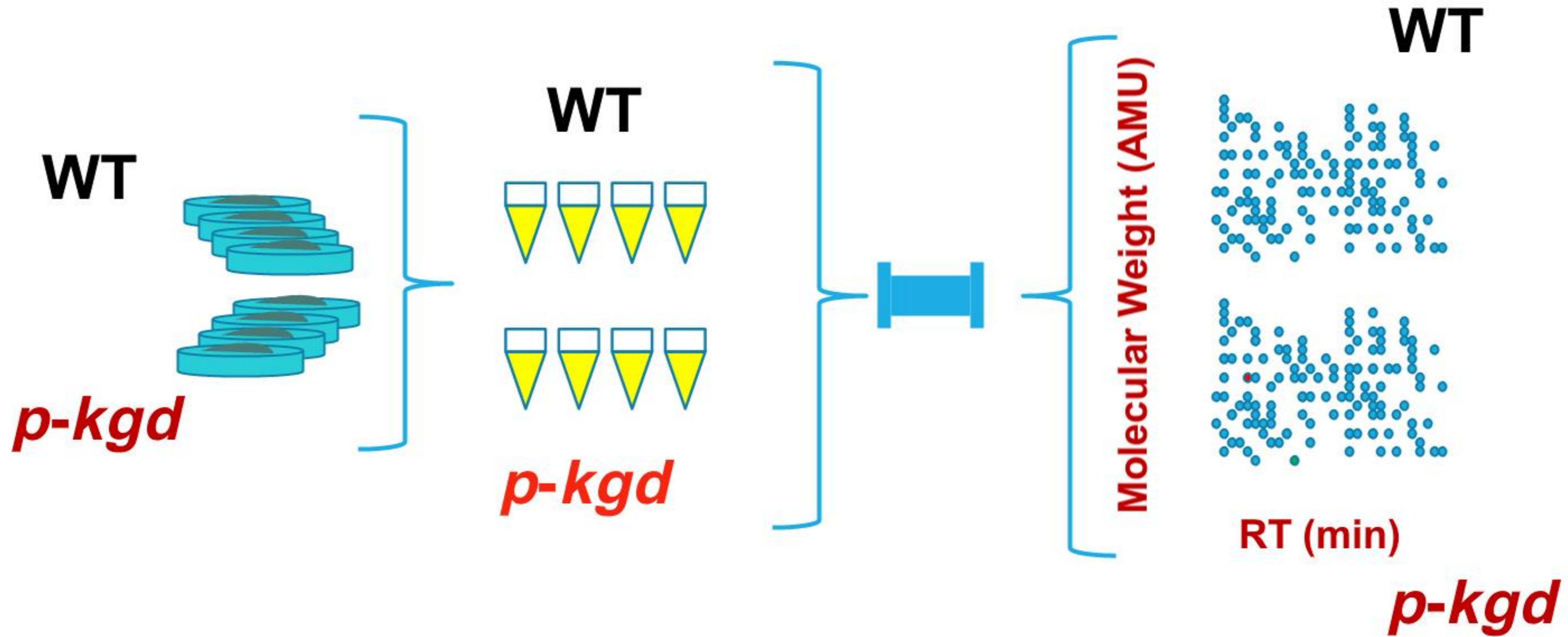


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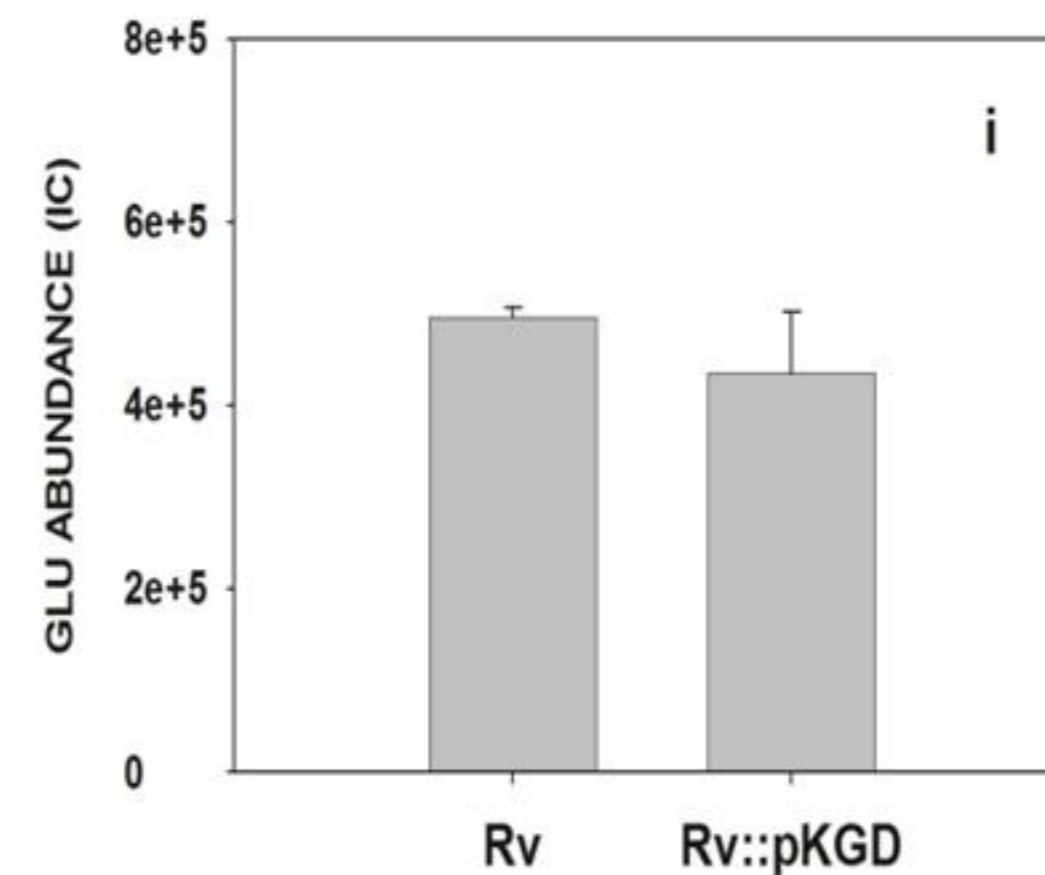
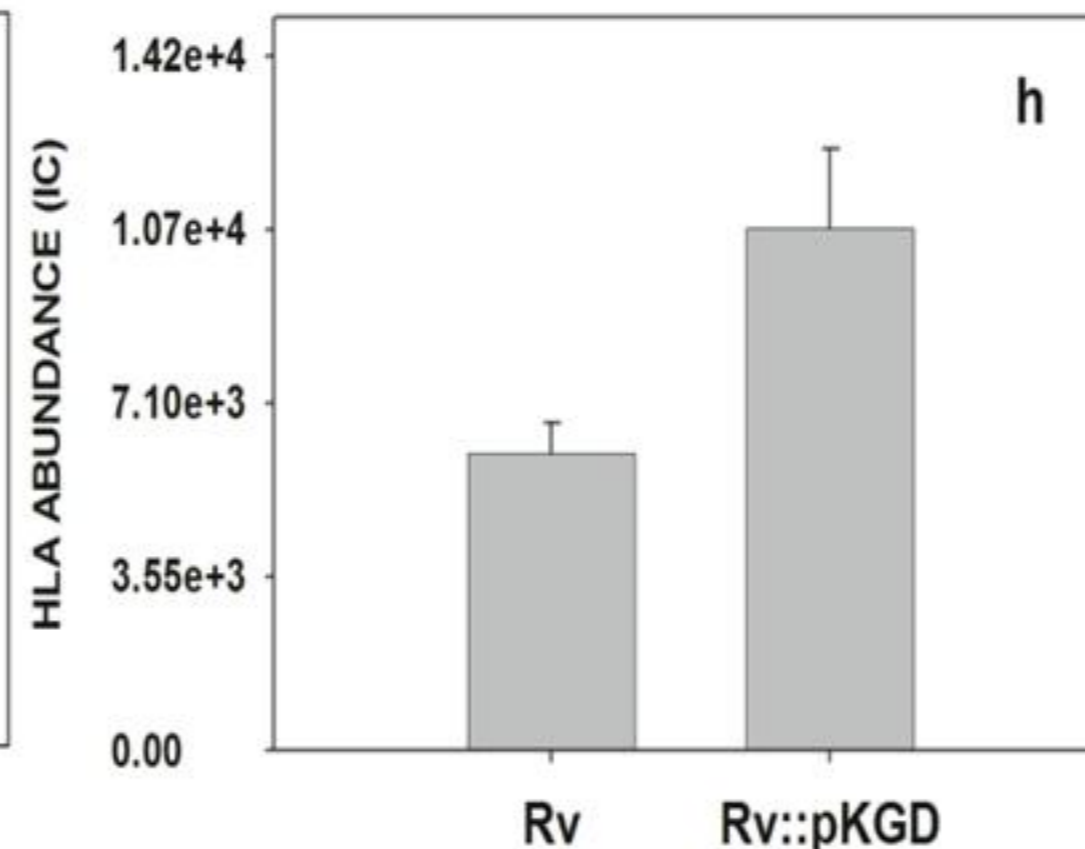
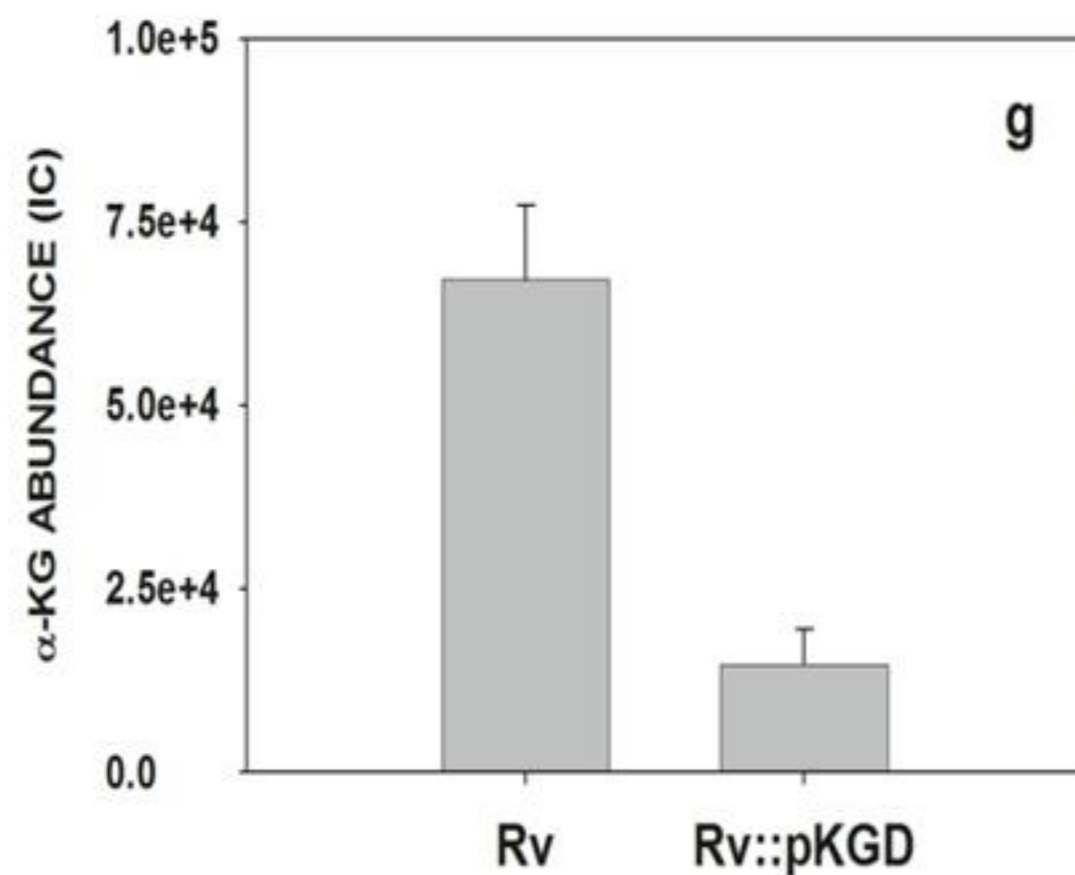
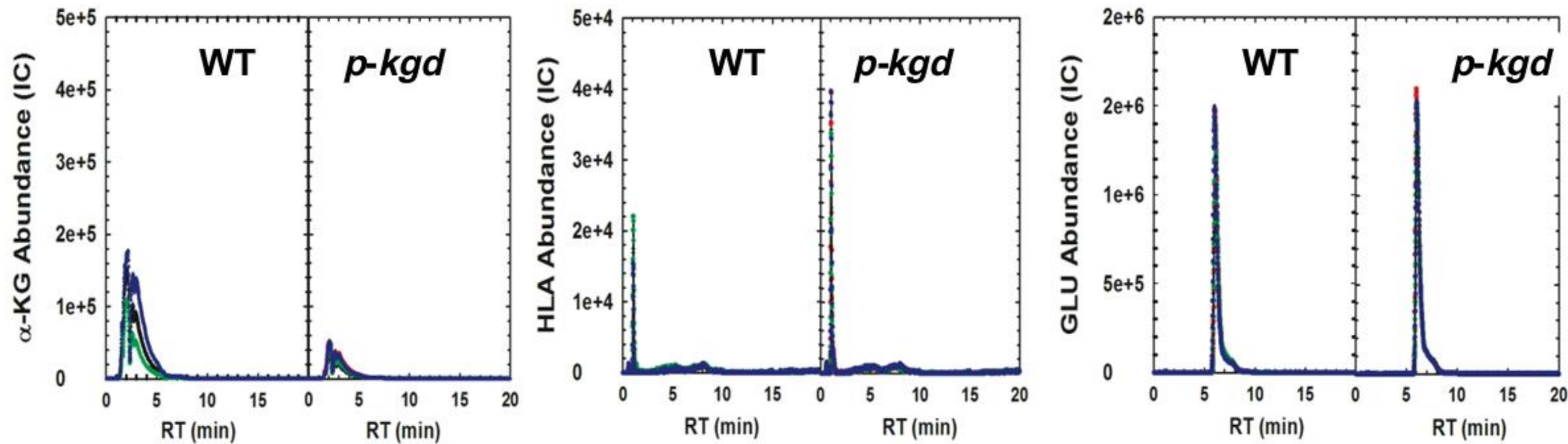
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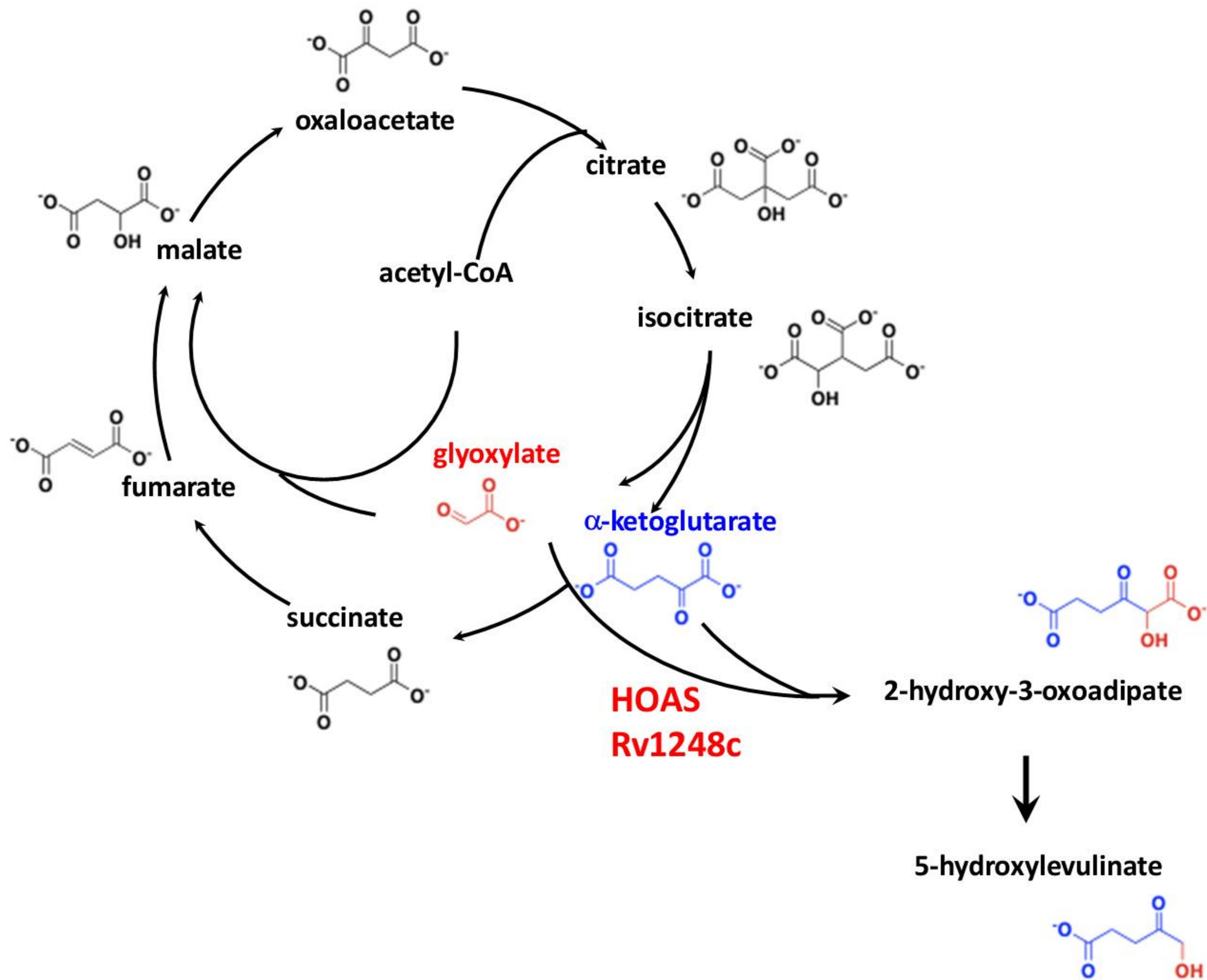


IN VIVO CONFIRMATION

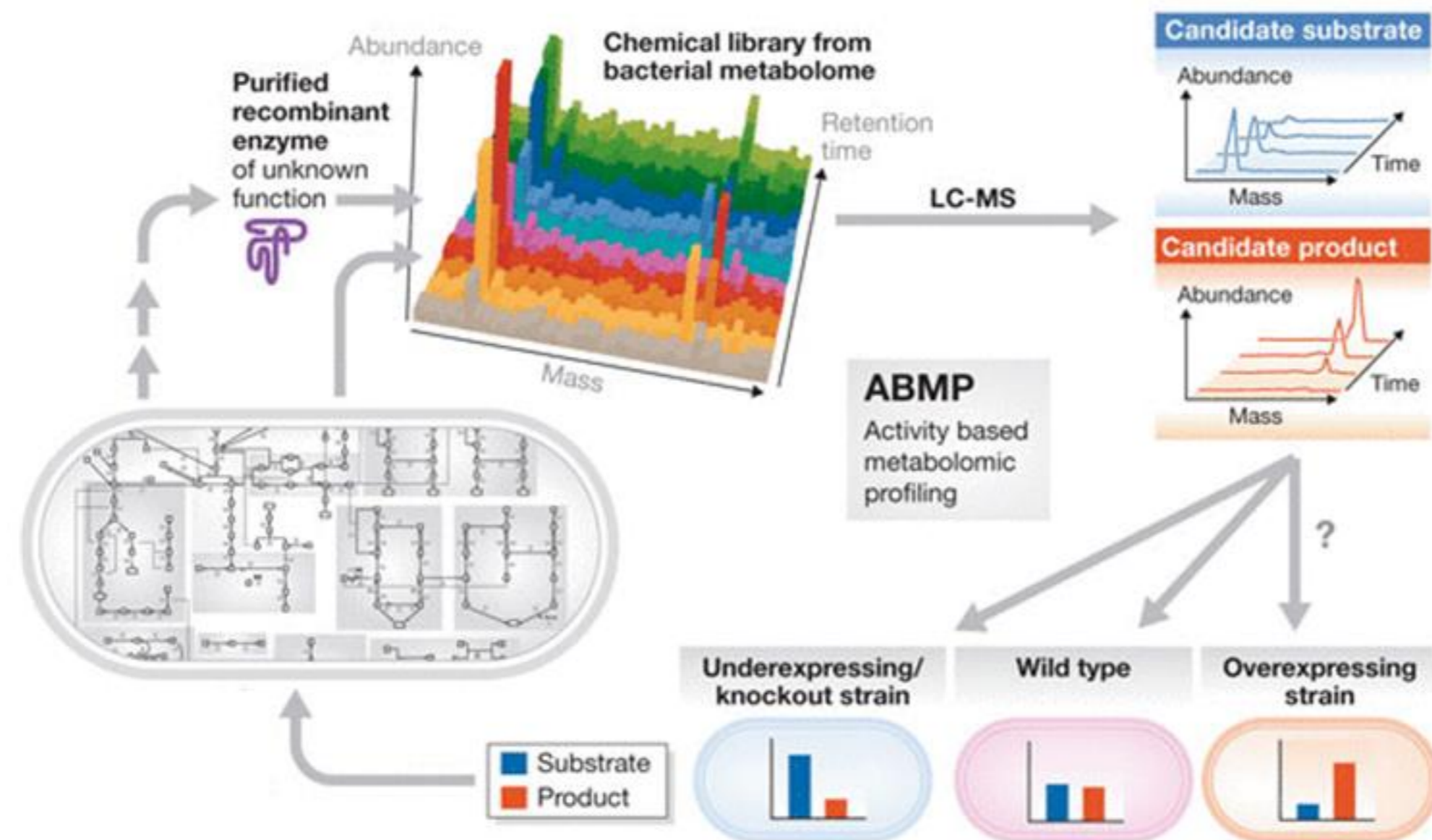


IN VIVO CONFIRMATION





ABMP WORKFLOW



ACKNOWLEDGEMENTS

- Hyungjin Eoh
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- Sumit Chakraborty, Madhumitha Nandakumar, Zhe Wang
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- Dirk Schnappinger
- Helena Boshoff, Clif Barry (NIH)
- Eric Rubin, HSPH

- ID division



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