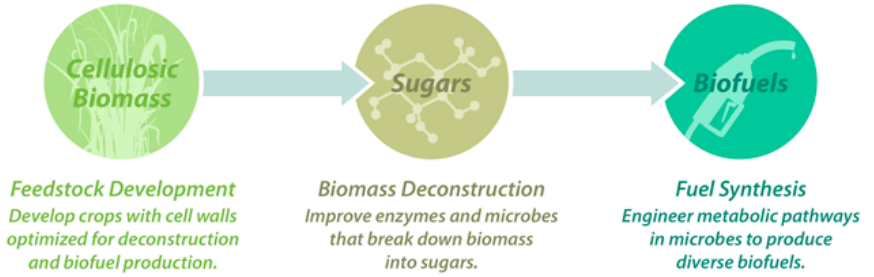
JGI JOINT GENOME INSTITUTE
UNITED STATES DEPARTMENT OF ENERGY

Metabolomics Technologies at JGI

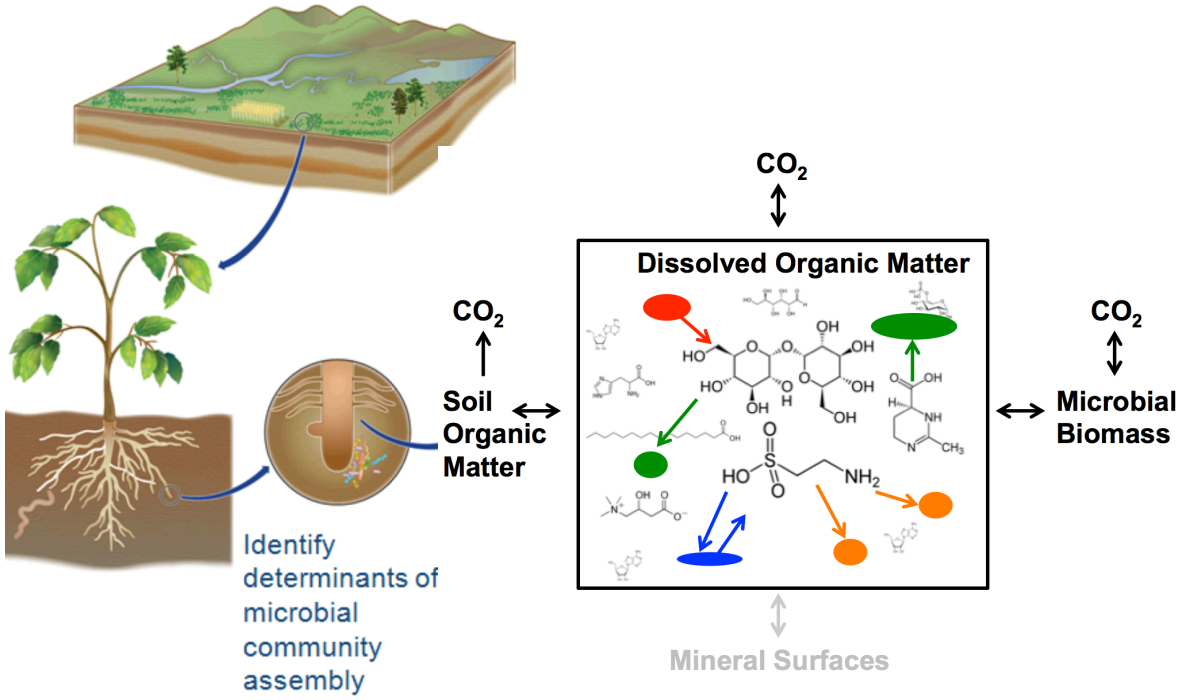
Trent Northen

Metabolism is central to DOE missions in Genomics

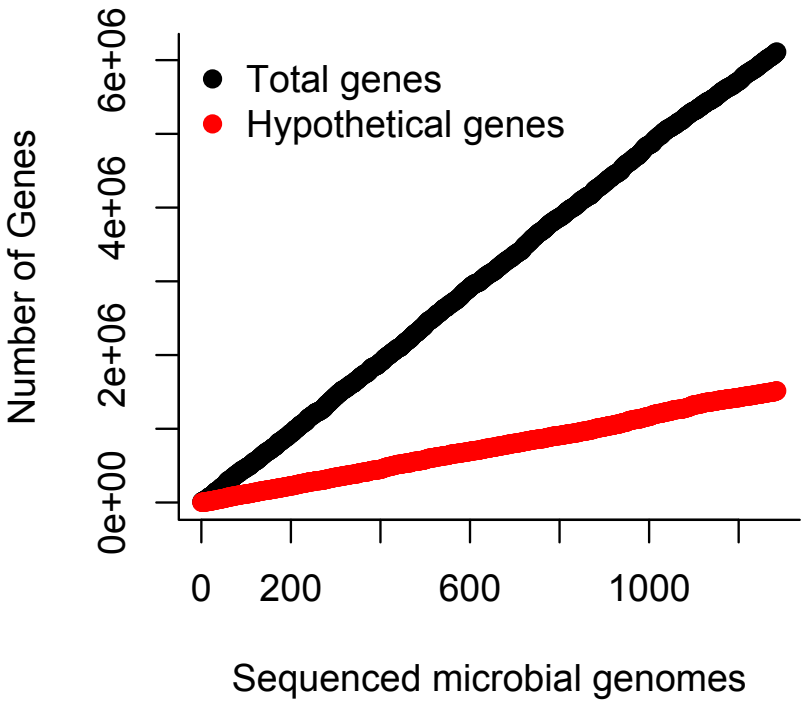
- Bioenergy
- Contaminant Bioremediation
- Understanding Earth's biogeochemical cycles

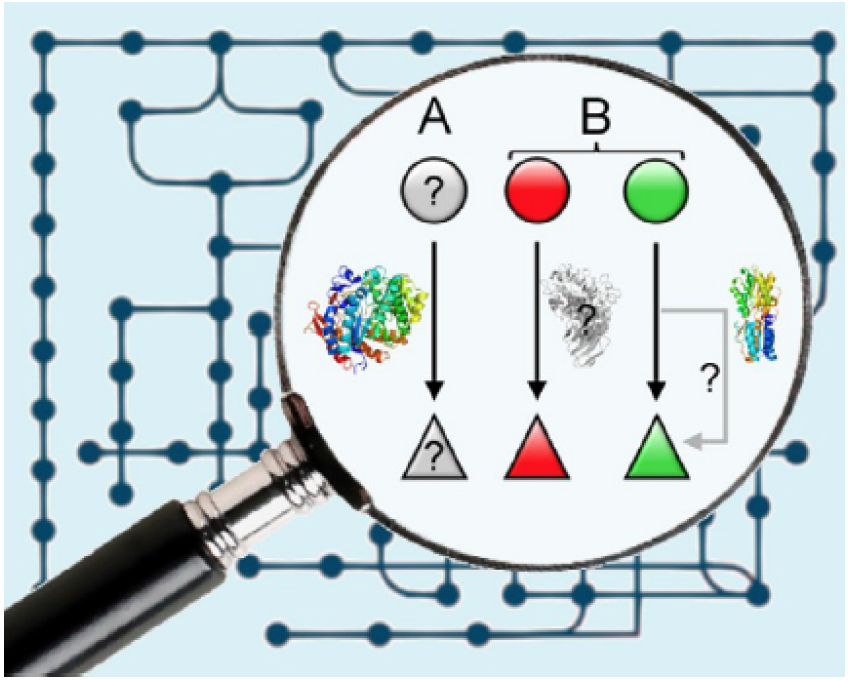


DOE Bioenergy Research Centers



The need: Sequencing is now fast and easy but assigning function remains tedious and slow.





Median bacterial genome:

3261 **protein coding genes** and

971 **"hypothetical"** genes

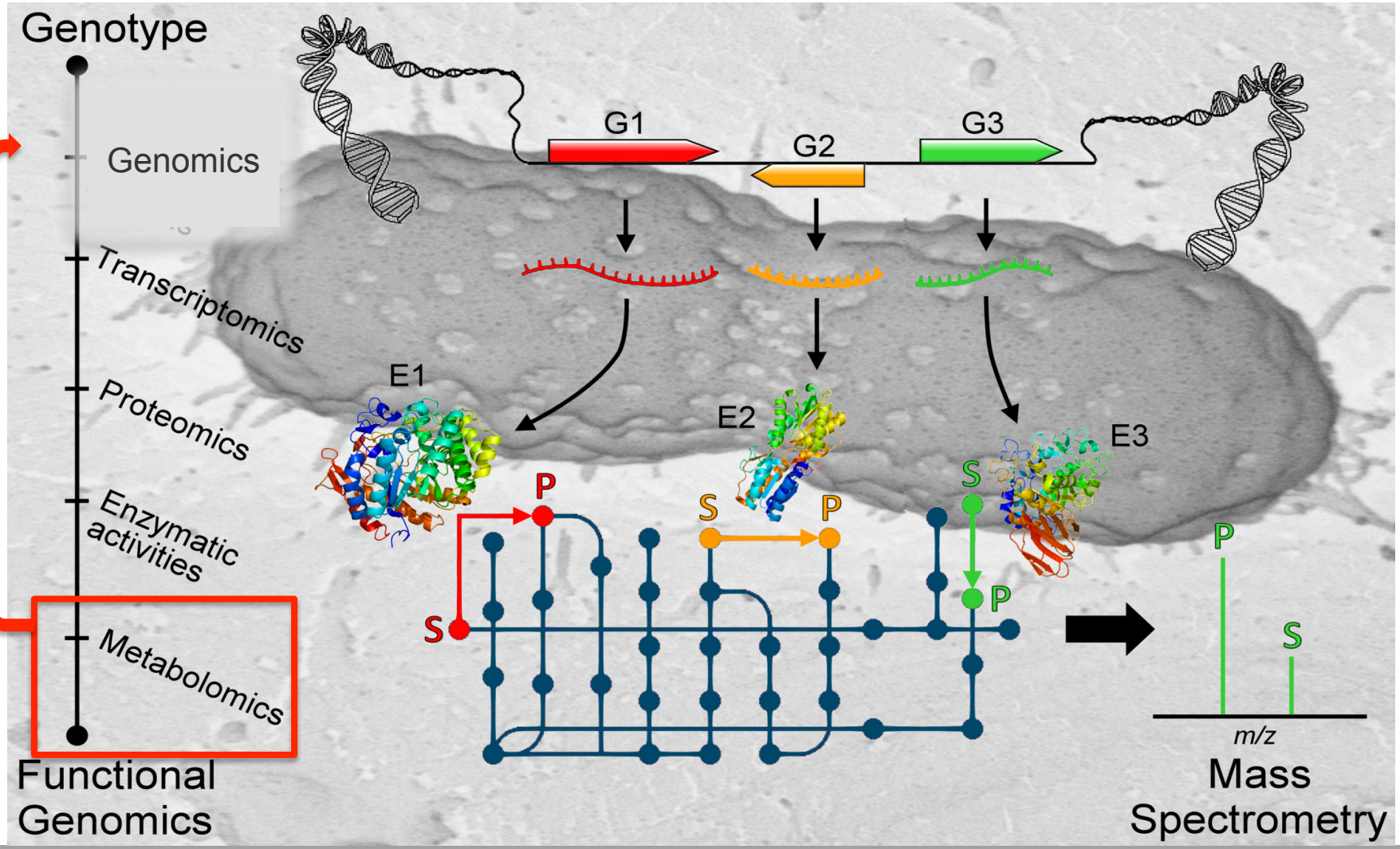
Determining the function of this 'genomic dark matter' is one of the grand challenges in microbiology.

Tremendous advances in sequencing

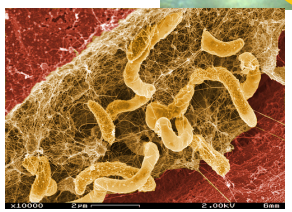
Classical methods are still used for functional assignment: 1 gene, 1 protein= 1 PhD

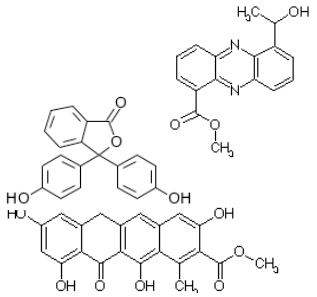
Metabolomics provides a functional complement genomics

Mass spectrometry based metabolomics provides a direct functional readout that can be linked to gene function



LC-MS/MS Workflow

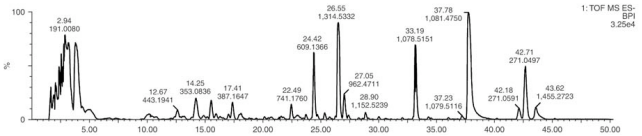


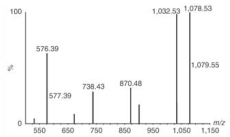


metabolite solvent extraction

HPLC
(C18; hilic)

Sample independent: suitable for unsequenced organisms and communities





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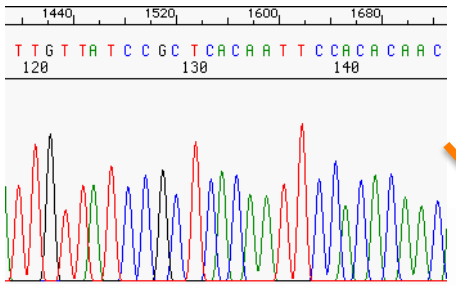
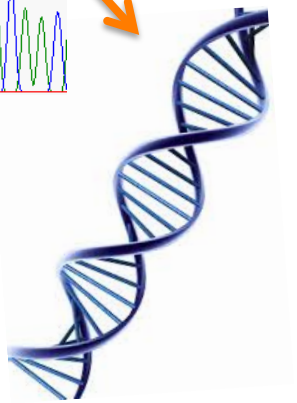
C18NEG/270.82471/1.34821/
C18NEG/168.88735/1.29241/

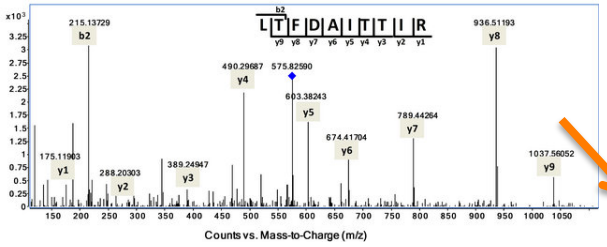
MS/MS

Metabolite identification
&
Quantification

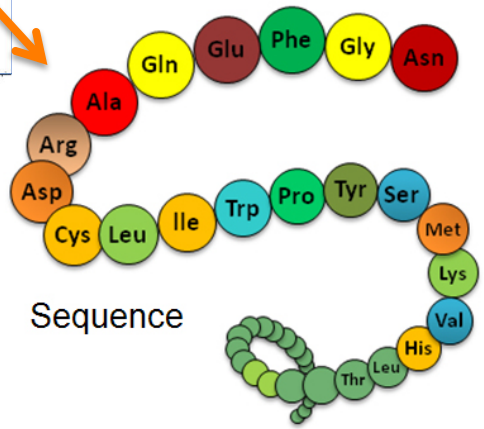
Metabolite profiling has unique challenges vs. DNA sequencing and proteomics



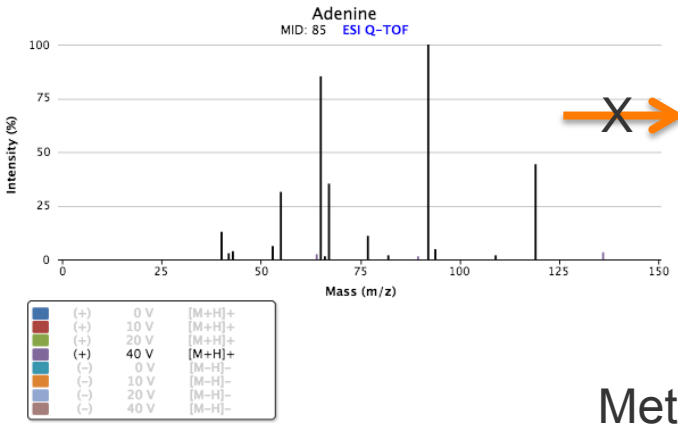


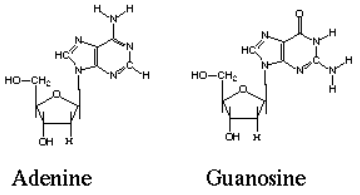


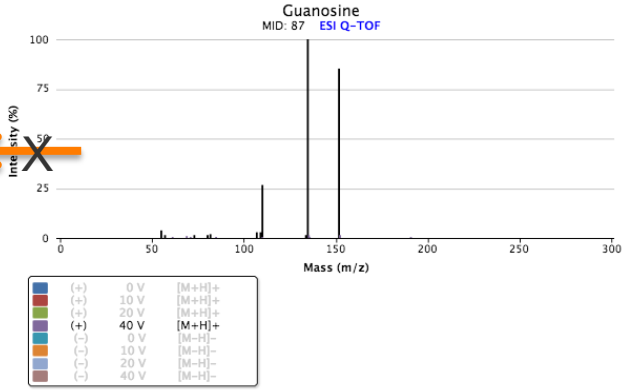
Peptide fragment analysis



Can't yet assign metabolite structure *de novo*







Metabolite fragment analysis

Metabolite identification is ideally based on comparison with authentic standards

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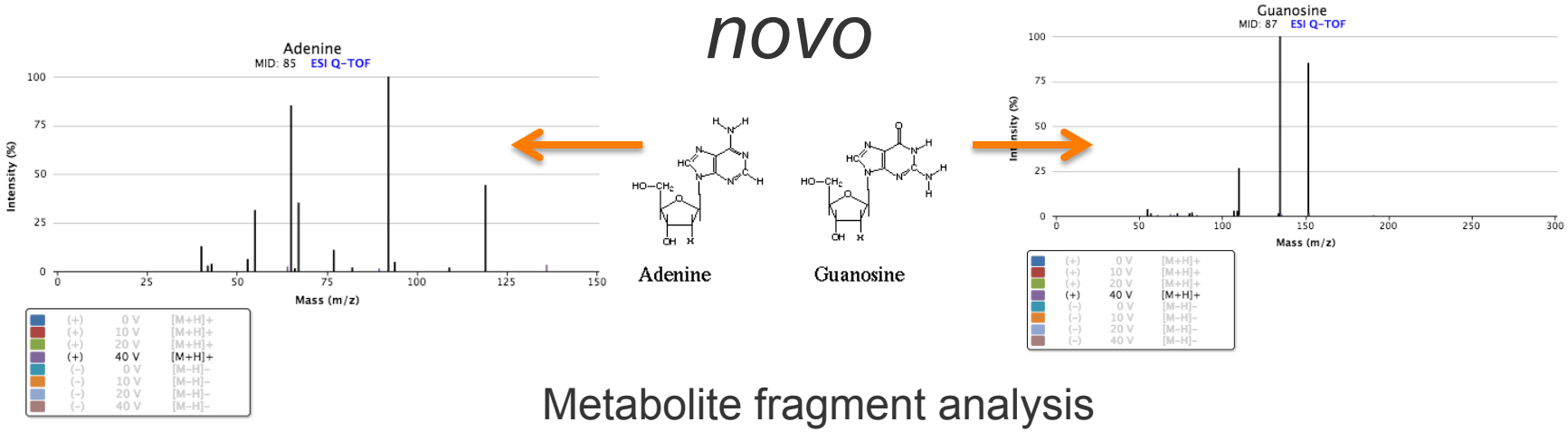
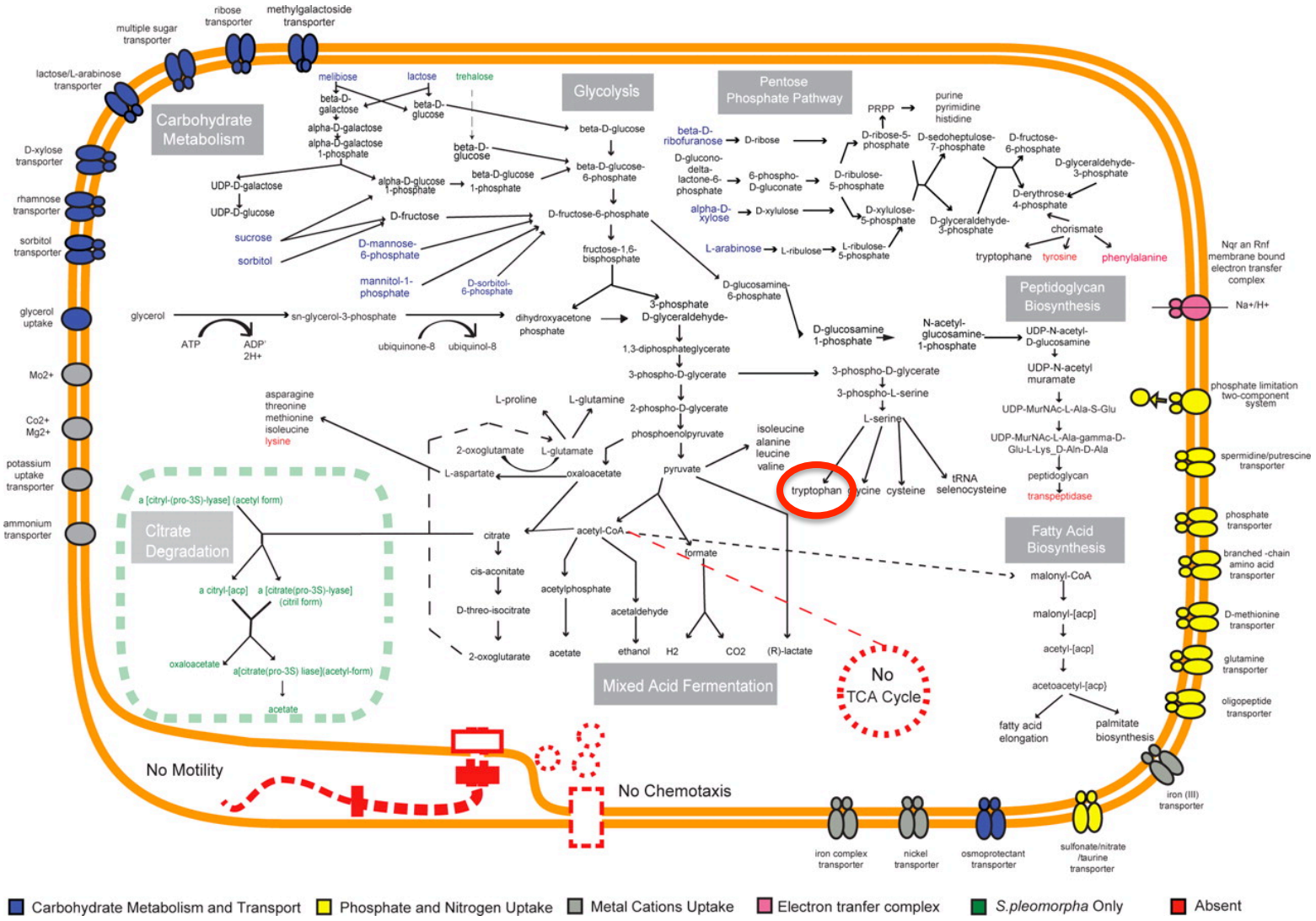


Metabolite fragment analysis

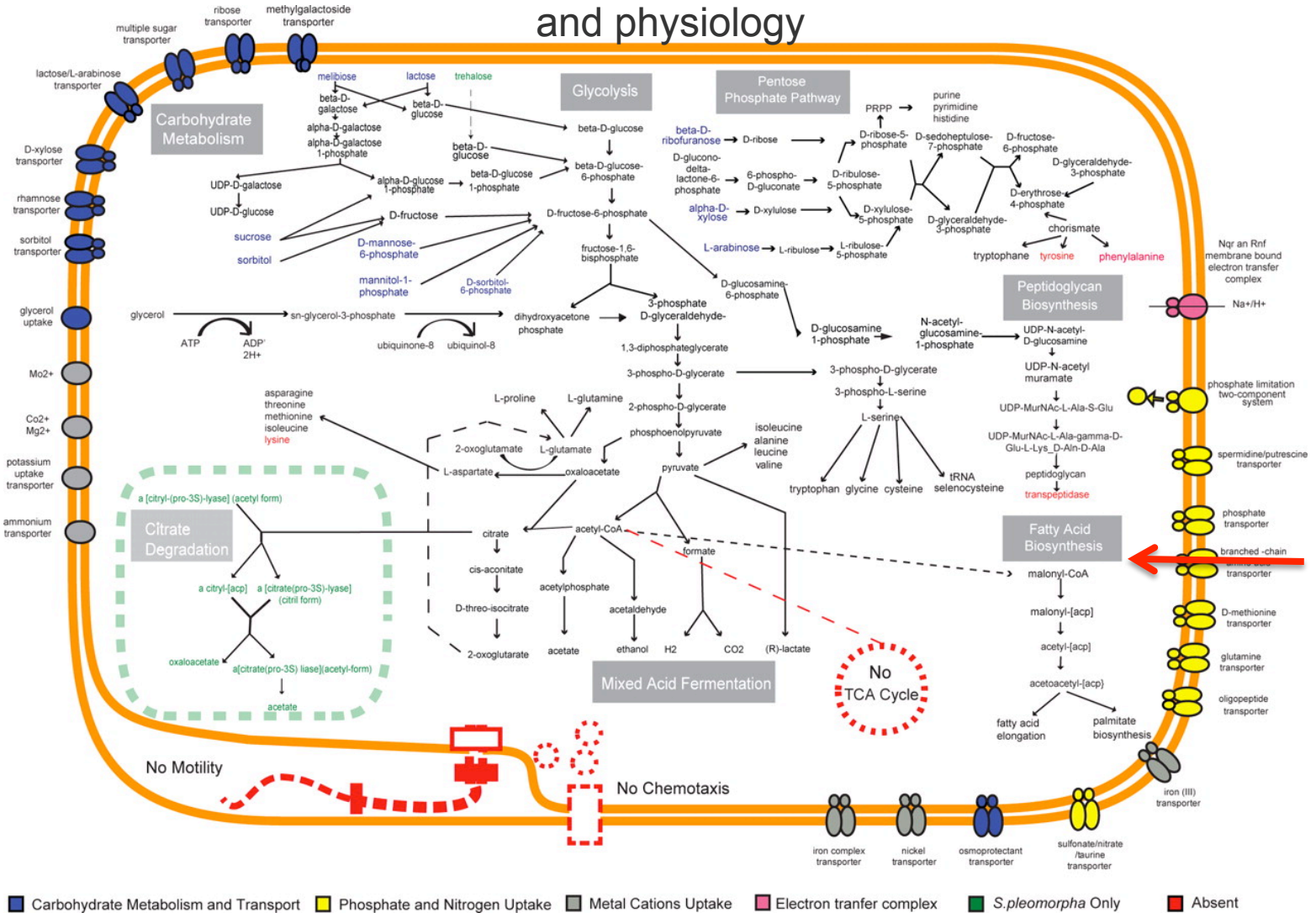
Endometabolomics

Detection of intracellular metabolites provides rich information on biosynthetic capacities

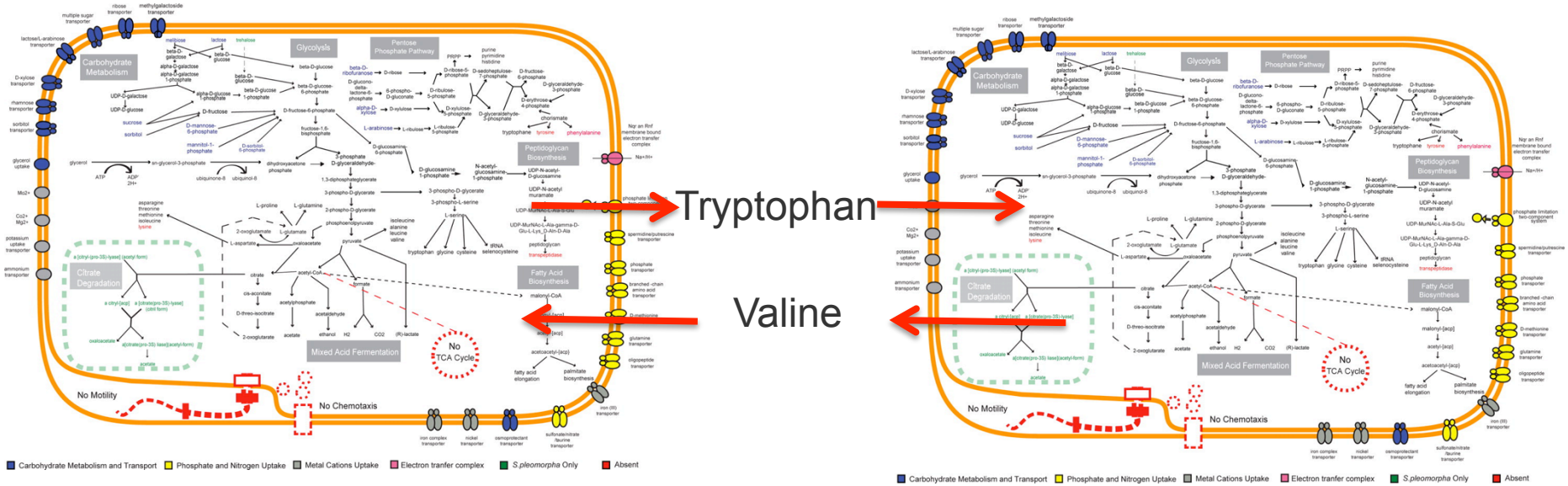


Exometabolomics (A.K.A. metabolic footprinting)

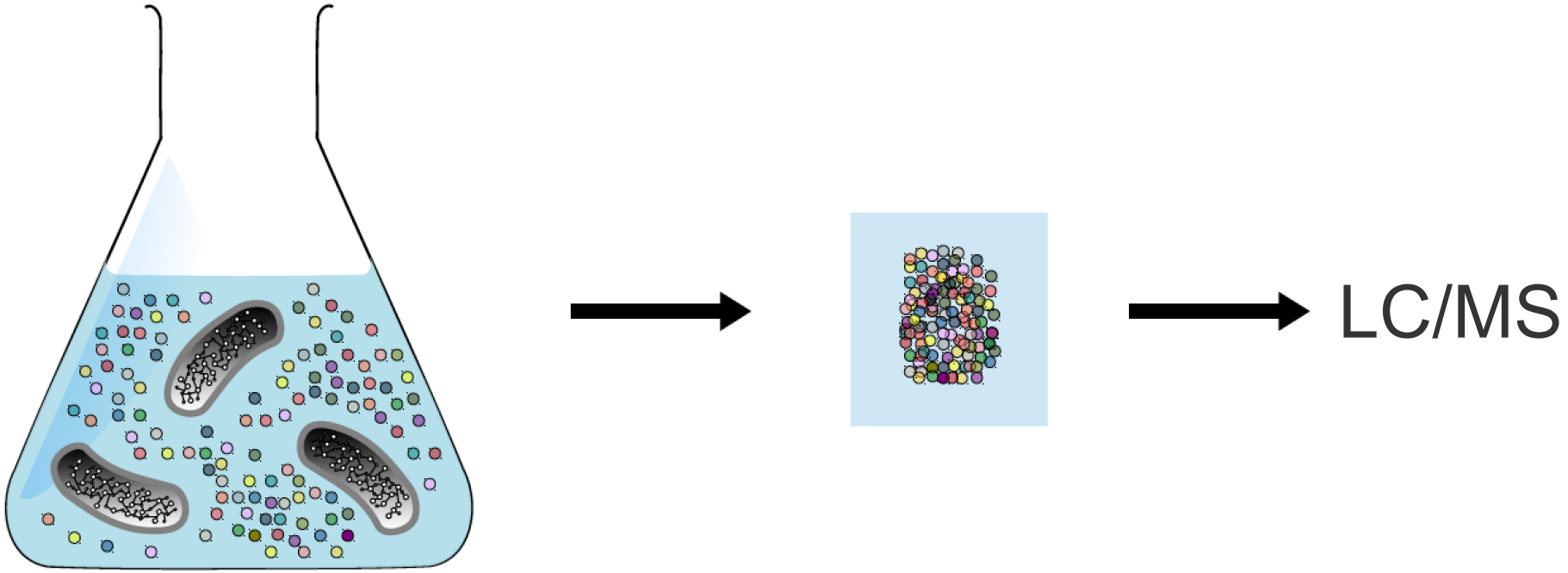
Detection of the uptake or release of metabolites provides information on nutrient cycling and physiology



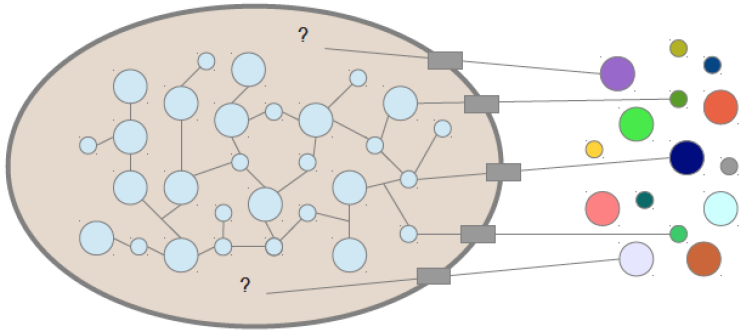
Exometabolomics to deconstruct intraspecific interactions



Exometabolomics approach



Measuring changes in a defined pool of metabolites (including unknowns) after culture growth is a tractable approach well suited to complement sequencing efforts



nature biotechnology

21 (2003) 692-696

High-throughput classification of yeast mutants for functional genomics using metabolic footprinting

Jess Allen[1], Hazel M Davey[1], David Broadhurst[1], Jim K Heald[1], Jem J Rowland[2], Stephen G Oliver[3] & Douglas B Kell[1]

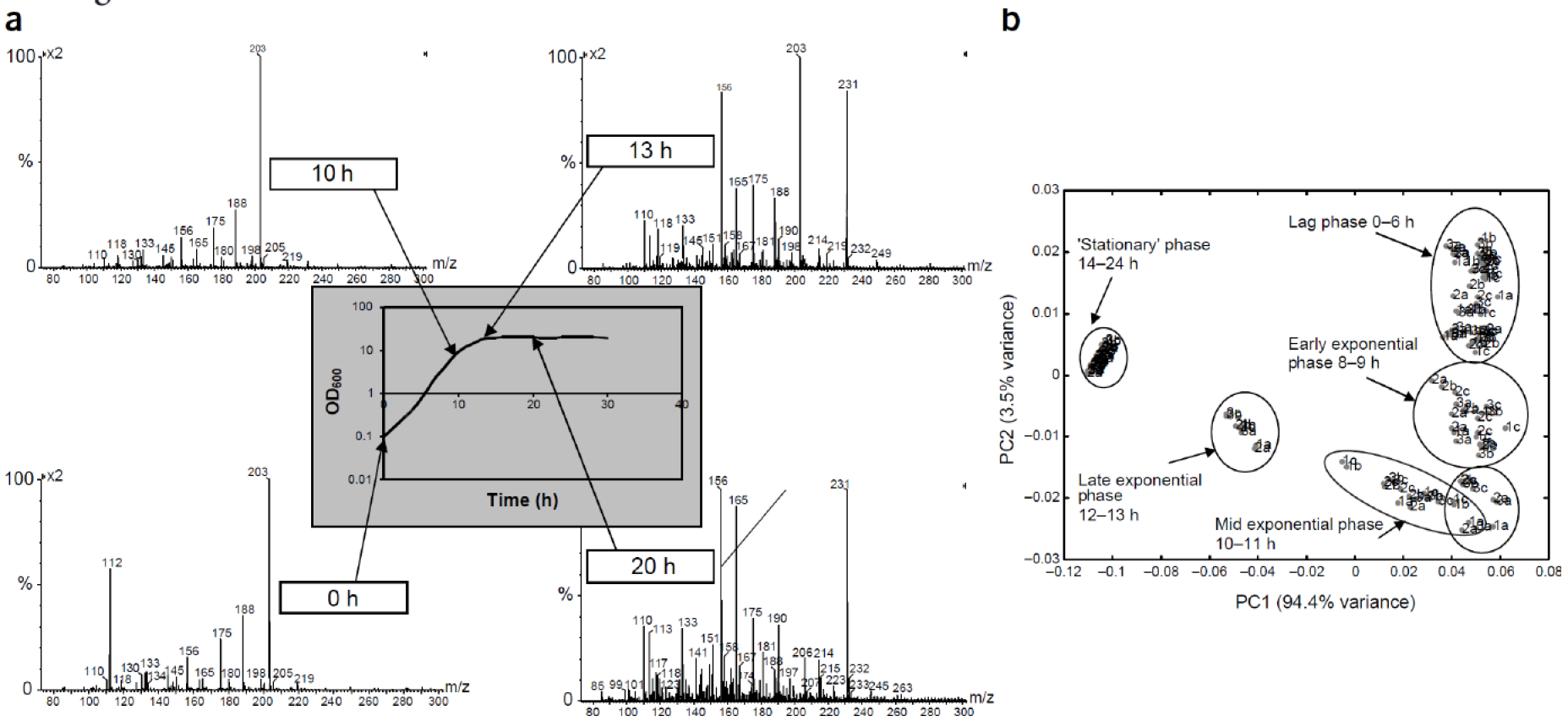


Figure 1 Metabolic footprinting of *Saccharomyces cerevisiae*. Cultures were grown on minimal medium supplemented with a metabolite cocktail (**Supplementary Table 1** online). Samples were removed from batch culture throughout the fermentation and prepared for mass spectral analysis (positive ionization, only *m/z* 65–300 displayed) as described in the text. TOF, time of flight. (**a**) Representative spectra. (**b**) Principal components analysis (PCA) of the data in **a**.

Direct coupling to genetics

nature biotechnology

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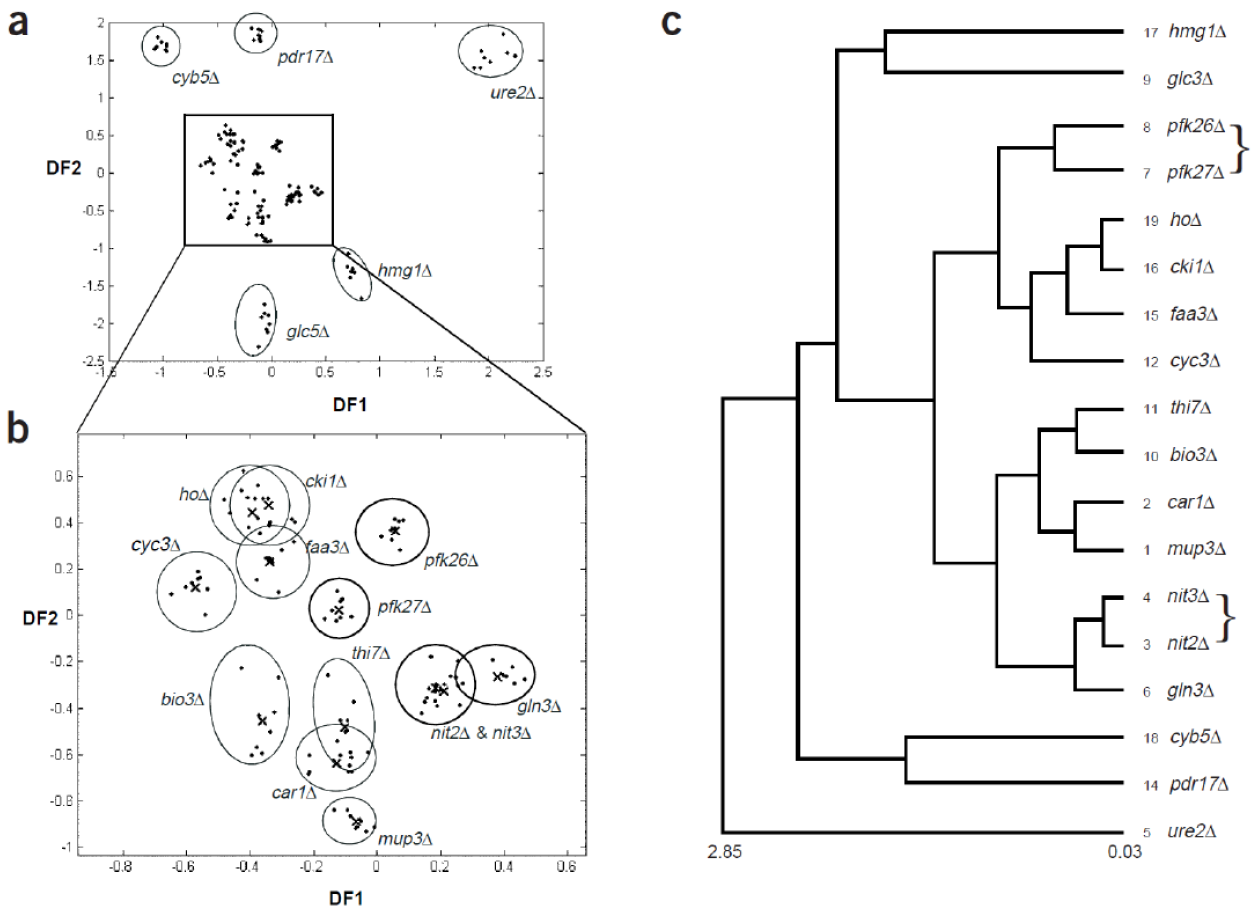
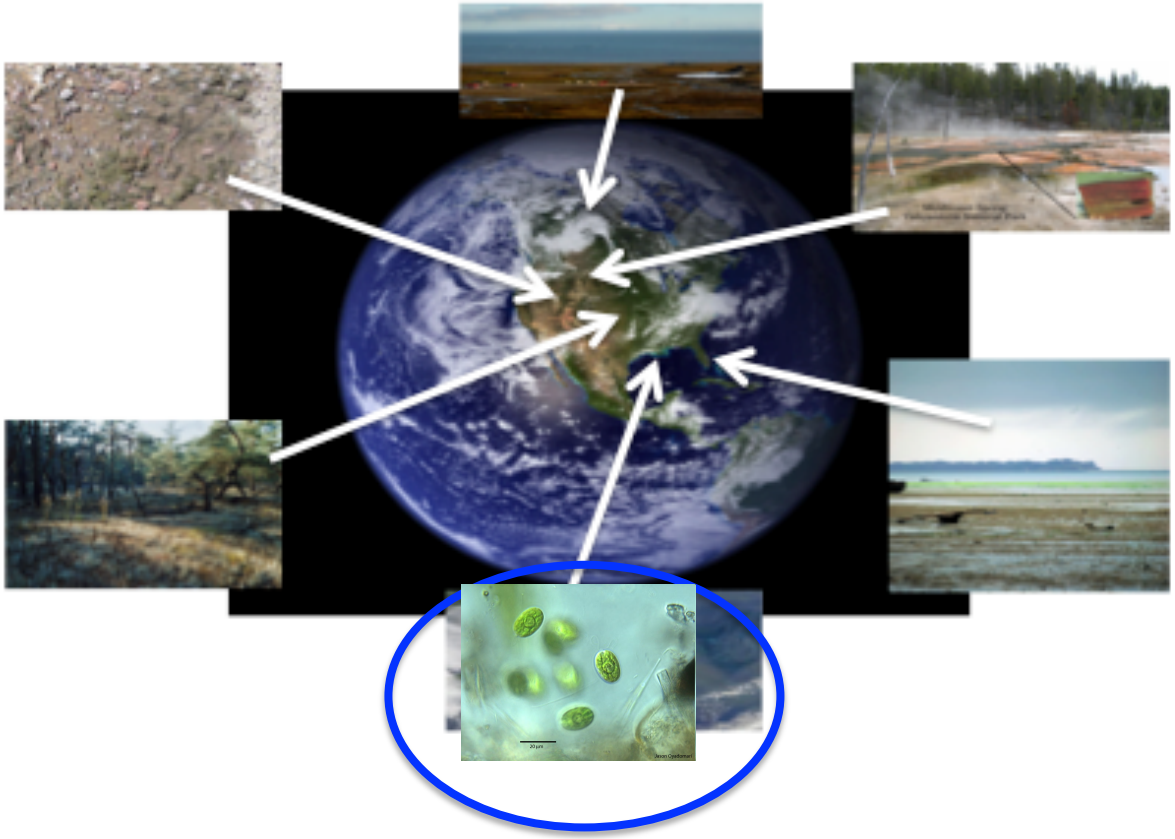


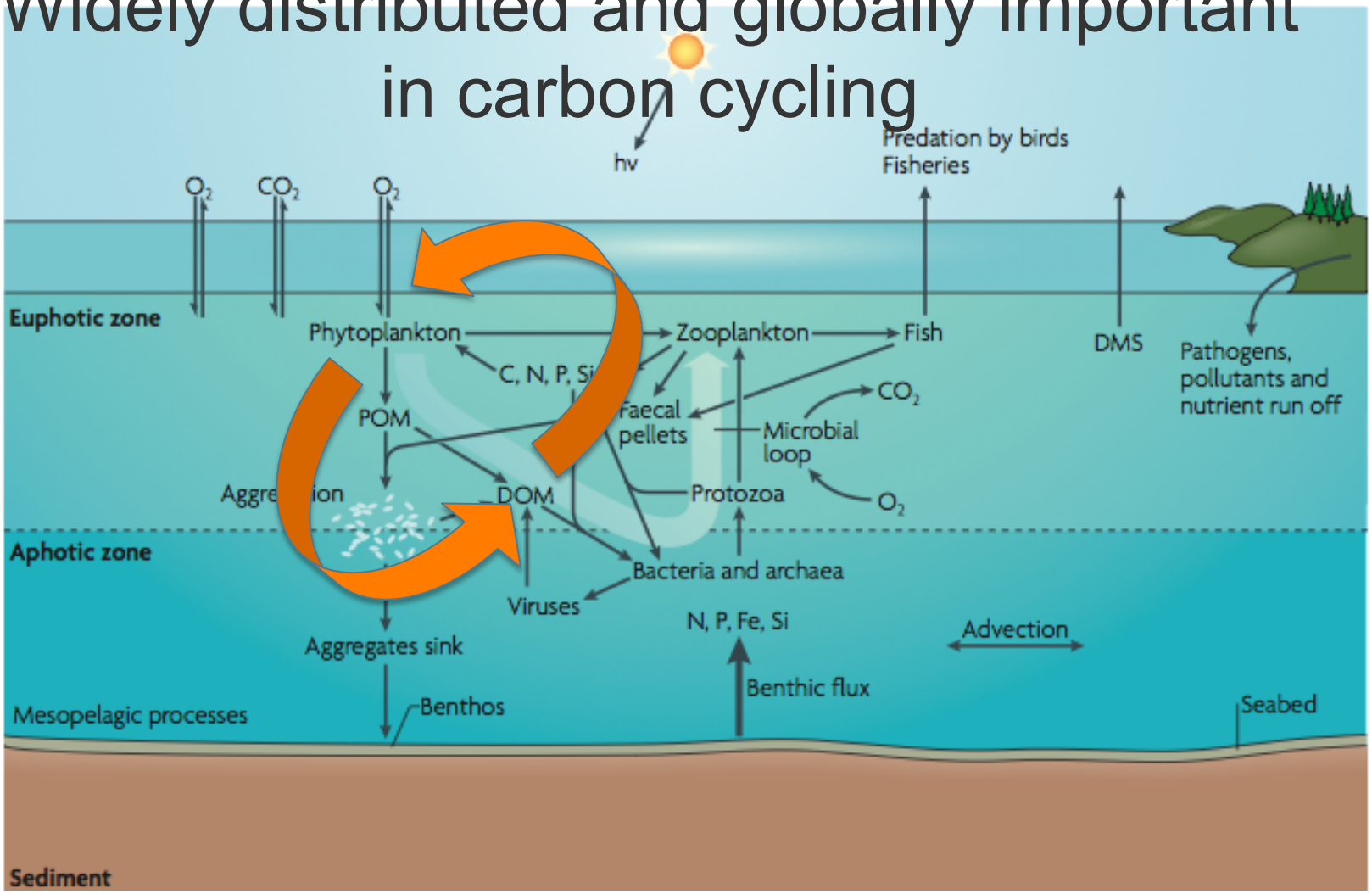
Example: Cyanobacteria govern a large fraction of global carbon cycling



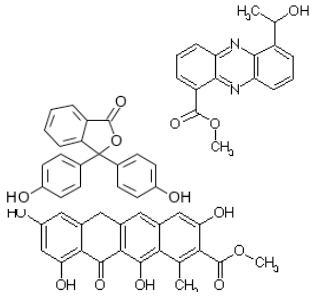
Marine Cyanobacteria

Example: Examination heterotrophic capabilities of *Synechococcus sp*

Widely distributed and globally important in carbon cycling



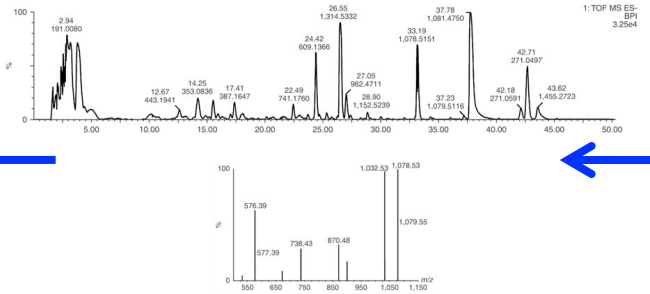
Endometabolomics on *Synechococcus* sp. PCC7002



metabolite solvent extraction

HPLC
(C18; hilic)

Sample independent: suitable for unsequenced organisms and communities



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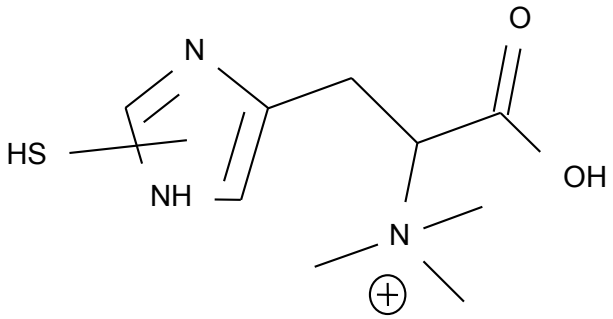
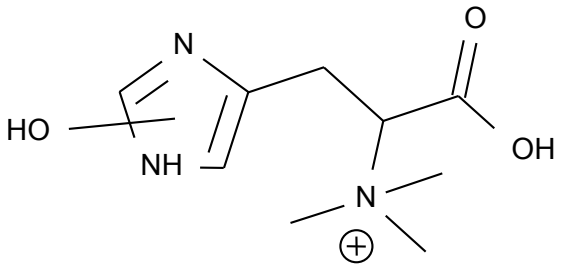
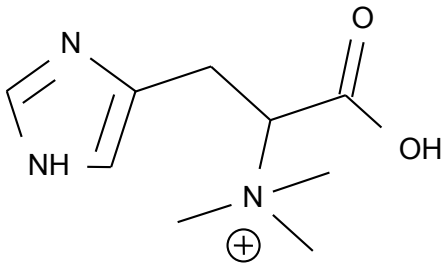
MS/MS

Metabolite identification
&
Quantification

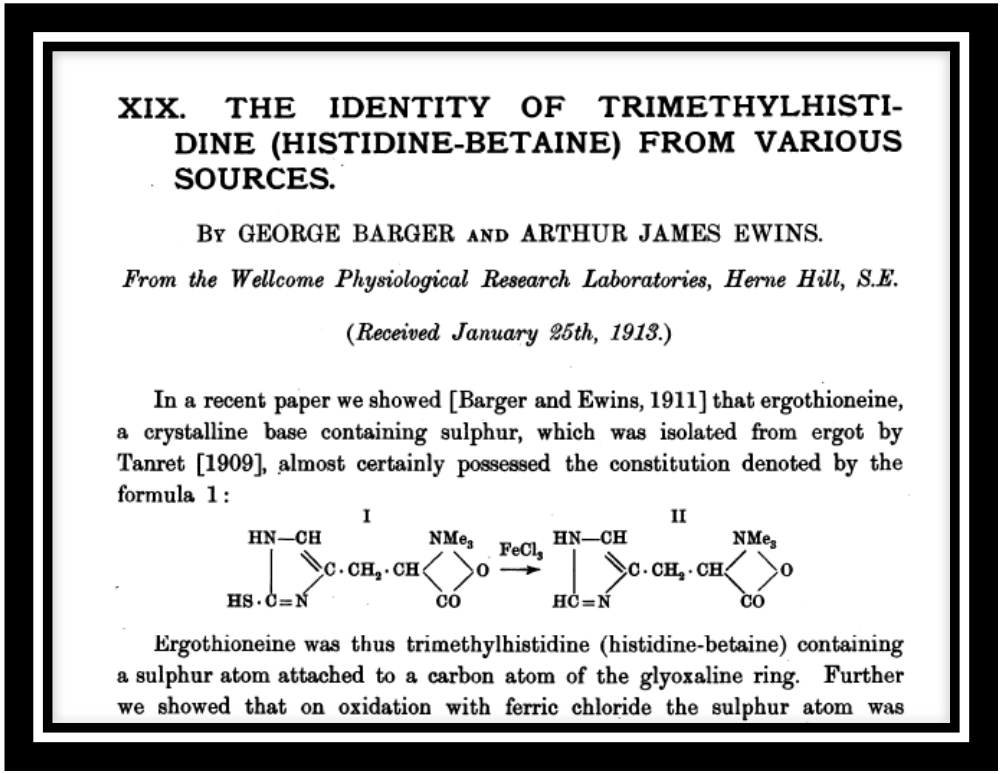
Detect many ‘novel’ metabolites







- Previously only to attributed to non-yeast-fungi and *Actinomycetales* bacteria
- The thiol form is called Ergothioneine (named for the ergot fungus from which it was isolated in 1909)
- Scavenges hydroxyl radicals, hypochlorous acid, inhibits radical production by metals
- It is not made by humans but is transported by a specific transporter ETT, abundant in some tissues.
- Mutation in ETT are associated with Crohn’s disease and rheumatoid arthritis.



XIX. THE IDENTITY OF TRIMETHYLHISTIDINE (HISTIDINE-BETAINE) FROM VARIOUS SOURCES.

By GEORGE BARGER and ARTHUR JAMES EWINS.

From the Wellcome Physiological Research Laboratories, Herne Hill, S.E.

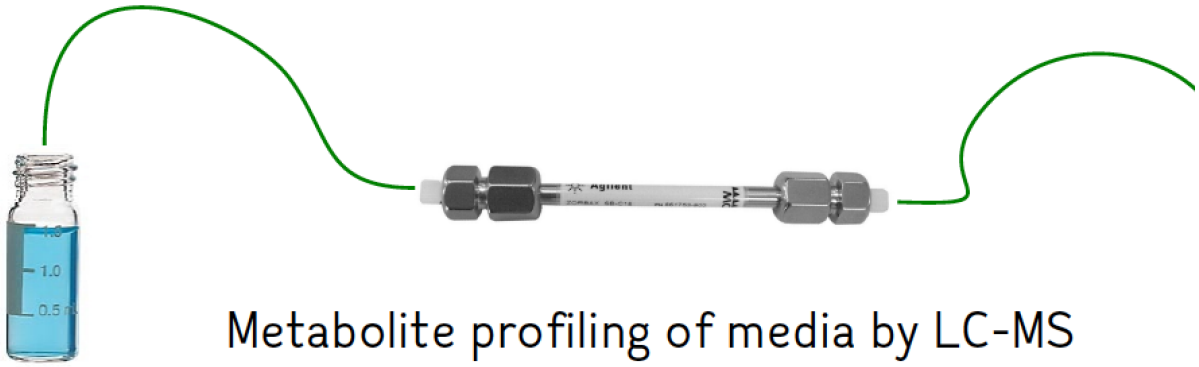
(*Received January 25th, 1913.*)

In a recent paper we showed [Barger and Ewins, 1911] that ergothioneine, a crystalline base containing sulphur, which was isolated from ergot by Tanret [1909], almost certainly possessed the constitution denoted by the formula 1:

I $\xrightarrow{FeCl_3}$ II

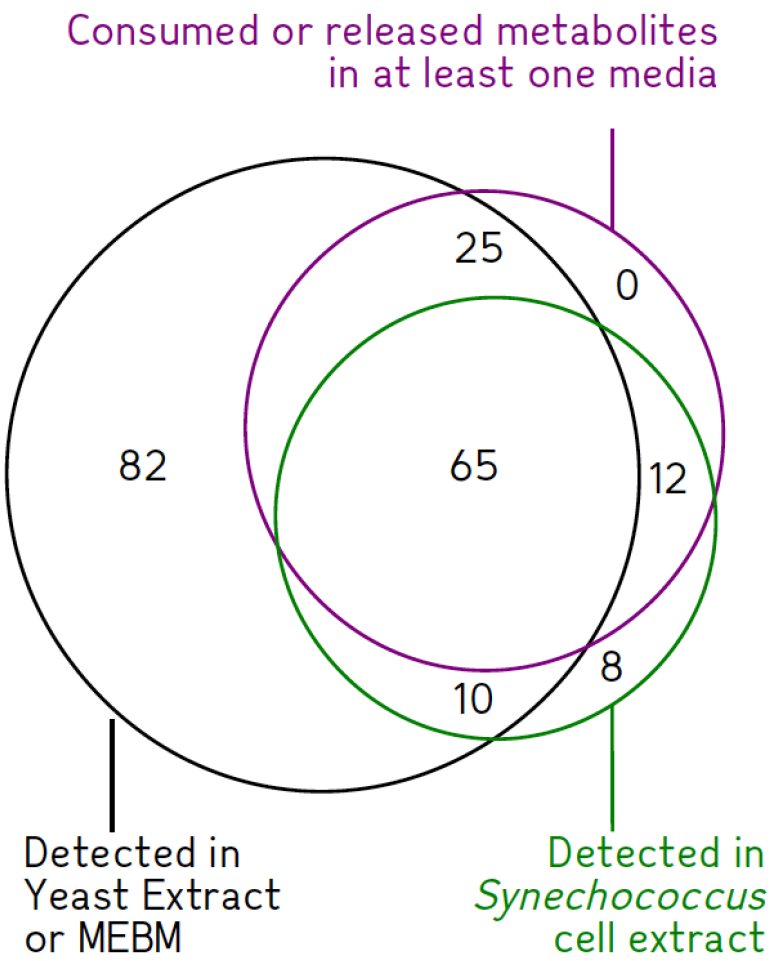
Ergothioneine was thus trimethylhistidine (histidine-betaine) containing a sulphur atom attached to a carbon atom of the glyoxaline ring. Further we showed that on oxidation with ferric chloride the sulphur atom was

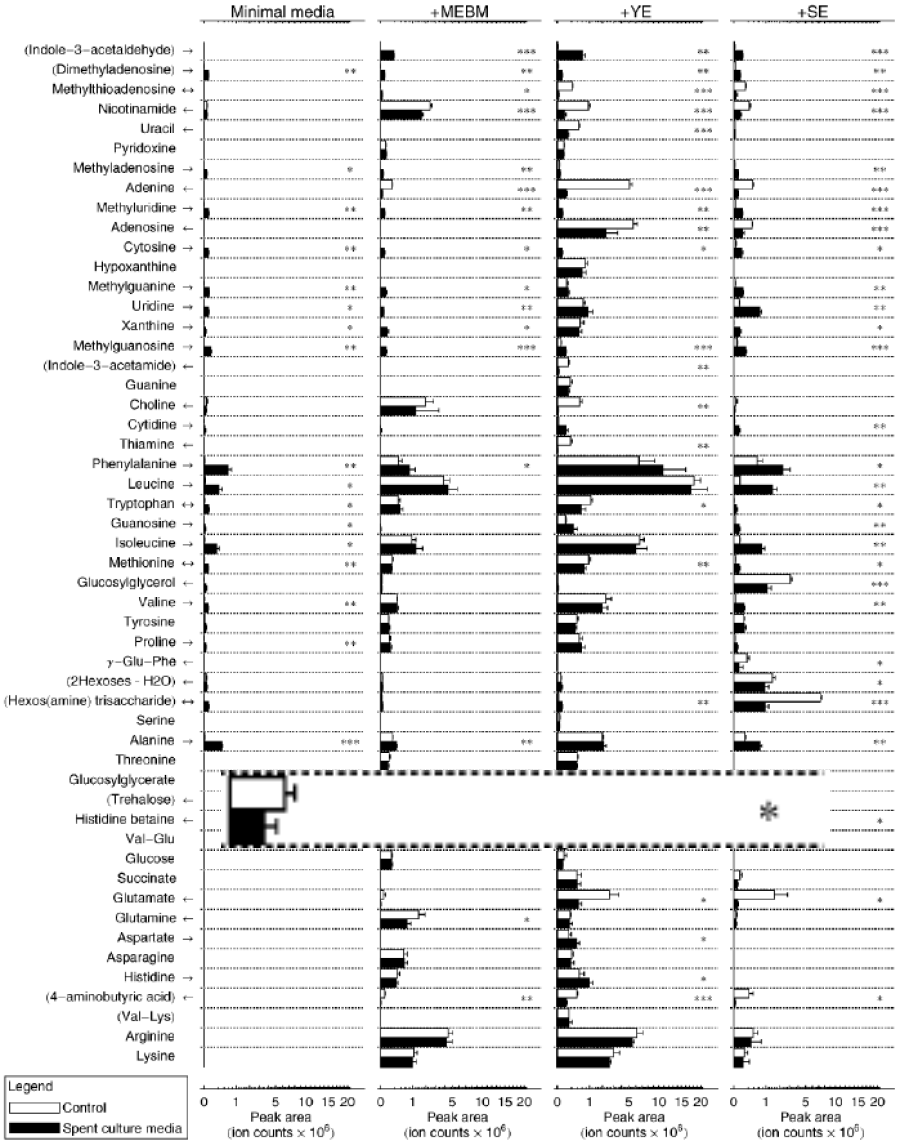
Approach: Compare metabolites taken up from rich media



Metabolite profiling of media by LC-MS

Found broad heterotrophic capabilities esp. for 'self' metabolites

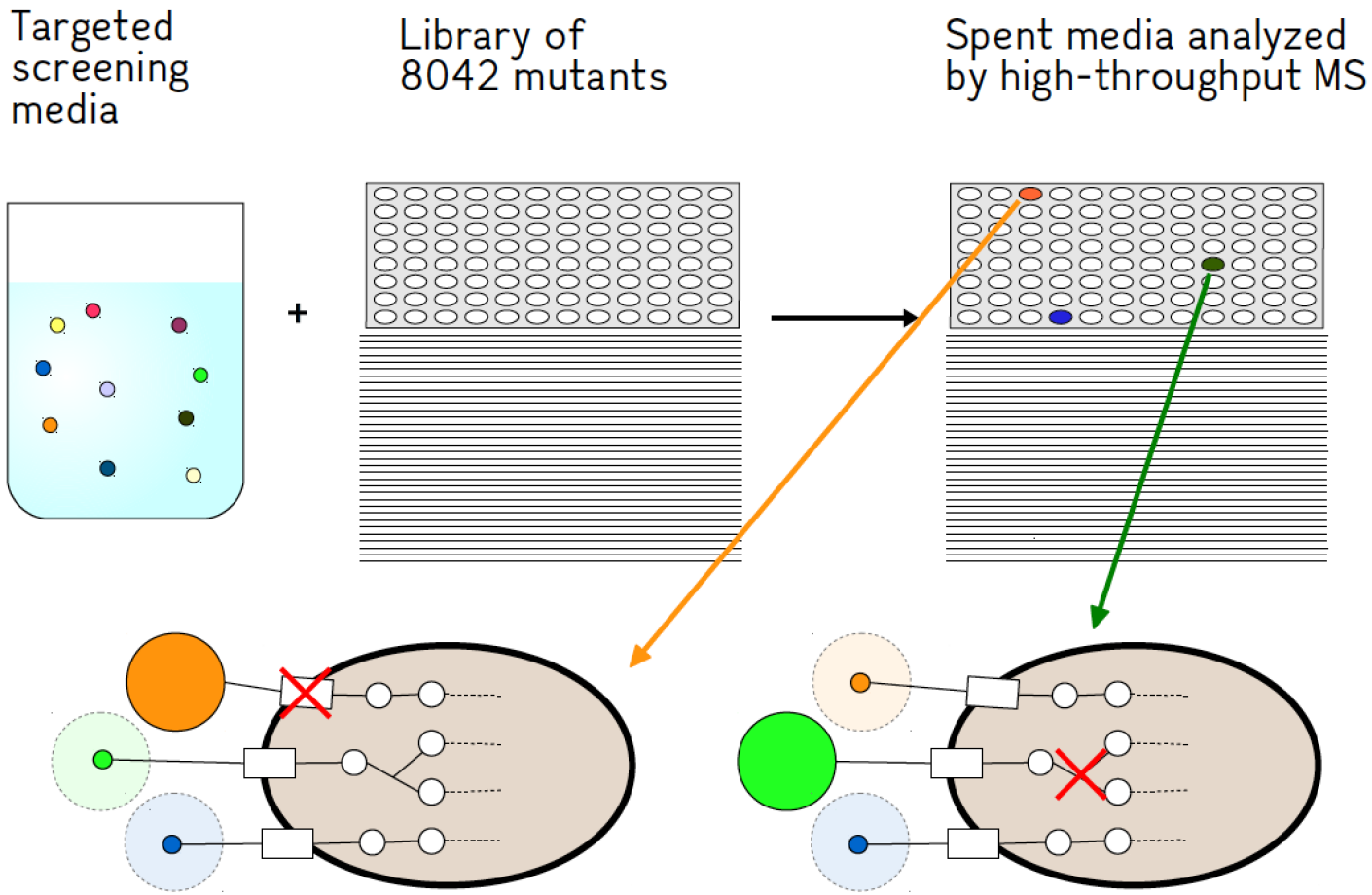




Baran R *et al* (2011) *Mol BioSyst* **7**: 3200-3206

Example: Integrating KO's and exometabolomics for Enzyme/ Transporter discovery

Screen libraries of bacterial mutants for defects in metabolite uptake



With Adam Deutschbauer and Adam Arkin (LBNL)

Results: Obtained direct biochemical support for both known and novel genes

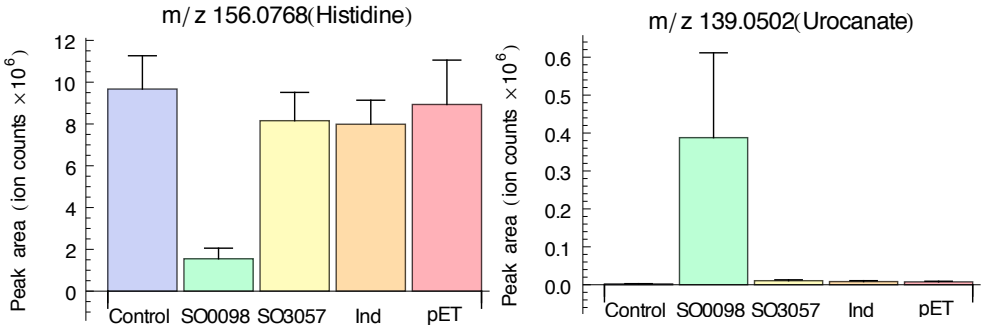
Organism	Gene(s)	Affected metabolite	Note
E. coli	*pfs*	MTA	5'-methylthioadenosine/S-adenosylhomocysteine nucleosidase
E. coli	*pncA*	Nicotinamide	pyrazinamidase/nicotinamidase
E. coli	*manX*, *manY*, *manZ*	Glucosamine	subunits of mannose PTS permease
E. coli	*nagB*	Glucosamine	glucosamine-6-phosphate deaminase
E. coli	*anmK* (*ydhH*)	ahMurNAc	anhydro-N-acetylmuramic acid kinase
E. coli	*argE*	Citrulline	acetylornithine deacetylase
S. oneidensis	SO3749	Citrulline	Non-homologous functional analog of *argE*
S. oneidensis	SO1043, SO1044	Citrulline	subunits of an ABC transporter
S. oneidensis	SO3057	Ergothioneine	Predicted Pal/Histidase
S. oneidensis	SO1313, SO1314	ahMurNAc	

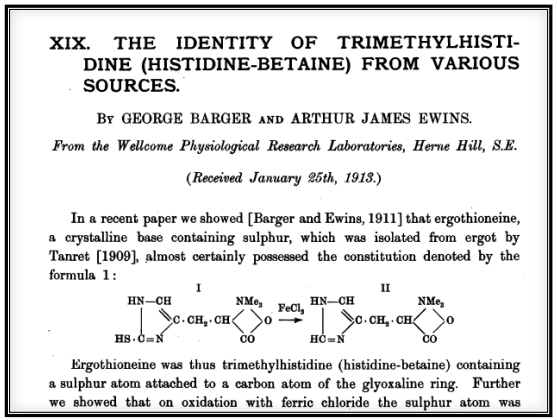
Baran R *et al* (2013) *ACS Chemical Biology* **8**(1): 189–199

Validation of activities and specificity

- SO0098 required for the utilization of histidine as a nitrogen source
- SO3057 (paralog of SO0098) required for the utilization of ergothioneine
- Cloned and purified proteins and showed predicted activities and substrate specificities *in vitro*

a - incubation with histidine





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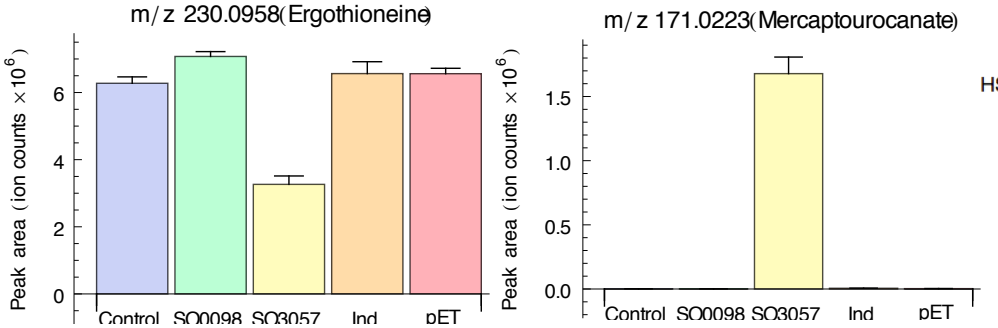
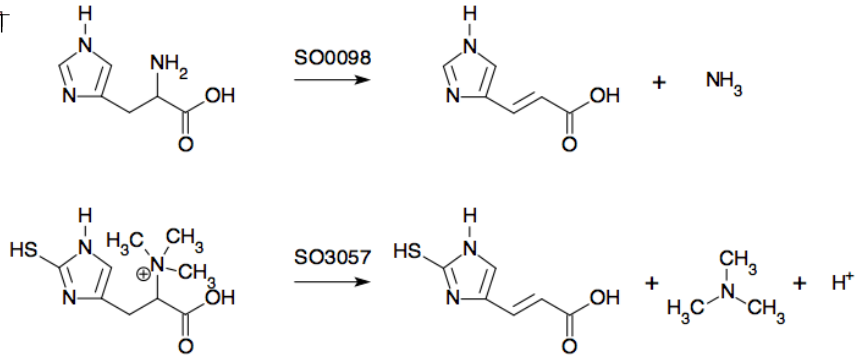
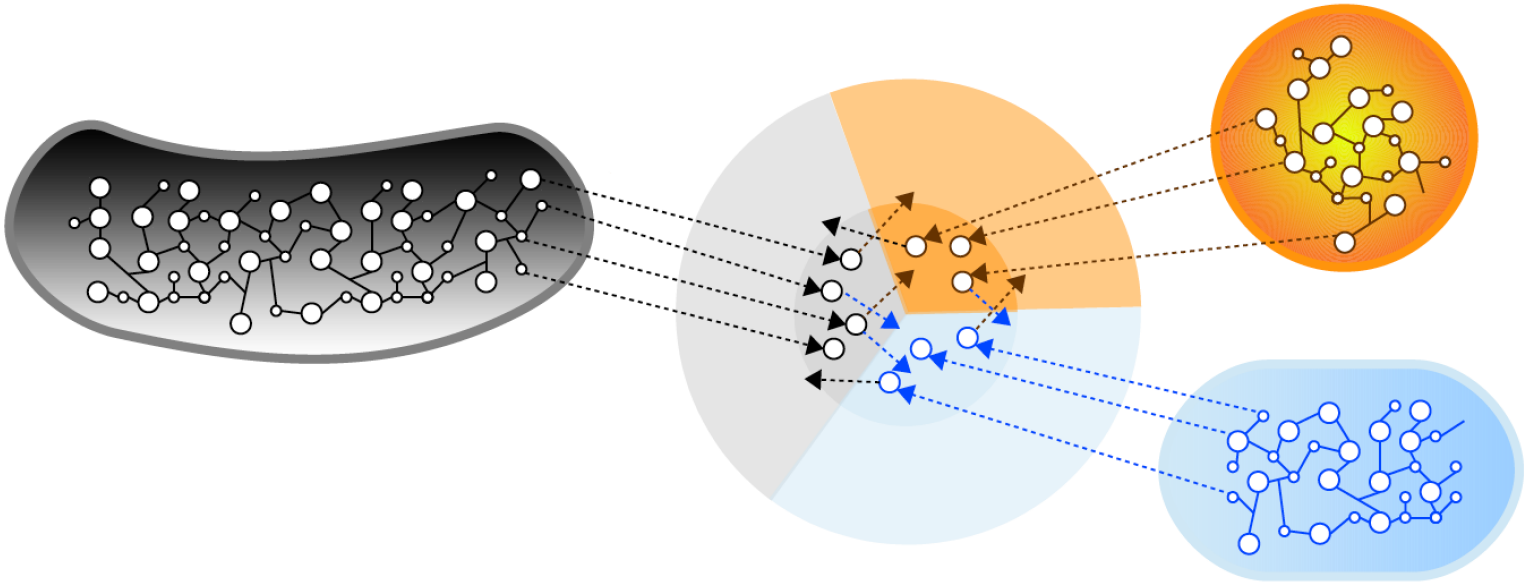
b - incubation with ergothioneine

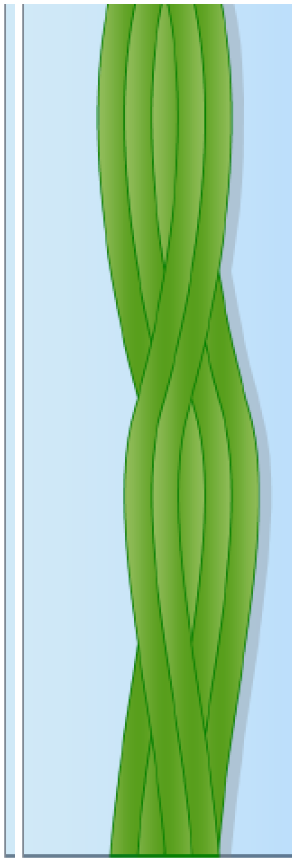


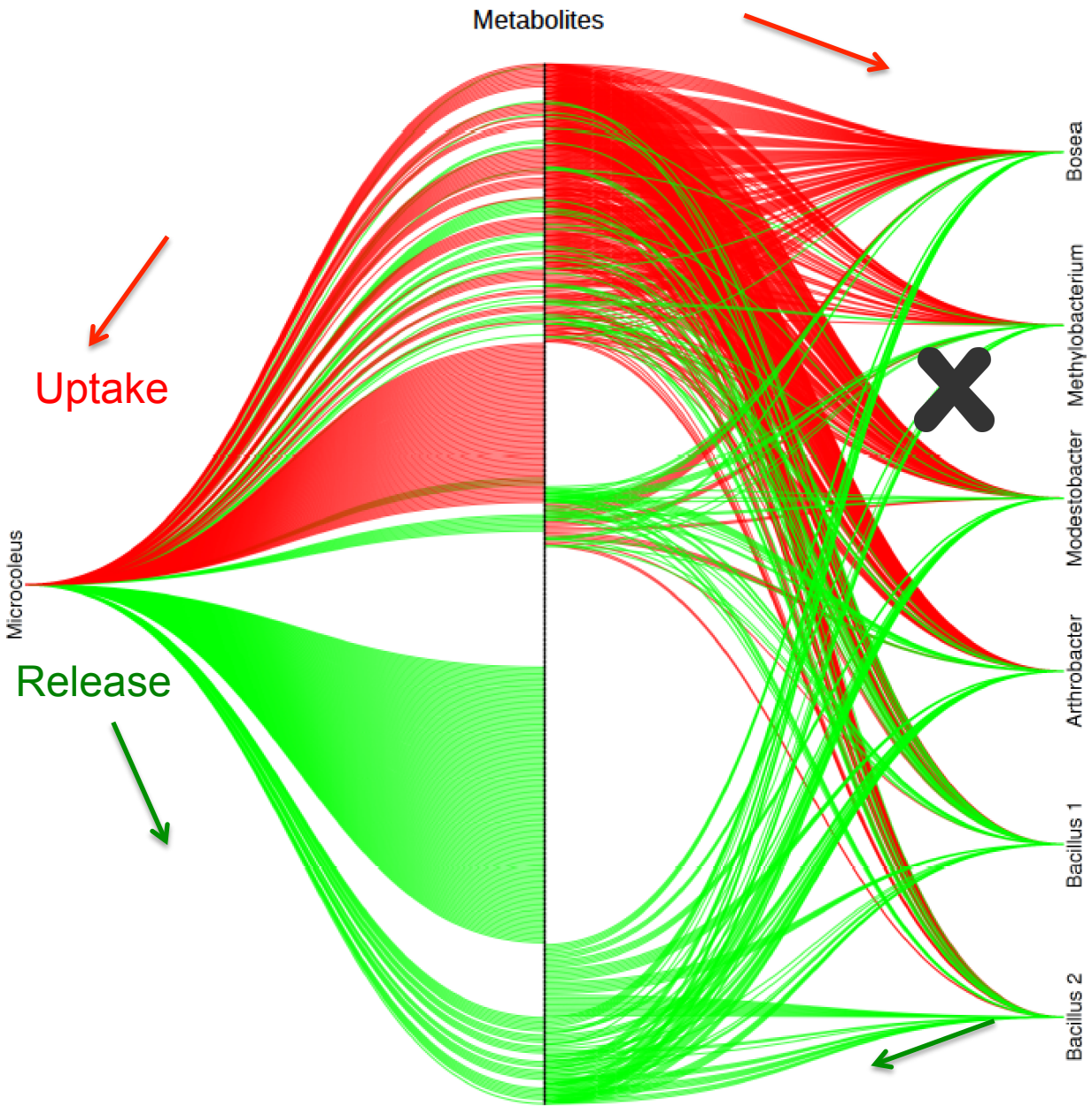


Exo-metabolomics to study nutrient cycling in biological soil crusts

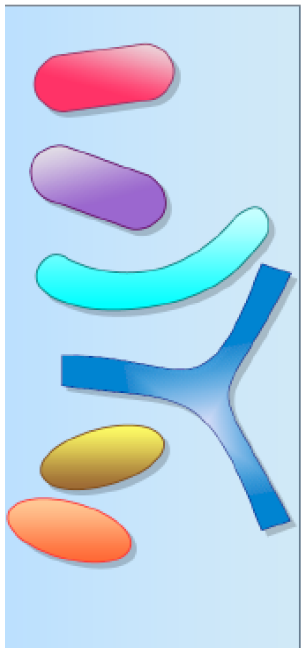


Exometabolite investigation of metabolite webs



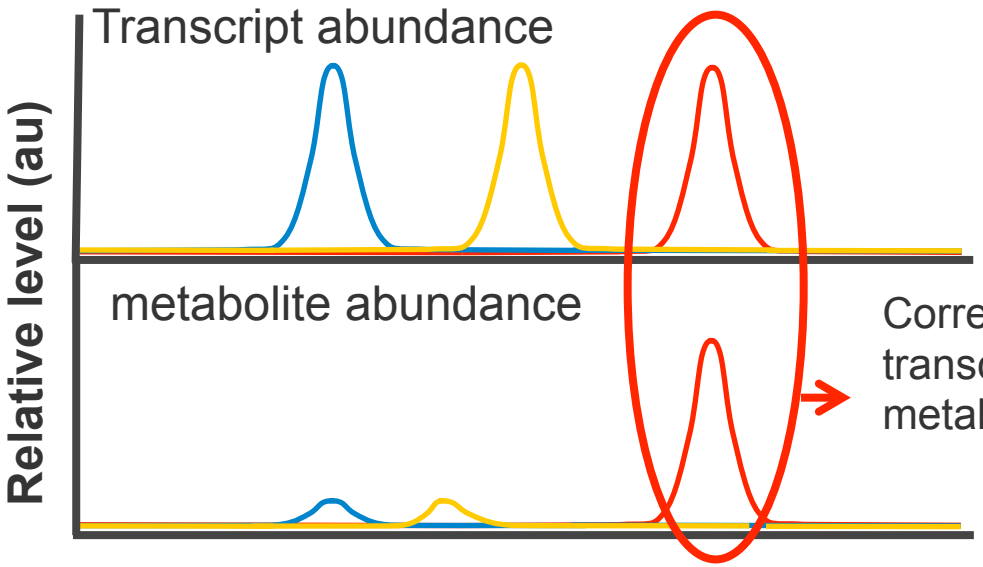


Heterotrophs

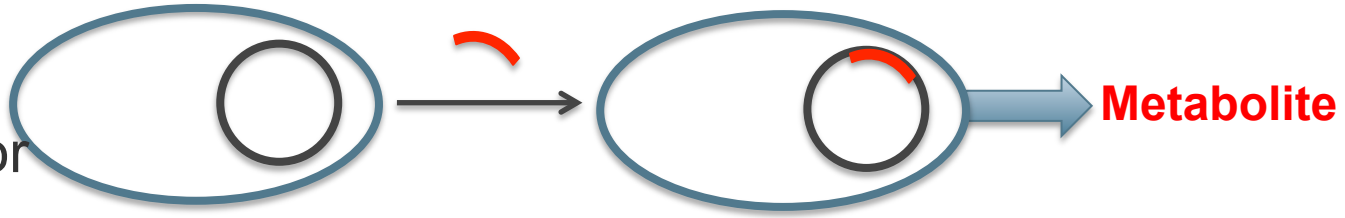


Additional opportunities for integrating exometabolomics with JGI genomics

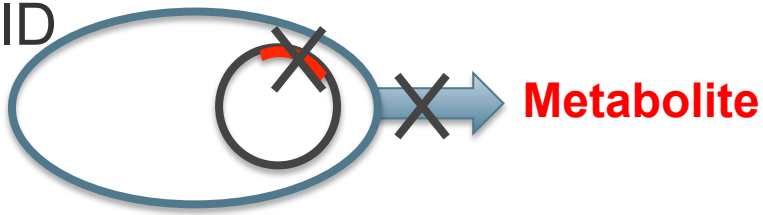
- Investigation of plant-microbe interactions.
- Integration of transcriptomics with metabolomics.
- Exometabolomic analysis of gene knockouts (e.g. FEBA).
- Sequencing and exometabolomic analysis of mutant or strain collections.
- DNA synthesis + exometabolomics to ID gene function



DNA synthesis + exometabolomics



DNA mutagenesis + exometabolomics



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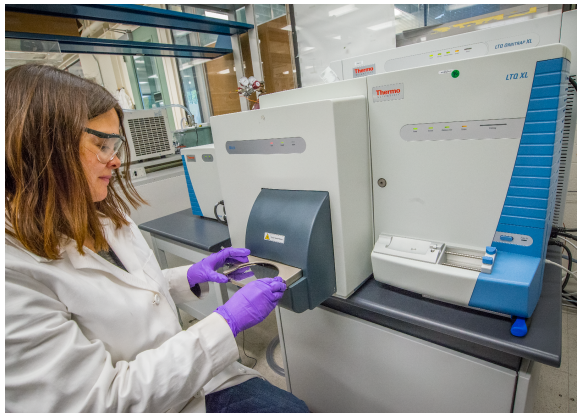

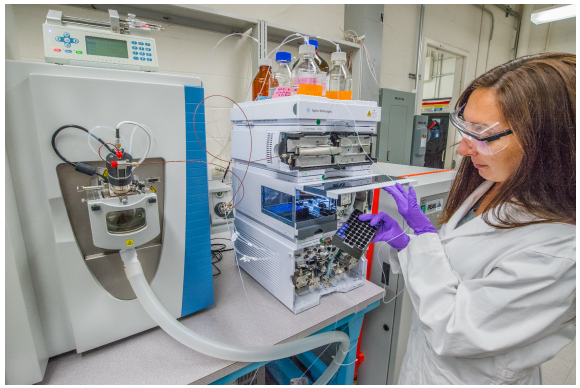

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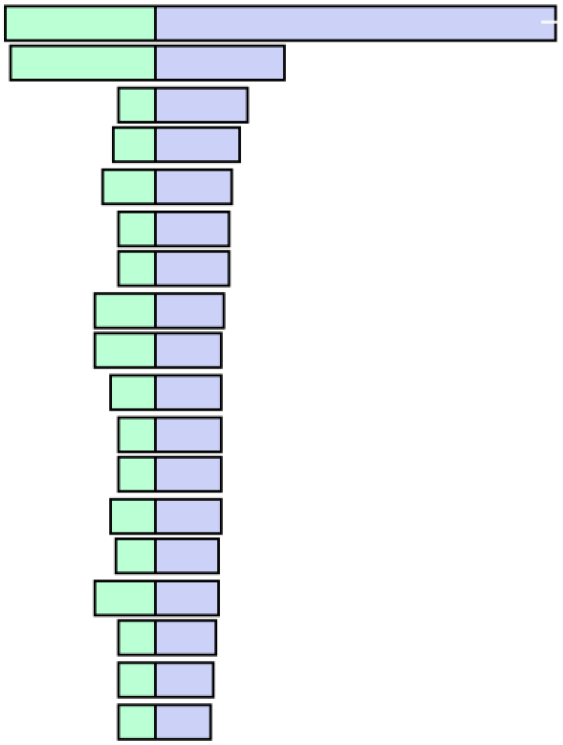
Example of results: MTA/SAH Nucleosidase

f m/z 298.0968 (Methylthioadenosine) in spent media of E. coli mutants



OD	Peak Area	Gene	Annotation
0	JW0155	pfs
1	JW1632	ydhH
7	JW0710	gltA
7	JW3584	secB
7	JW2500	yfgA
7	JW3985	pgi
7	JW0663	nagA
7	JW3143	glmM
7	JW0204	rnhA
7	JW1844	lpxM
7	JW4002	dgkA
7	JW0145	mrcB
7	JW2576	rluD
7	JW3906	priA
7	JW2788	recB
7	JW4326	dnaT
7	JW3606	rfaG
7	JW3039	rpoD