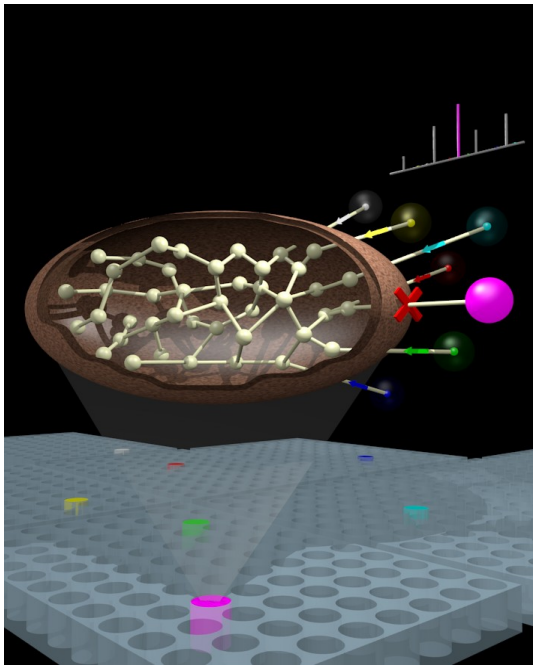


# *Mapping Microbial Metabolism Using Metabolomics*

Richard Baran
(Northen Lab)

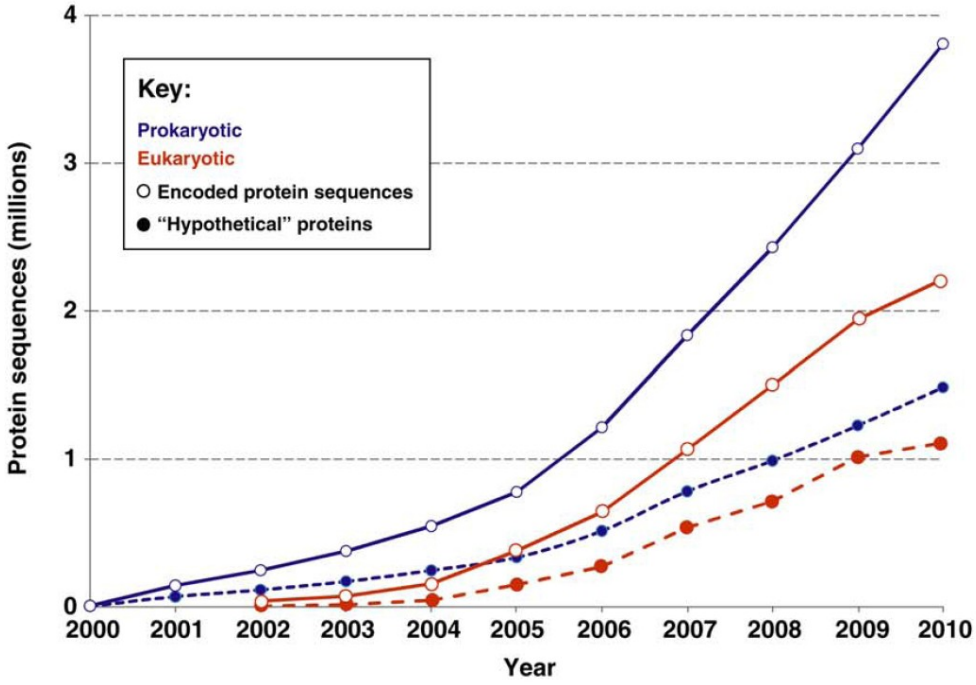
Lawrence Berkeley National Laboratory

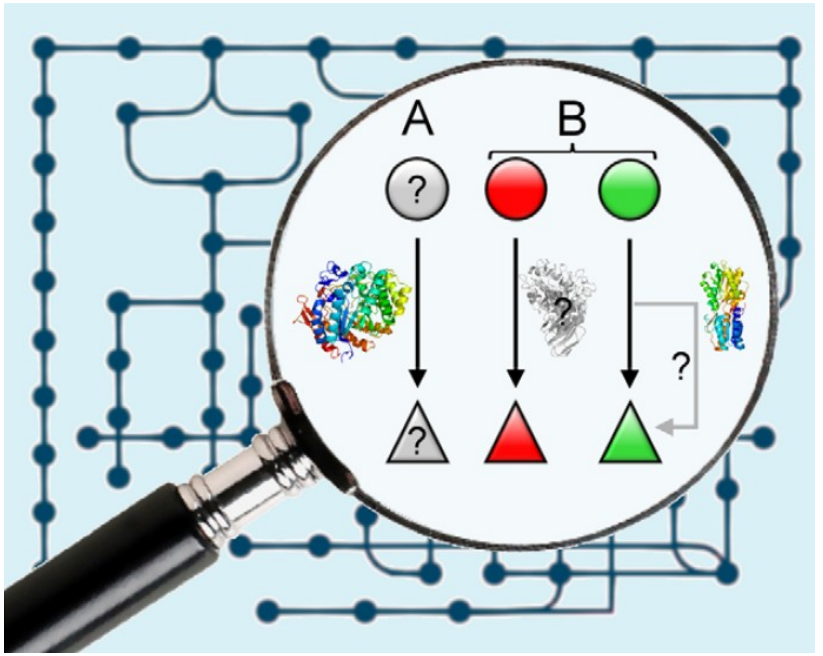
March 6, 2013

U.S. DEPARTMENT OF ENERGY | Office of Science

# Motivation

Many unknowns in microbial metabolism and interactions with the environment




Galperin MY & Koonin EV (2010) *Trends Biotechnol* **28**: 398-406
Baran R *et al* (2009) *Curr Opin Microbiol* **12**: 547-552

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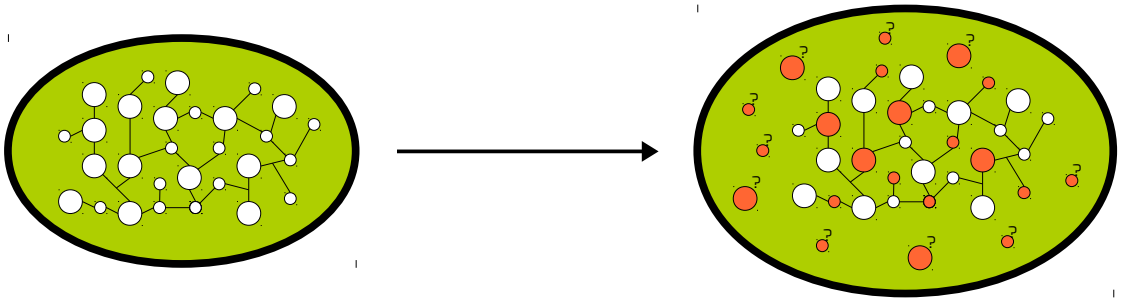
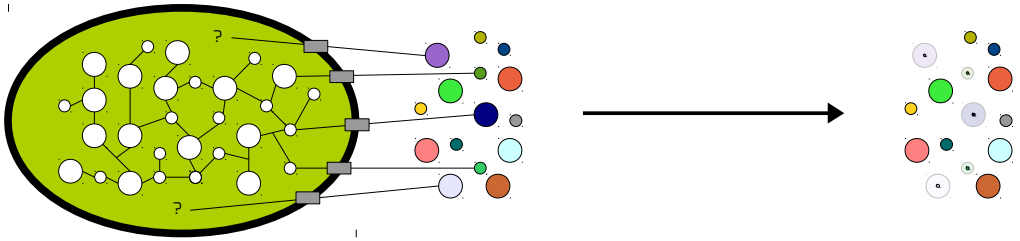
# Outline

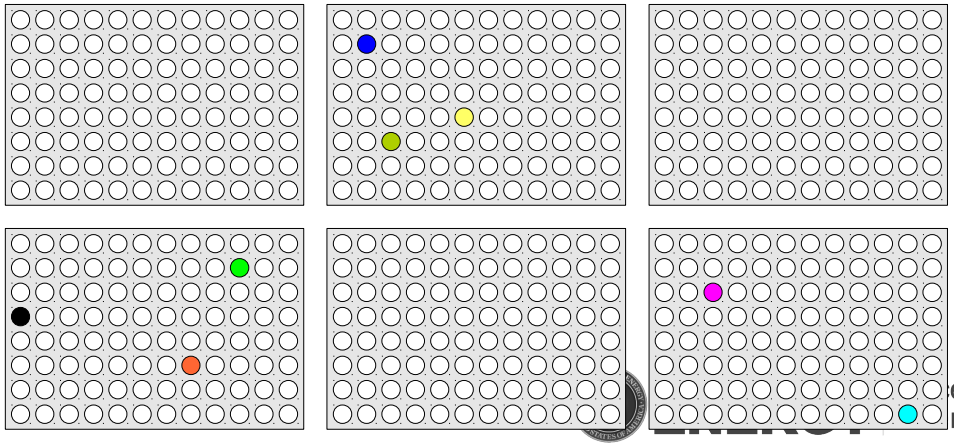
- **Metabolite Profiling**
  metabolite identification



- **Footprinting**
  uptake/release
  of metabolites

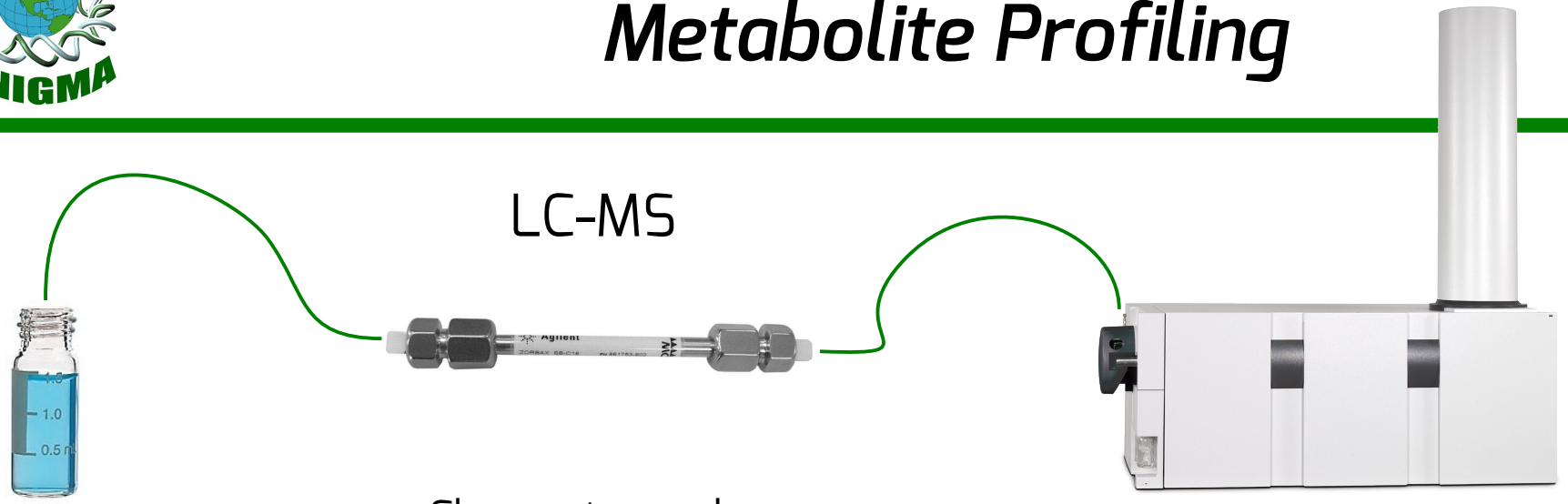


- **High-throughput**
  screening of mutant libraries
  for enzyme and transporter
  discovery/annotation

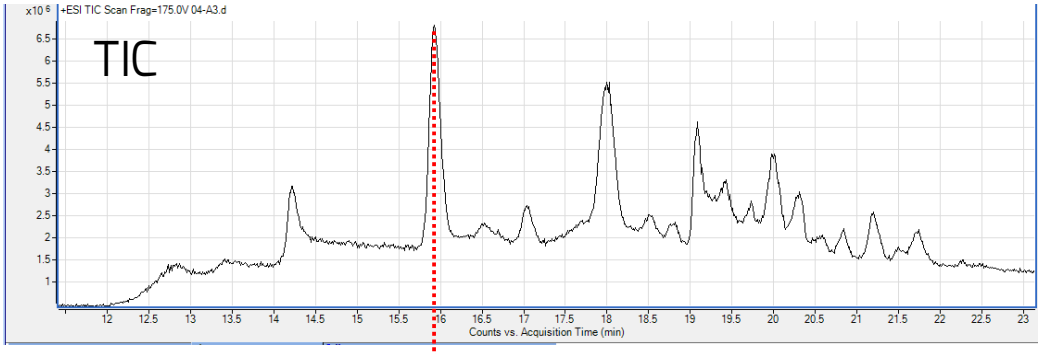


# Metabolite Profiling

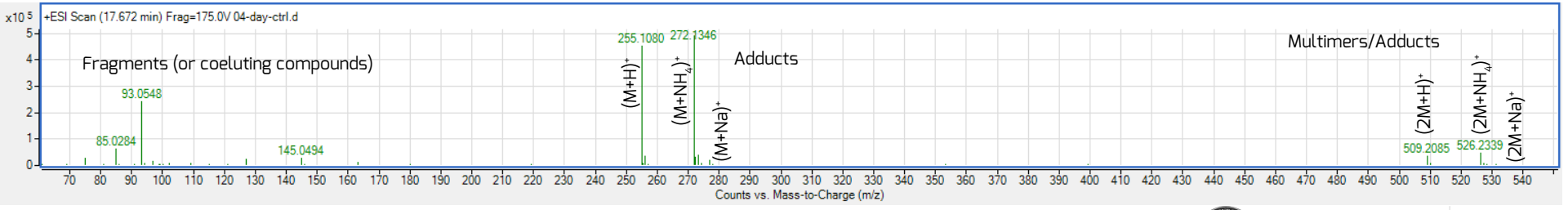
## LC-MS



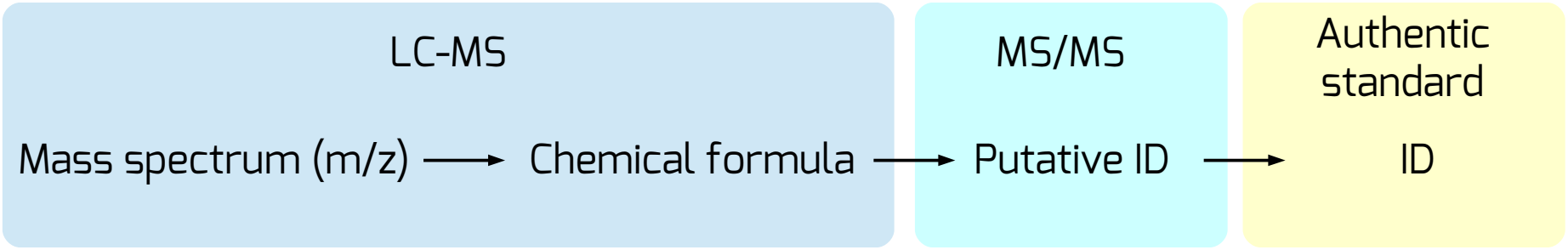
## Chromatography




## Mass spectrum



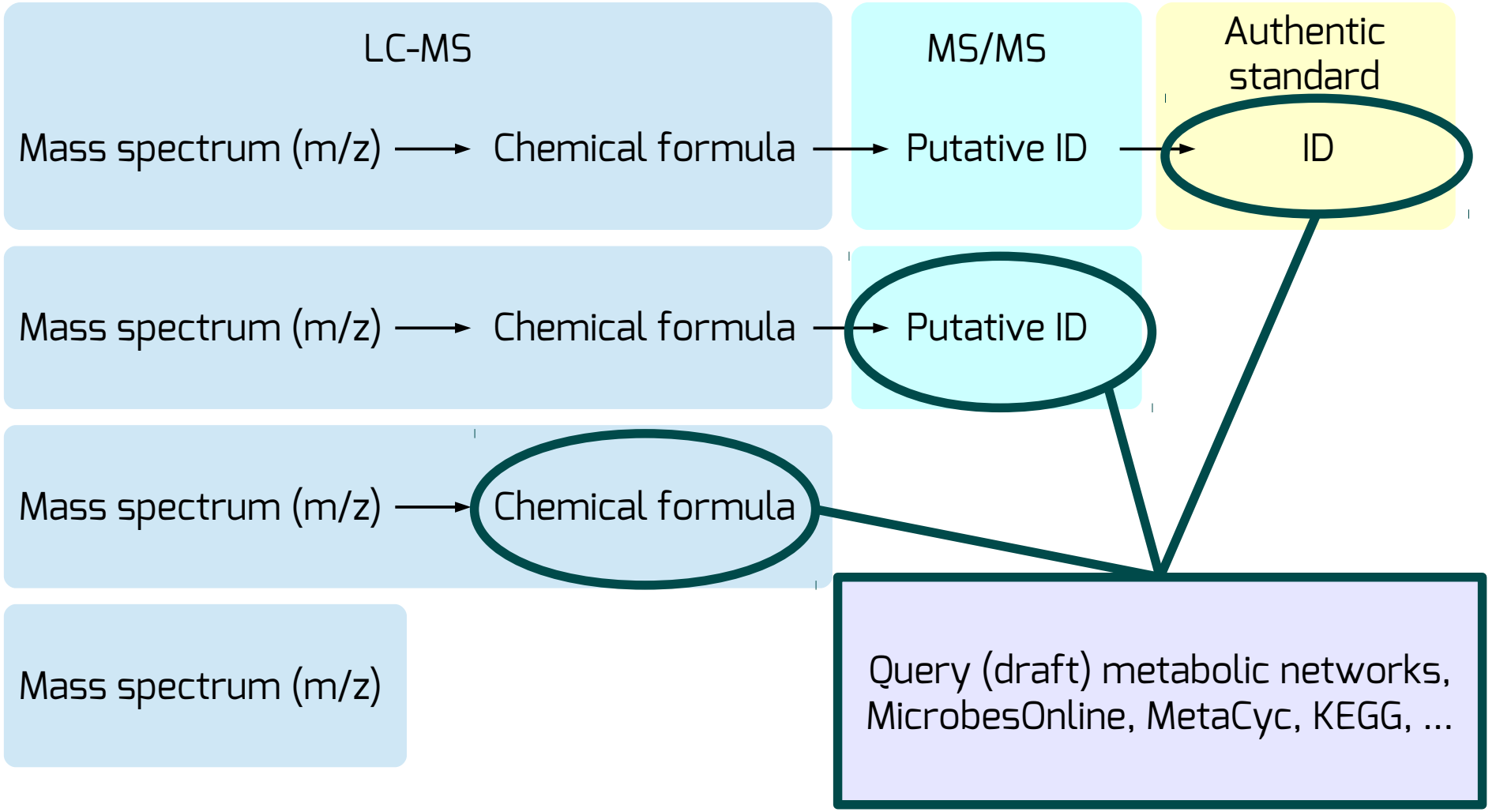
# Metabolite Identification



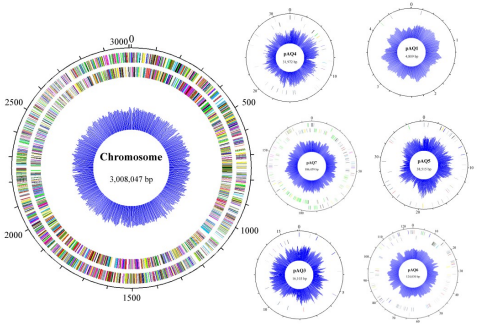
# Metabolite Identification

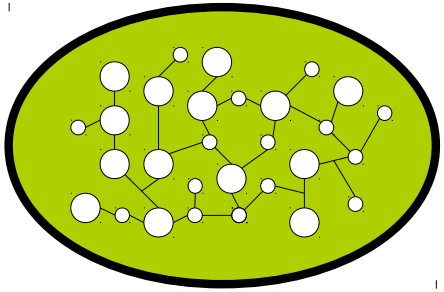
| LC-MS | MS/MS | Authentic standard |
| --- | --- | --- |
| Mass spectrum (m/z) → Chemical formula → Putative ID → ID | | |
| Mass spectrum (m/z) → Chemical formula → Putative ID | | |
| Mass spectrum (m/z) → Chemical formula | | |
| Mass spectrum (m/z) | | |

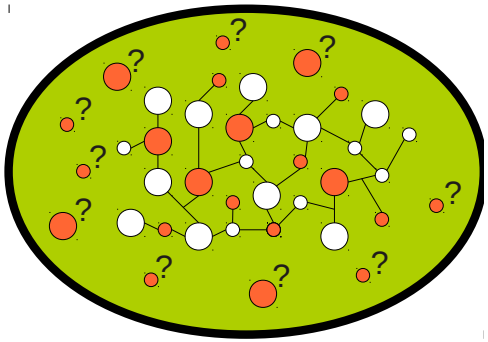
# Metabolite Identification

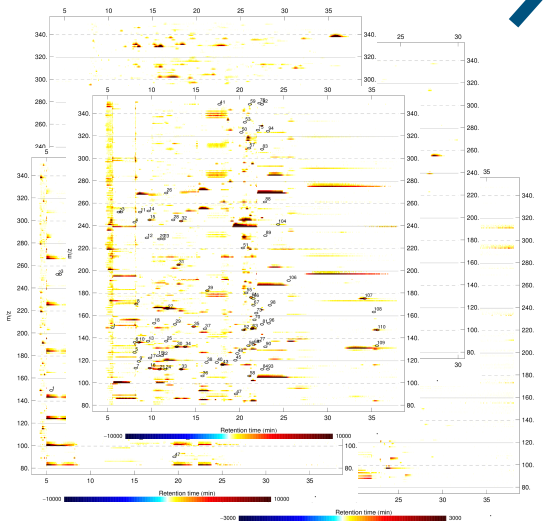


# Predicted vs. Measured Metabolites



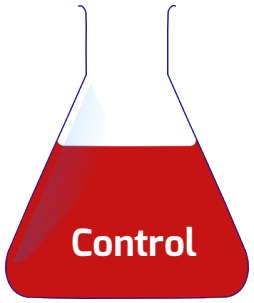



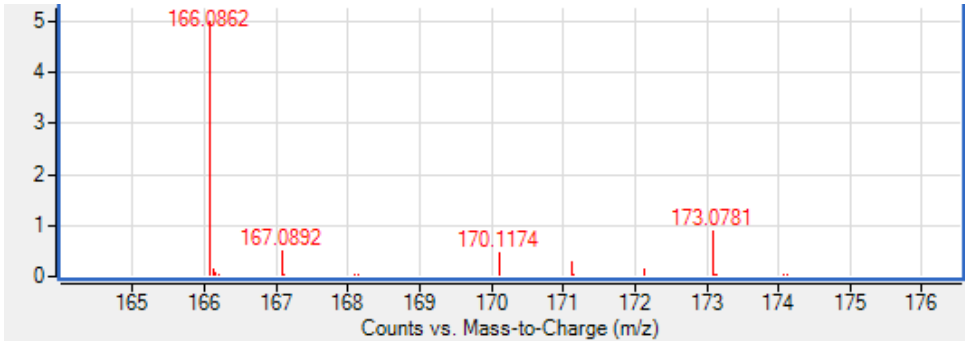




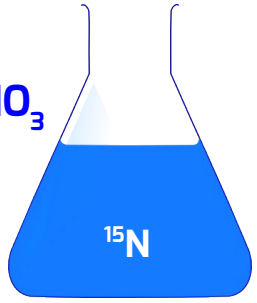
*Synechococcus sp.* PCC 7002

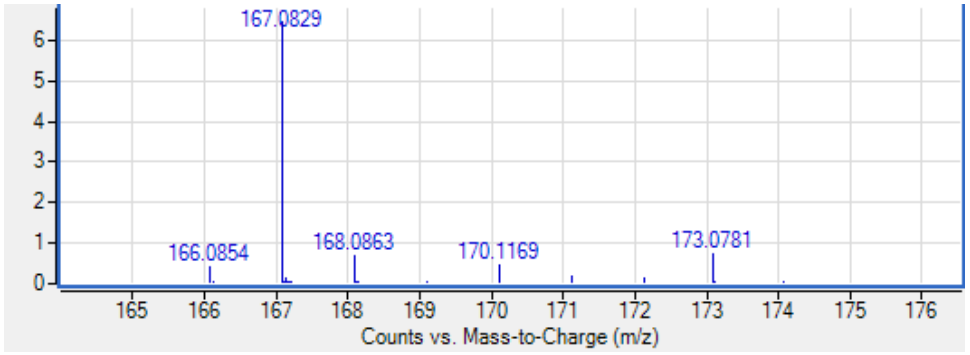
# Uniform Stable Isotope Labeling



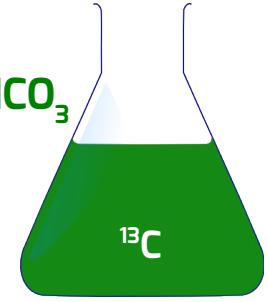



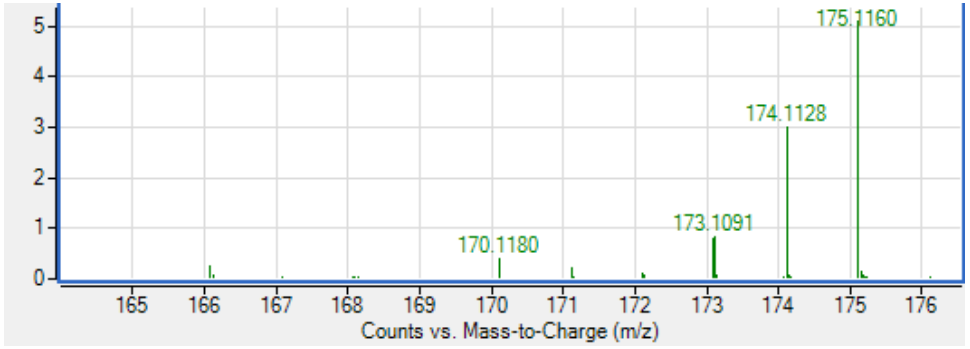

$(^{15}N)NaNO_3$



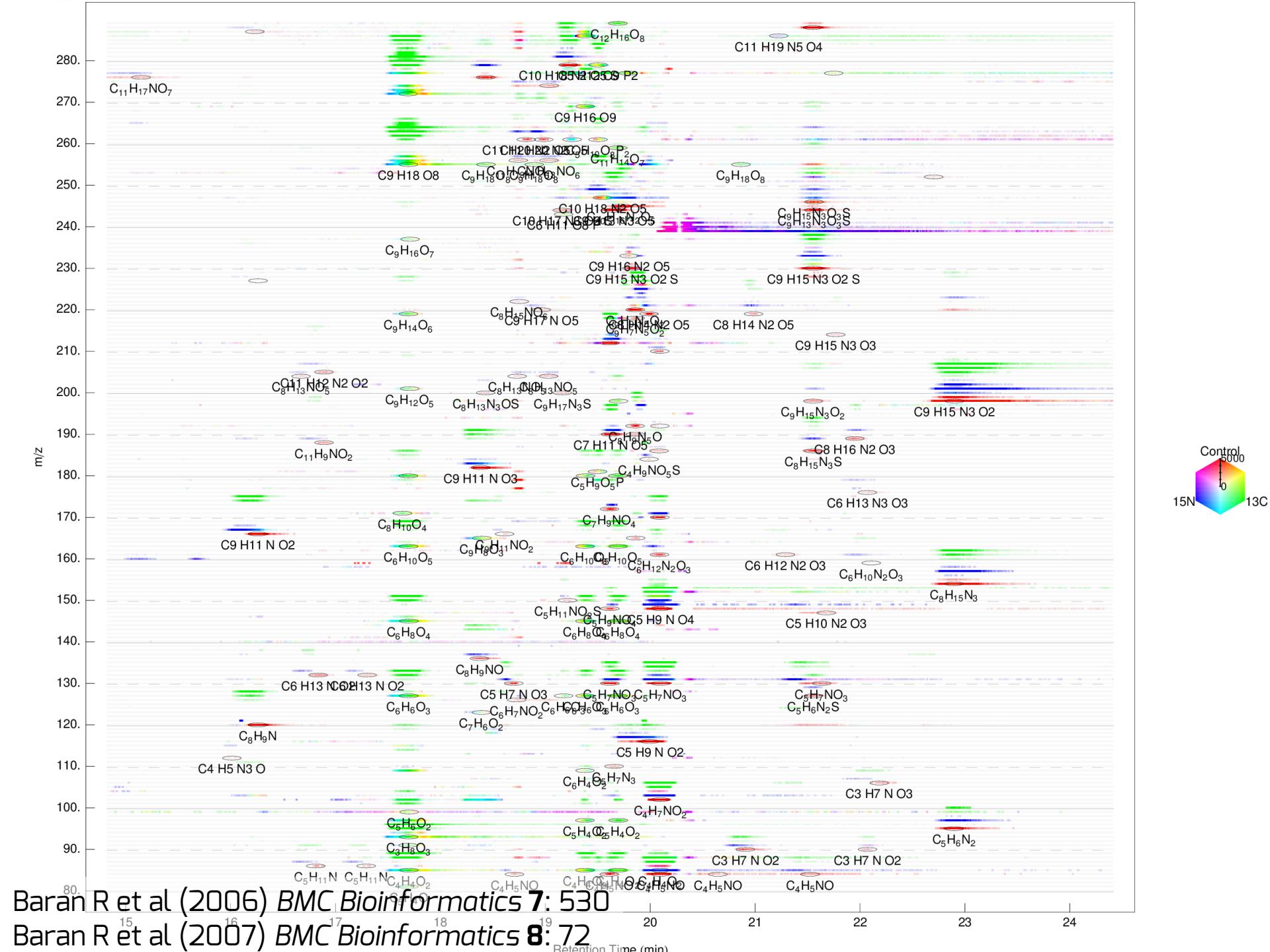




$(^{13}C)NaHCO_3$



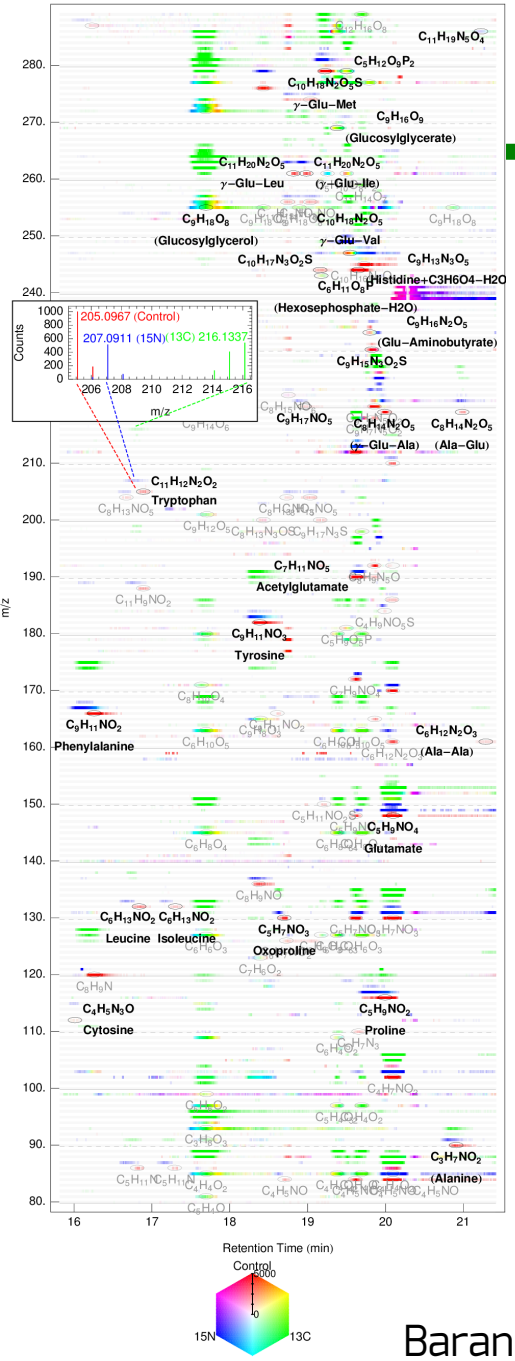




Hegeman AD *et al* (2007) *Anal Chem* **79**: 6912-6921
Giavalisco P *et al* (2009) *Anal Chem* **81**: 6546-6551

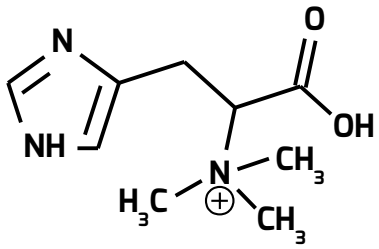


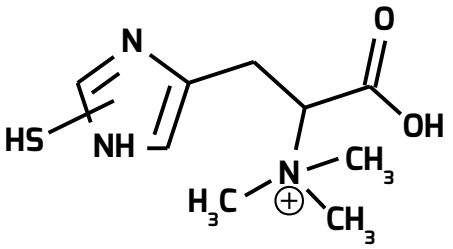

Baran R et al (2006) *BMC Bioinformatics* **7**: 530
Baran R et al (2007) *BMC Bioinformatics* **8**: 72

# Metabolite Profiling Results




- ~100 distinct metabolites detected
- 82 assigned chemical formulas
  - 74 unique
  - 45 outside of Syn7002Cyc
  - **24 outside of MetaCyc or KEGG!**
- 54 identified or putatively identified metabolites
  - Using authentic standards or MS/MS

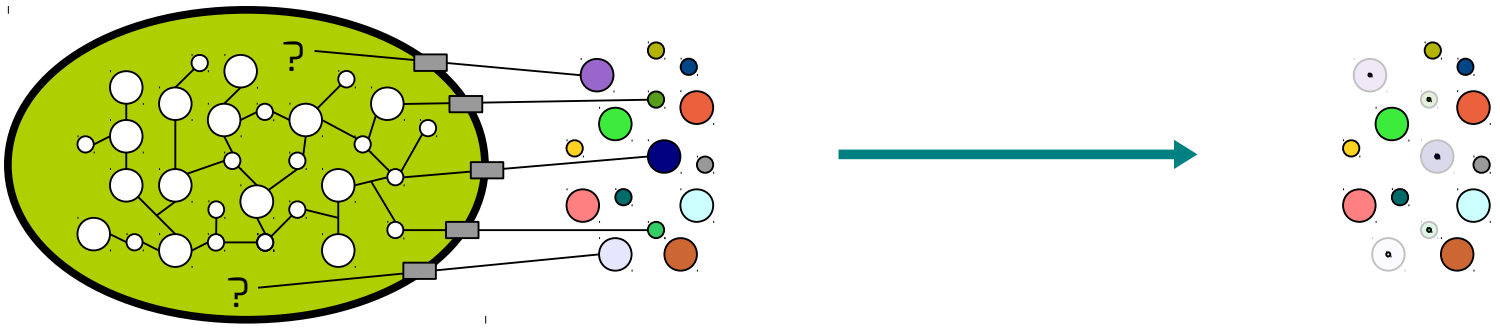



(Ergothioneine?)

Baran R *et al* (2010) *Anal Chem* **82**(21): 9034–9042

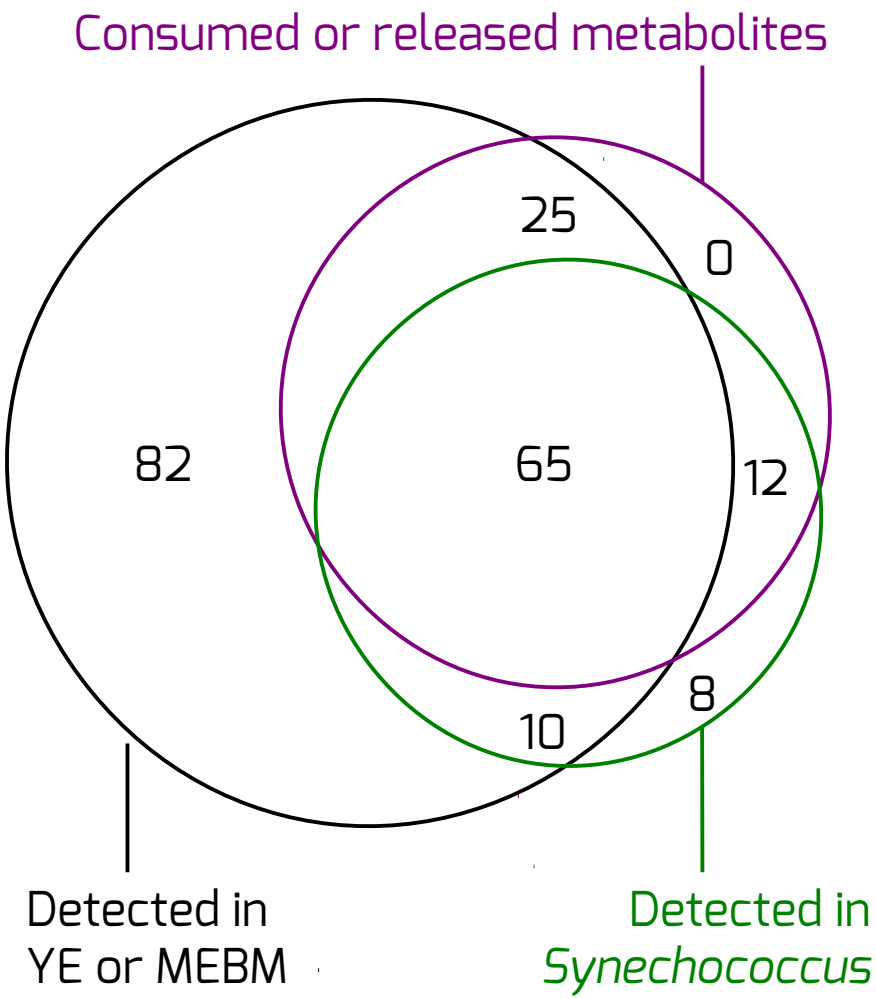
# Metabolic Footprinting




- Growing *Synechococcus* in different complex media
- Untargeted profiling of metabolite uptake/release
- Photoheterotrophic repertoire of cyanobacteria has not been studied systematically
- Current knowledge limited to glycerol, glucose, amino acids

Allen J et al (2003) *Nat Biotechnol* **21**, 692-696
Kell DB et al (2005) *Nat Rev Microbiol* **3**, 557-565

# Metabolic Footprinting Results



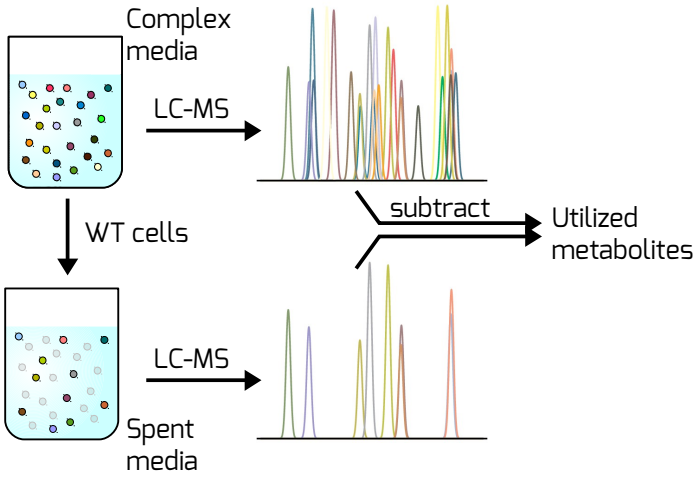

- 202 metabolites detected
- 95 identified or putatively identified
- 45 taken up
- 55 released

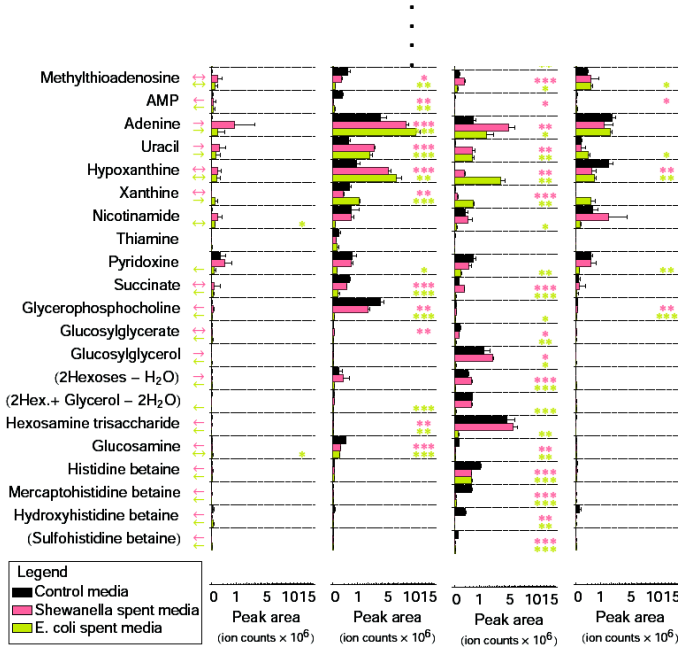
- ***Synechococcus sp.* PCC 7002 uptakes most of its intracellular metabolites – including the unusual ones!**

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

# Enzyme and Transporter Discovery

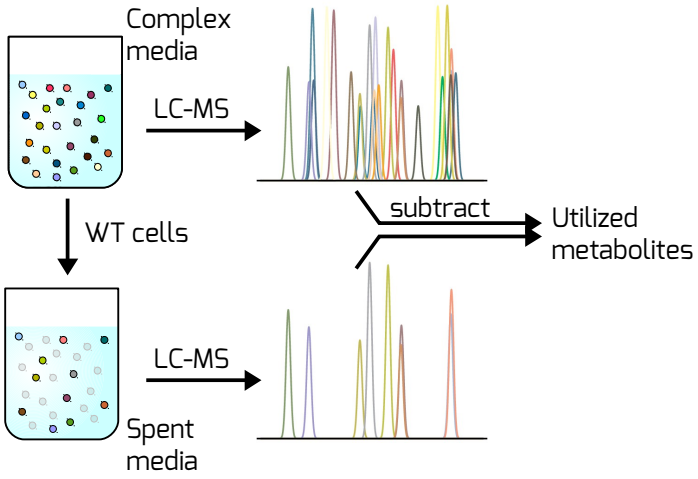
## 1. Identify utilized metabolites

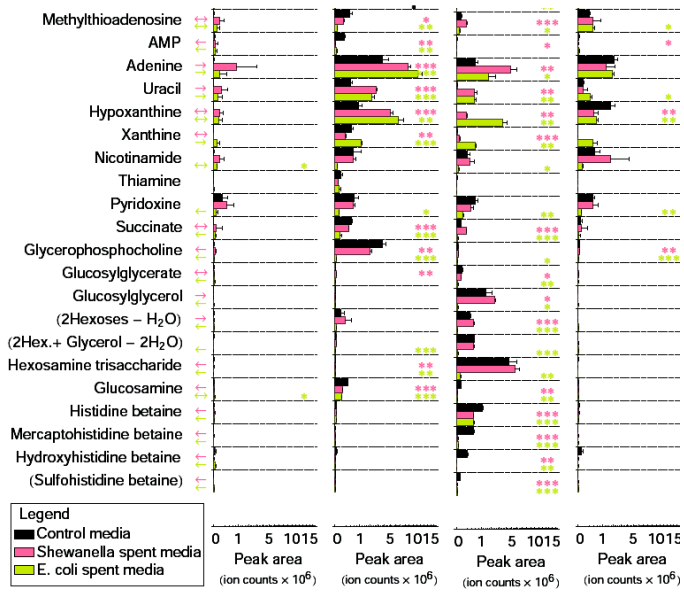


# Enzyme and Transporter Discovery

## 1. Identify utilized metabolites



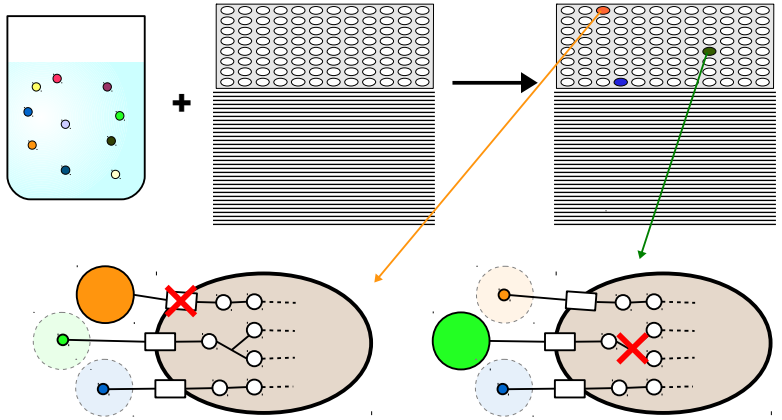



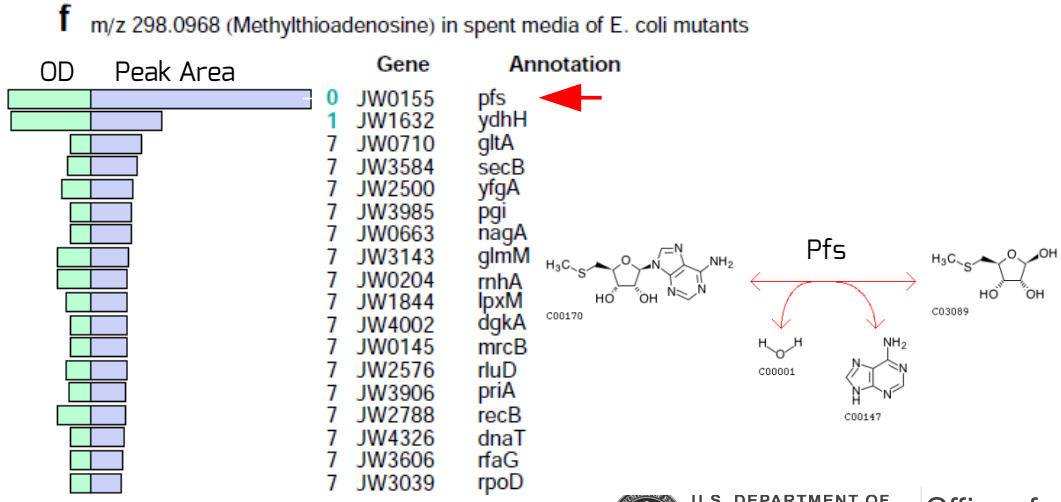

## 2. Screen mutants for defects in utilization

Targeted screening media

Library of 8042 mutants

Spent media analyzed by high-throughput MS





# Enzyme and Transporter Discovery

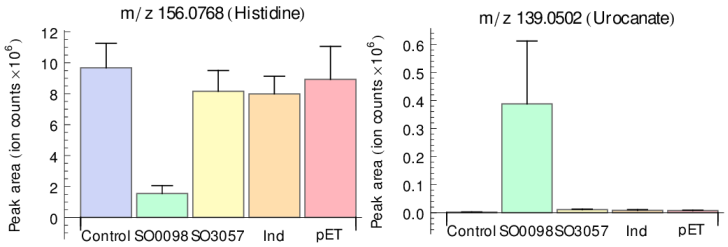
| Organism | Gene(s) | Affected metabolite | Note |
|---|---|---|---|
| *E. coli* | *pfs* | MTA | 5'-methylthioadenosine/S-adenosylhomocysteine nucleosidase |
| *E. coli* | *pncA* | Nicotinamide | pyrazinamidase/nicotinamidase |
| *E. coli* | *manX*, *manY*, *manZ* | Glucosamine | subunits of mannose 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 | |

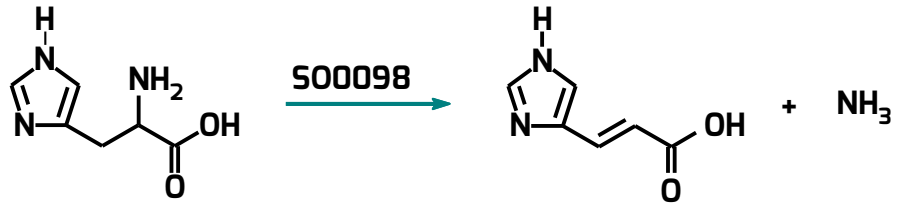
Baran R *et al* (2013) *ACS Chemical Biology* **8**(1): 189–199

# *S. oneidensis* Histidases

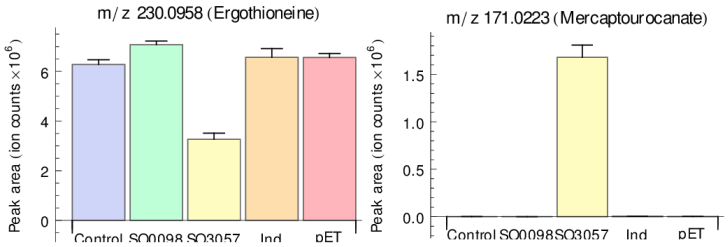
- Pal/Histidase SO0098 required for the utilization of histidine as a nitrogen source (Deutschbauer at al, 2011)
- Mutant in orthologous SO3057 unable to utilize ergothioneine
- *In vitro* assays validate activity and show strict substrate specificity

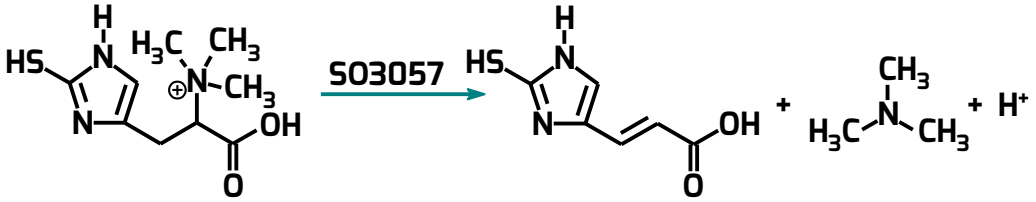
**a** –incubation with histidine



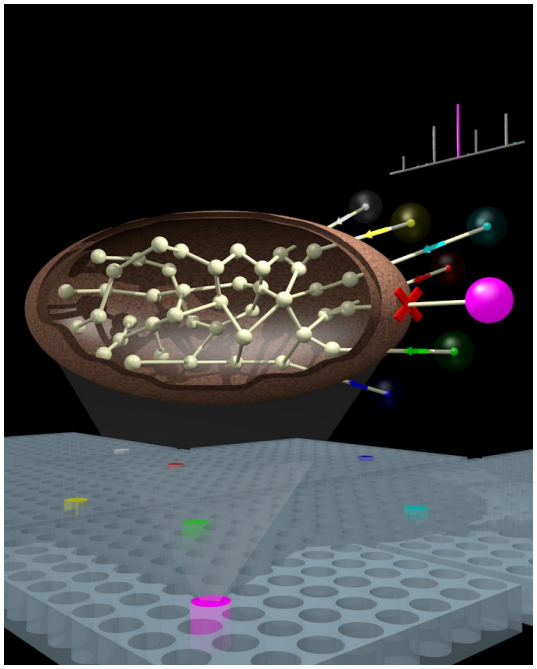

**b** –incubation with ergothioneine




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

# Thank you!



Trent Northen
Ben Bowen
Xiaoliang Cheng
Katherine Louie

Nicholas Bouskill
Eoin Brodie
Steve Yannone

Adam Deutschbauer
Morgan Price
Kelly Wetmore
Jennifer Kuehl
Jayashree Ray
Paul Adams
Adam Arkin

Muriel Gugger (PCC)

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