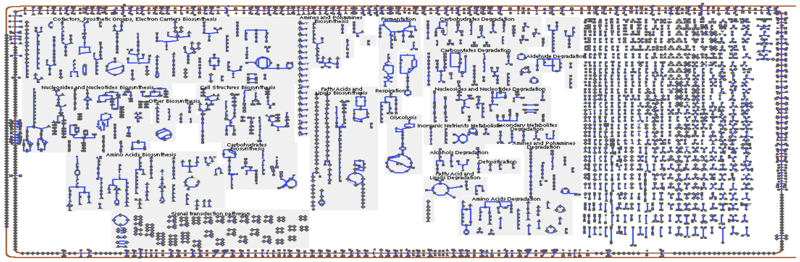


# The Omics Dashboard for Metabolomics Data

Peter D. Karp, Suzanne Paley, Paul O'Maille

SRI International

ecocyc.org
biocyc.org
metacyc.org

BioCyc Database Collection

# Overview of Omics Dashboard

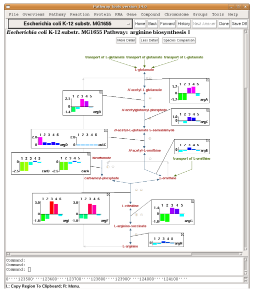
- New interactive tool for analysis of omics data
  - Metabolomics
  - Gene expression
  - Proteomics
- Use cases:
  - Quickly survey how all cellular systems are responding to a stimulus
  - Examine specific pathways, subsystems of interest
  - Gauge relative metabolite levels

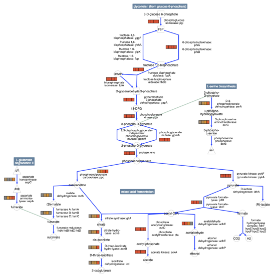
- Available at BioCyc.org, and with downloadable Pathway Tools software

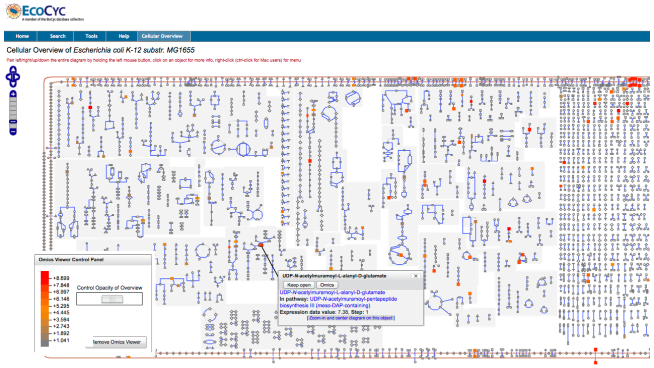
- Paley et al., *Nucleic Acids Research* 2017

# BioCyc.org Overview

- 11,000 Pathway/Genome Databases for sequenced organisms
  - Predicted metabolic reactions, pathways, metabolomes
  - Atom mappings, metabolic models
  - Curated from 66,000 publications
- Extensive bioinformatics tools for metabolomics analysis
  - Paint metabolomics data onto individual pathways, multi-pathway diagrams, full metabolic maps
  - SmartTables to manipulate metabolite sets (e.g., map to pathways)
  - Karp et al, *Metabolites* 5:291 2015







Cellular Overview of *Escherichia coli K-12 substr. MG1655*

Pan left/right/up/down the entire diagram by holding the left mouse button, click on an object for more info, right-click (ctrl-click for Mac users) for menu

**UDP-*N*-acetylmuramoyl-L-alanyl-D-glutamate**

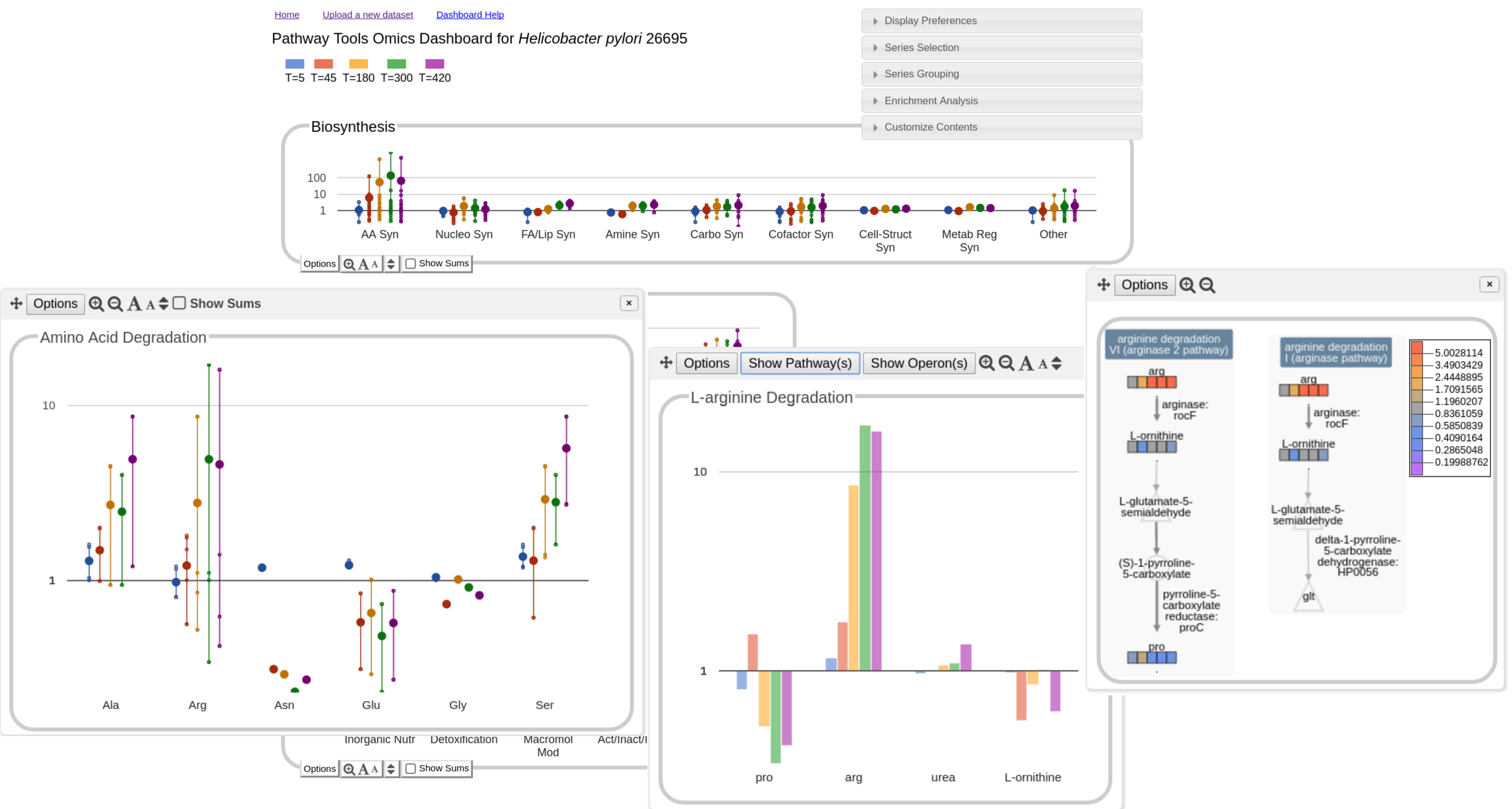
UDP-*N*-acetylmuramoyl-L-alanyl-D-glutamate

**In pathway:** UDP-*N*-acetylmuramoyl-pentapeptide biosynthesis III (*meso*-DAP-containing)

**Expression data value:** 7.38, **Step:** 1

# Organization of Omics Dashboard

- **Panels** summarize omics data for multiple cellular systems
- Each panel contains a set of **plots** (subsystems)


Home
Upload a new dataset
Dashboard Help
Pathway Tools Omics Dashboard for *Helicobacter pylori* 26695
T=5 T=45 T=180 T=300 T=420
Display Preferences
Series Selection
Series Grouping
Enrichment Analysis
Customize Contents
Biosynthesis
100
10
1
AA Syn
Nucleo Syn
FA/Lip Syn
Amine Syn
Carbo Syn
Cofactor Syn
Cell-Struct Syn
Metab Reg Syn
Other
Options
☐ Show Sums
Amino Acid Degradation
10
1
Ala
Arg
Asn
Glu
Gly
Ser
Inorganic Nutr
Detoxification
Macromol Mod
Act/Inact/I
Options
Show Pathway(s)
Show Operon(s)
L-arginine Degradation
10
1
pro
arg
urea
L-ornithine
arginine degradation VI (arginase 2 pathway)
arg
arginase: rocF
L-ornithine
L-glutamate-5-semialdehyde
(S)-1-pyrroline-5-carboxylate
pyrroline-5-carboxylate reductase: proC
pro
arginine degradation I (arginase pathway)
arg
arginase: rocF
L-ornithine
L-glutamate-5-semialdehyde
delta-1-pyrroline-5-carboxylate dehydrogenase: HP0056
glt
5.0028114
3.4903429
2.4448895
1.7091565
1.1960207
0.8361059
0.5850839
0.4090164
0.2865048
0.19988762

# Omics Dashboard Panels

- Panels and plots available configured automatically for current organism and current dataset

- Plots defined from MetaCyc pathways and pathway classes, Gene Ontology terms
  - User can add or remove plots from any panel
  - Many non-metabolic panels for gene-expression data

- Click on a plot to drill down for more information

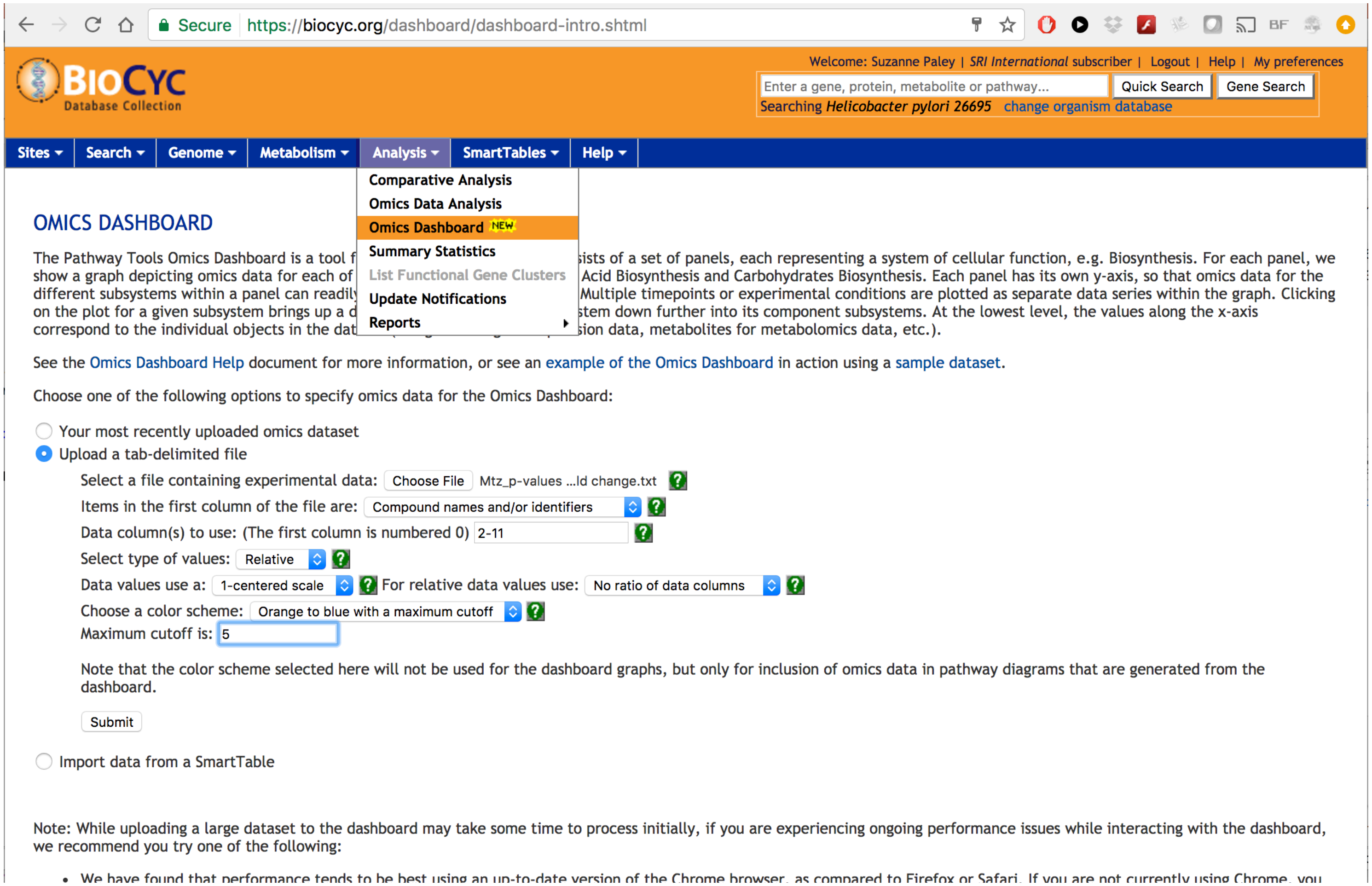
# Using the Dashboard

- Apply normalization and significance calculations before uploading data, if applicable

- Import data as a column-delimited file
  - Metabolite names/IDs in first column
  - Specify which other columns contain data of interest
  - Compare all metabolites with only significantly changed metabolites

- Types of data values
  - Fold change values
  - Absolute quantities (counts, areas, intensities)
  - P-values from replicate analysis (used for enrichment analysis)

# Invoking the Omics Dashboard



## OMICS DASHBOARD

The Pathway Tools Omics Dashboard is a tool f … sists of a set of panels, each representing a system of cellular function, e.g. Biosynthesis. For each panel, we
show a graph depicting omics data for each of … Acid Biosynthesis and Carbohydrates Biosynthesis. Each panel has its own y-axis, so that omics data for the
different subsystems within a panel can readil … Multiple timepoints or experimental conditions are plotted as separate data series within the graph. Clicking
on the plot for a given subsystem brings up a d … stem down further into its component subsystems. At the lowest level, the values along the x-axis
correspond to the individual objects in the dat … sion data, metabolites for metabolomics data, etc.).

See the Omics Dashboard Help document for more information, or see an example of the Omics Dashboard in action using a sample dataset.

Choose one of the following options to specify omics data for the Omics Dashboard:

○ Your most recently uploaded omics dataset
● Upload a tab-delimited file

Select a file containing experimental data: Choose File Mtz_p-values ...ld change.txt
Items in the first column of the file are: Compound names and/or identifiers
Data column(s) to use: (The first column is numbered 0) 2-11
Select type of values: Relative
Data values use a: 1-centered scale For relative data values use: No ratio of data columns
Choose a color scheme: Orange to blue with a maximum cutoff
Maximum cutoff is: 5

Note that the color scheme selected here will not be used for the dashboard graphs, but only for inclusion of omics data in pathway diagrams that are generated from the dashboard.

Submit

○ Import data from a SmartTable

Note: While uploading a large dataset to the dashboard may take some time to process initially, if you are experiencing ongoing performance issues while interacting with the dashboard, we recommend you try one of the following:

- We have found that performance tends to be best using an up-to-date version of the Chrome browser, as compared to Firefox or Safari. If you are not currently using Chrome, you

# *Helicobacter pylori* Dataset – MoA of antibiotics

- Unpublished data courtesy of Paul O'Maille (SRI Biosciences)
- Time series metabolomics investigation of metronidazole action against *H. pylori* (compared to DMSO control)
- Current MoA:
  - Metronidazole (prodrug) converted to radicals in anaerobic pathogens
  - Metronidazole radicals induce DNA breakage through random attack[1]
- Metabolomics and Dashboard – new tools, new insights

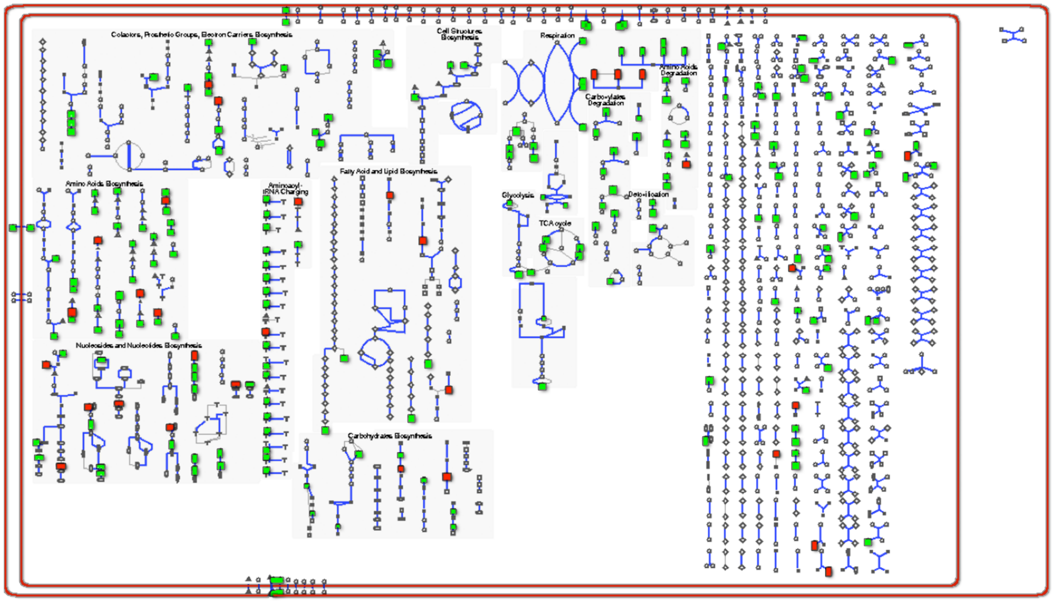
[1] Edwards DI (1993) Nitroimidazole drugs--action and resistance mechanisms. I. Mechanisms of action. *J Antimicrob Chemother* **31:** 9-20

# *Helicobacter pylori* Dataset – MoA of antibiotics

- Study design:
  - *H. pylori* cultures exposed to metronidazole (or DMSO control)
  - 5 biological replicates taken at T = 5, 45, 180, 300, 420 minutes
  - Metabolon data collection and processing:
    - Global (non-targeted) metabolite analysis of samples via LC/GC-MS platform
    - Replicates averaged, and fold changes computed relative DMSO control
    - Significance p-values computed using Two Way ANOVA with Contrasts

# *Helicobacter pylori* Dataset – Metabolome Coverage

HpyCyc: *Helicobacter pylori* 26695 PGDB



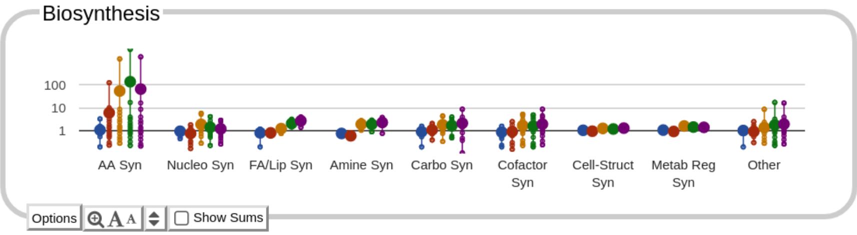
Cellular overview – colored nodes are experimentally measured metabolites where statistically significant (red) and non-significant (green) changes are indicated.

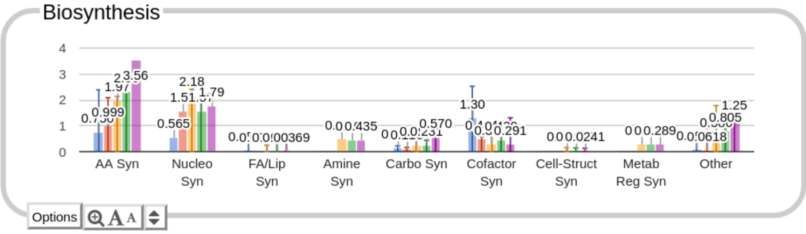
- 318 *H. pylori* metabolites identified
- 103 matches out of 602 total HpyCyc metabolites
- 17% coverage of *H. pylori* metabolome

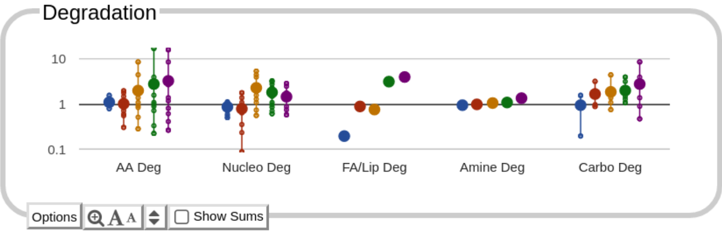
# Metabolite Enrichment Analysis

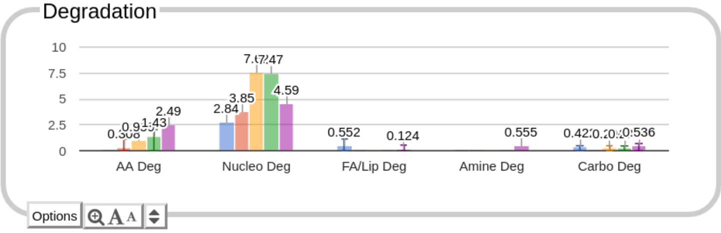
- Which pathways have more perturbed metabolites than expected by chance?
- User specifies
  - A p-value column associated with each timepoint
  - Threshold (0.05 in this example)
  - Multiple hypothesis correction function
- Compute enrichment score for every pathway
  - Fisher-exact hypergeometric test
  - Enrichment score for a pathway = $-\log_{10}$(p-value)
- Panels show
  - Enrichment scores for each pathway
  - Highest component subsystem score

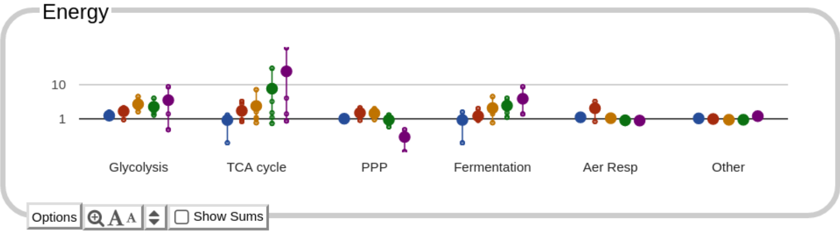
# Standard Display vs. Enrichment Mode Display

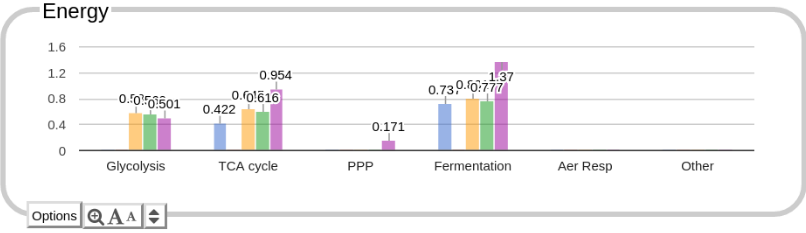

Biosynthesis

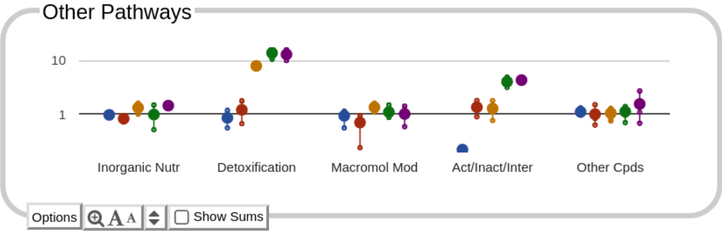

Biosynthesis

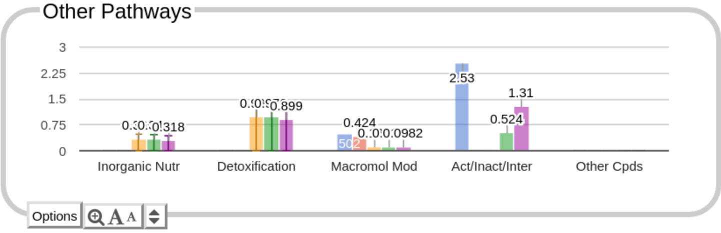

Degradation


Degradation

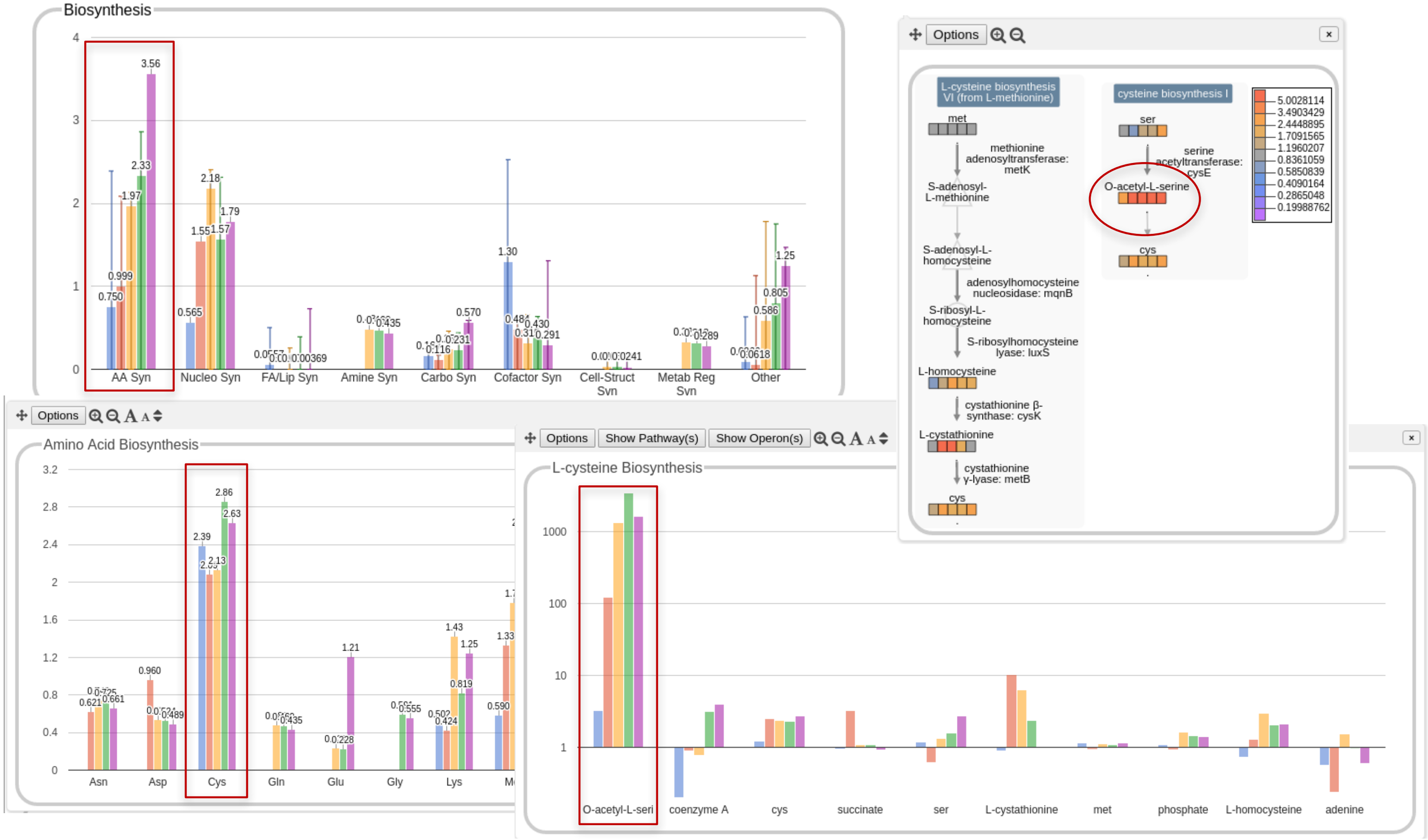

Energy

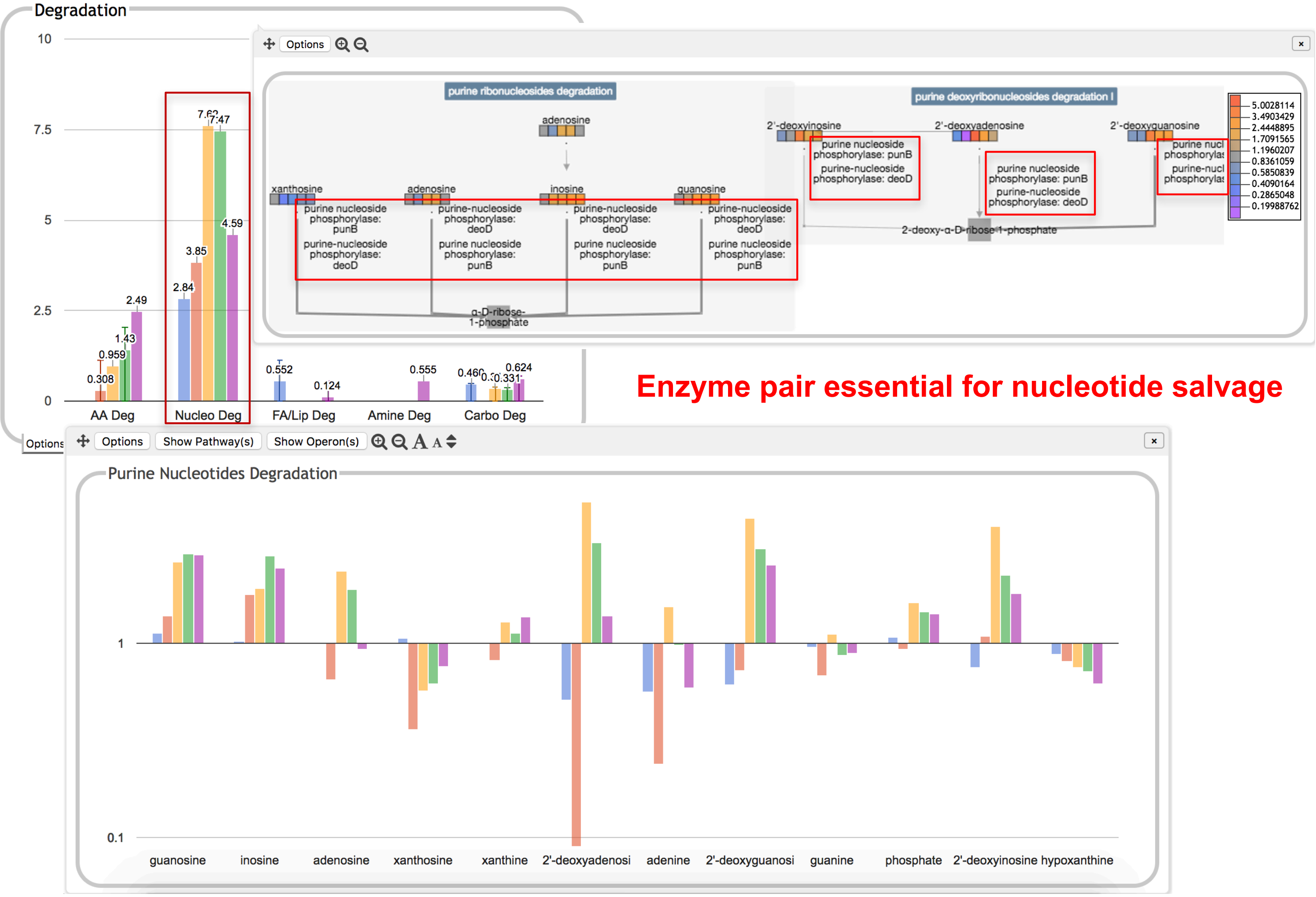

Energy


Other Pathways


Other Pathways

# Sulfur-Containing Amino Acids Disrupted During Early Phase Action

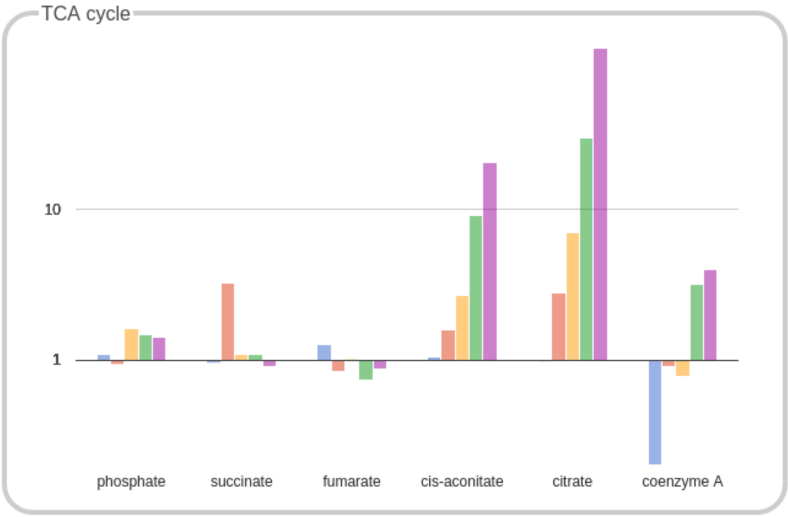


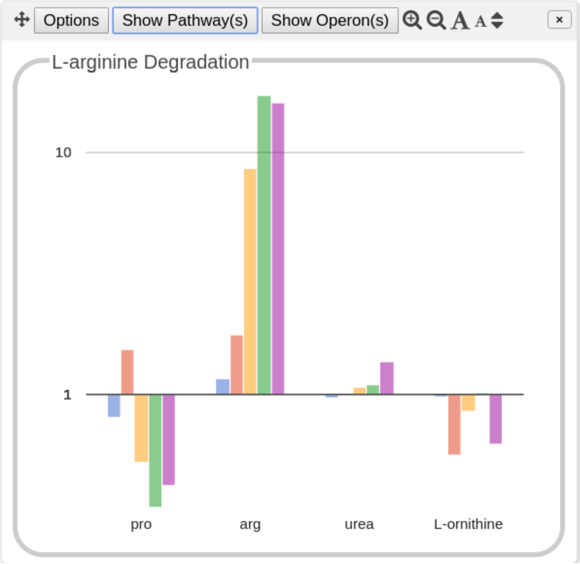


**Enzyme pair essential for nucleotide salvage**

# Late Phase Action Reveals Bacterial Death

- Accumulation of TCA cycle intermediates and arginine in later time points consistent with increasing morbidity





# New Insights from Dashboard Analysis of Metabolomics Mode of Action Study

- Early phase metronidazole action: disruption of sulfur amino acid biosynthesis and nucleolotide degradation pathways
- *H. pylori* critically depends on nucleotide salvage[1]
  - Nucleoside phosphorylase (chokepoint enzyme) evident in Dashboard
- Genome stability is tightly linked to nucleotide metabolism[2]
- Metabolic basis for DNA breakage

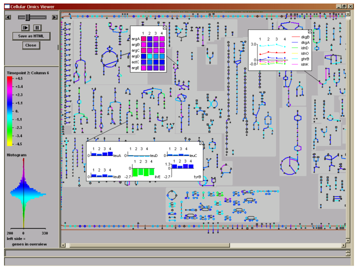
[1] Liechti G, Goldberg JB (2012) Helicobacter pylori relies primarily on the purine salvage pathway for purine nucleotide biosynthesis. *J Bacteriol* **194:** 839-854

[2] Kunz BA, Kohalmi SE, Kunkel TA, Mathews CK, McIntosh EM, Reidy JA (1994) International Commission for Protection Against Environmental Mutagens and Carcinogens. Deoxyribonucleoside triphosphate levels: a critical factor in the maintenance of genetic stability. *Mutat Res* **318:** 1-64

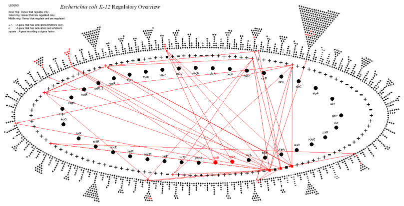
## Pathway Tools Software

- Create and maintain an organism database integrating genome, pathway, regulatory information
- Computational inference tools
- Interactive editing tools
- Query and visualization
- Generate metabolic flux models for organisms and organism communities
- Interpret omics datasets
- Comparative analysis
- Licensed by 7,000+ groups – free to academics

# Pathway Tools



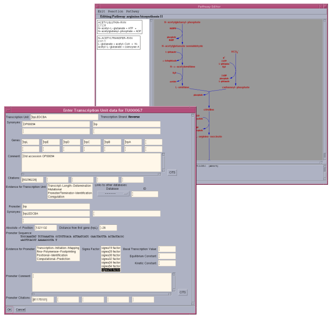
Annotated Genome + PathoLogic MetaCyc



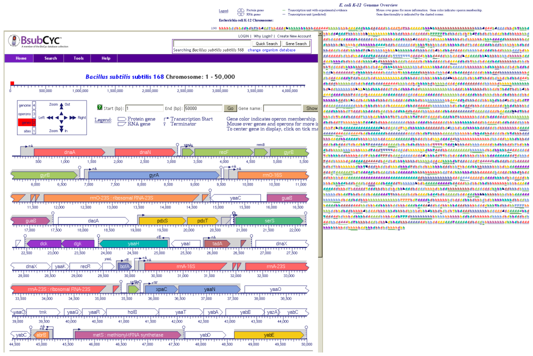
Pathway/Genome Database

MetaFlux

Pathway/Genome Navigator

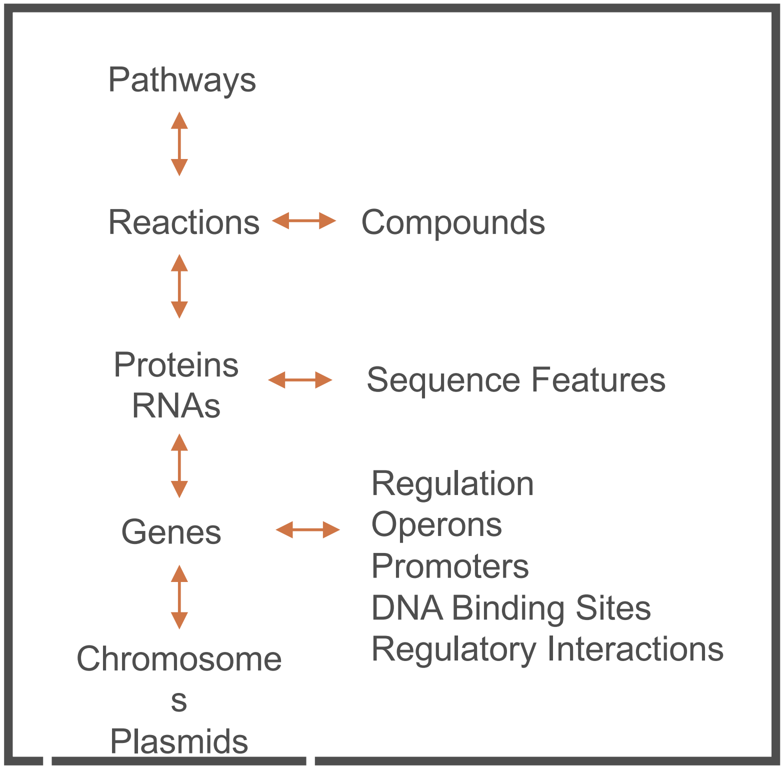


Pathway/Genome Editors



Licensed by 7,000+ Groups

# Pathway/Genome Database Organization



Pathways

Reactions ↔ Compounds

Proteins
RNAs ↔ Sequence Features

Genes ↔ Regulation
Operons
Promoters
DNA Binding Sites
Regulatory Interactions

Chromosomes
Plasmids

CELL

# BioCyc.org Overview

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  - Curated from 66,000 publications
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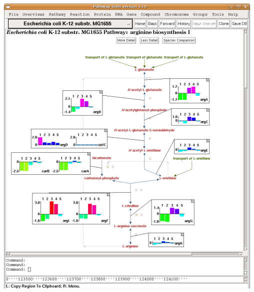
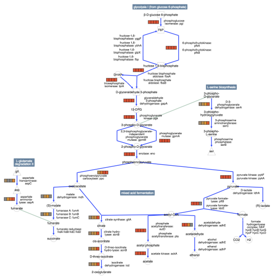
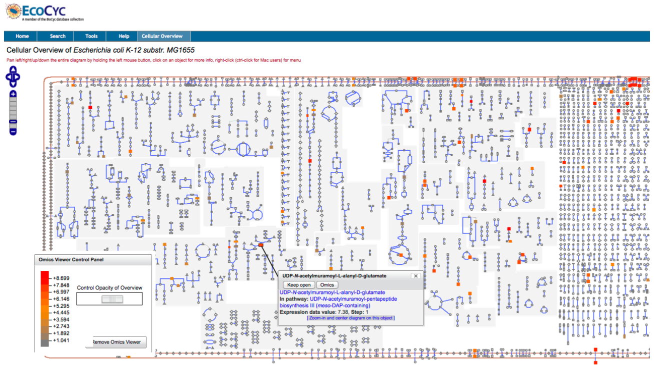


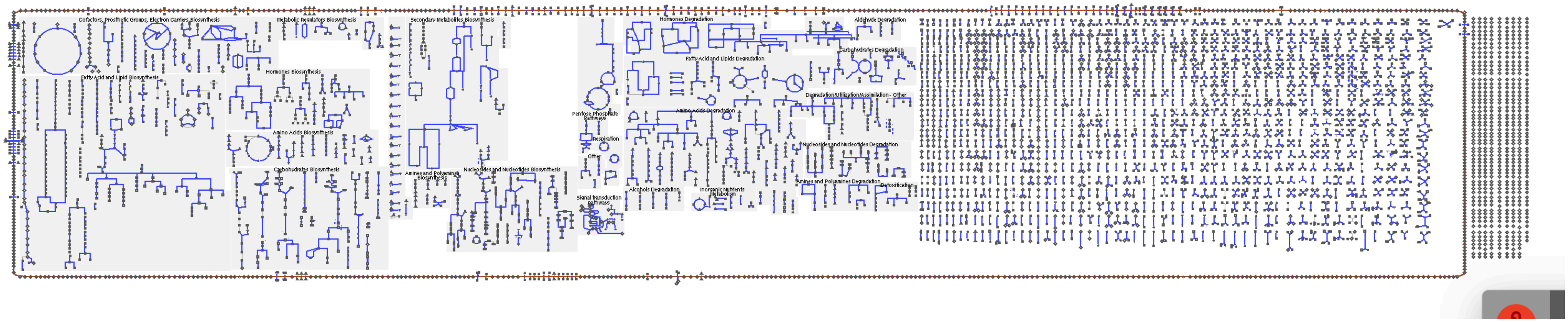


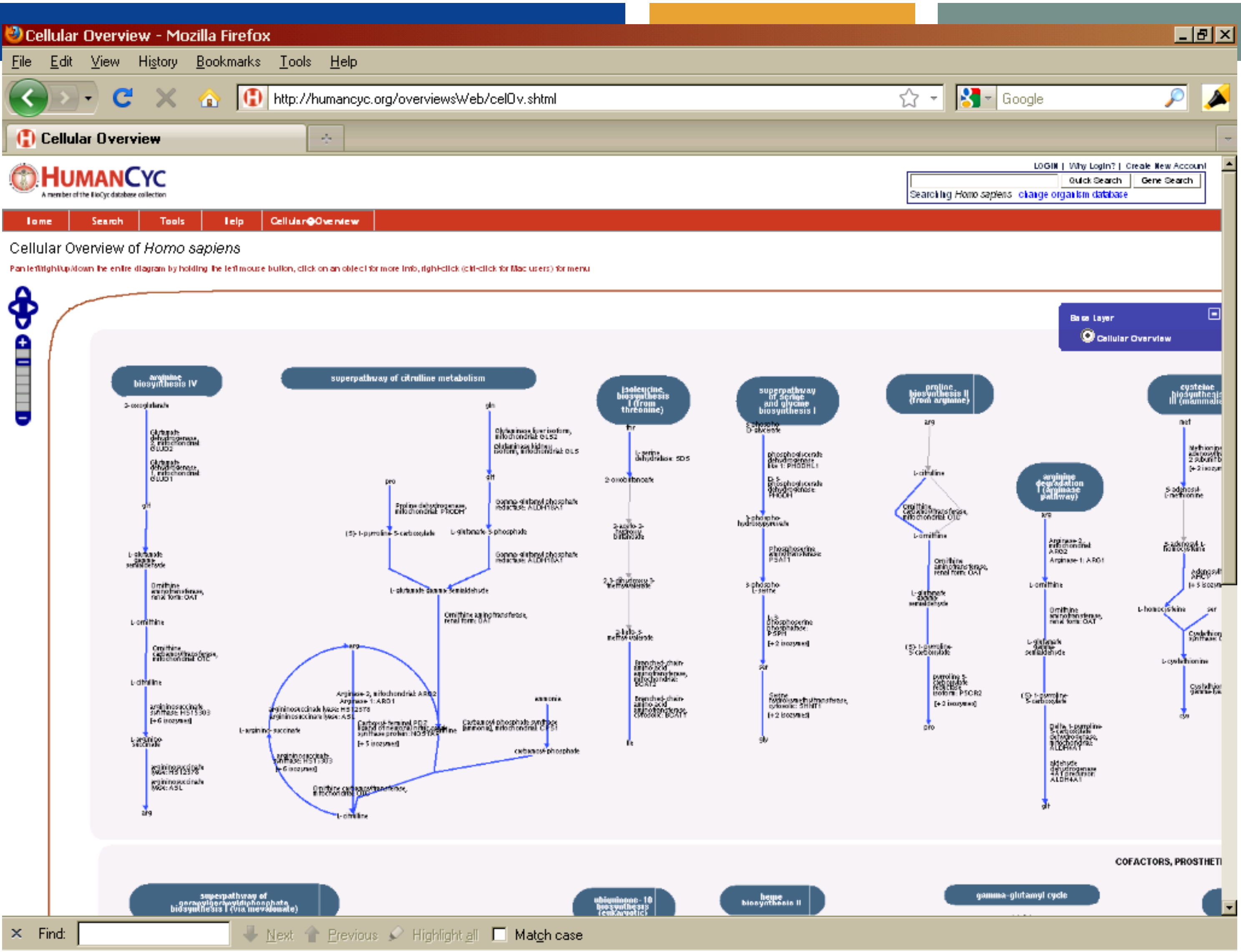


Cellular Overview of *Escherichia coli K-12 substr. MG1655*

Pan left/right/up/down the entire diagram by holding the left mouse button, click on an object for more info, right-click (ctrl-click for Mac users) for menu

# Cellular Overview for *Homo sapiens*





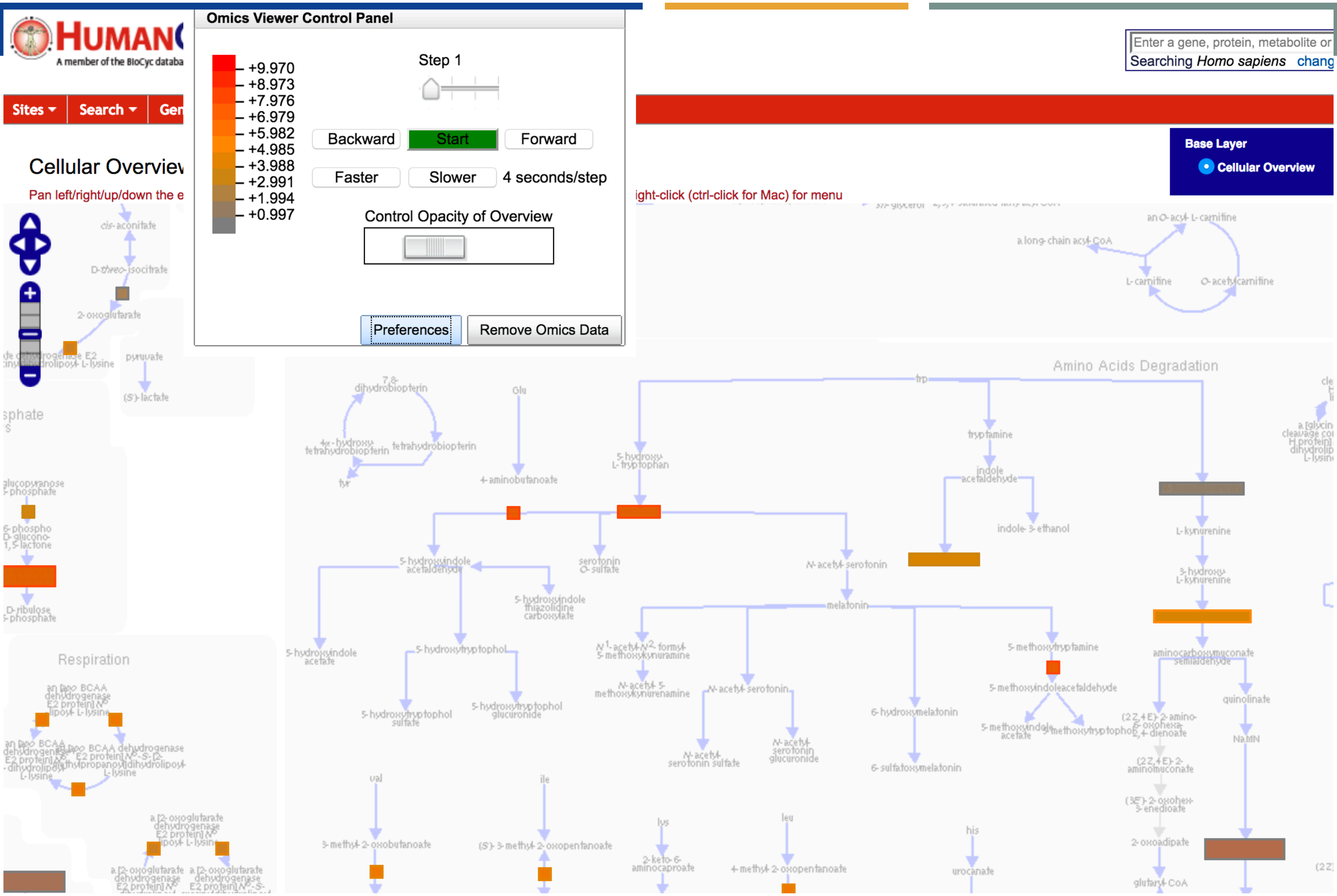
Cellular Overview of *Homo sapiens*

Pan left/right/up/down the entire diagram by holding the left mouse button, click on an object for more info, right-click (ctrl-click for Mac users) for menu

Cellular C

Pan left/right/up

more info, right-click (ctrl-click for Mac) for menu



Omics Viewer Control Panel

Step 1

4 seconds/step

Control Opacity of Overview

Cellular Overview

Pan left/right/up/down the e

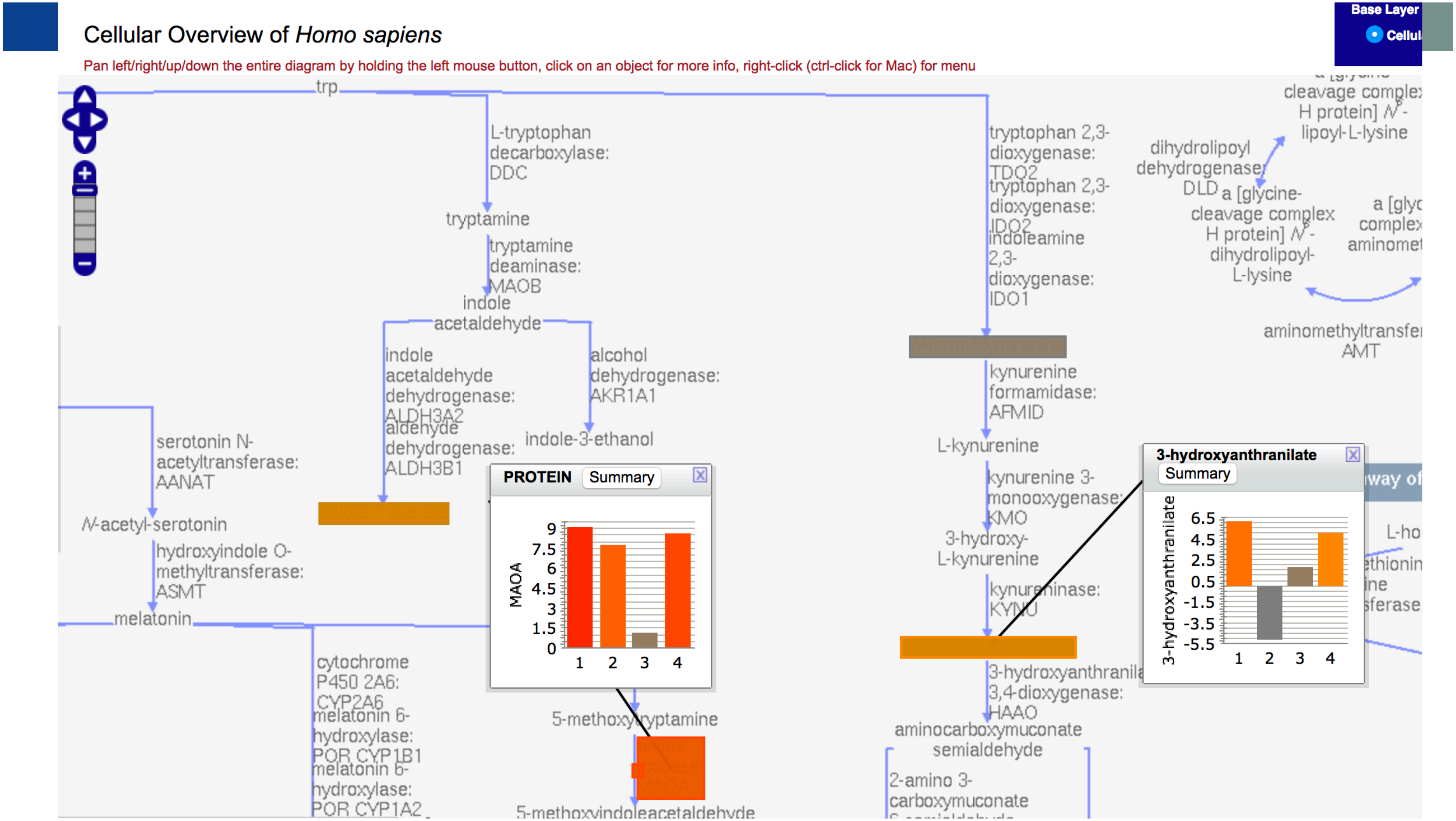
ight-click (ctrl-click for Mac) for menu

Enter a gene, protein, metabolite or

Searching *Homo sapiens* chang

**Base Layer**

● **Cellular Overview**



Cellular Overview of *Homo sapiens*

Pan left/right/up/down the entire diagram by holding the left mouse button, click on an object for more info, right-click (ctrl-click for Mac) for menu

# Summary

- Omics Dashboard provides top-down, organism-wide, system-level view of metabolomics results
- Quickly identify and drill down to cellular systems of interest
- Uniquely well-suited to visualization of enrichment analysis results
- Complements other forms of metabolomics data analysis

- Available at BioCyc.org
  - Free access to EcoCyc (*E. coli*) and MetaCyc
  - Subscription required for access to other organisms
- Also available within Pathway Tools software
  - Freely available for academic research

# Acknowledgements

- **Dashboard implemented by Suzanne Paley**
- ***H. pylori* dataset and analysis courtesy of Paul O’Maille**

- Funding sources:
  - **NIH National Institute of General Medical Sciences**

http://www.ai.sri.com/pkarp/talks/

BioCyc webinars:
biocyc.org/webinar.shtml