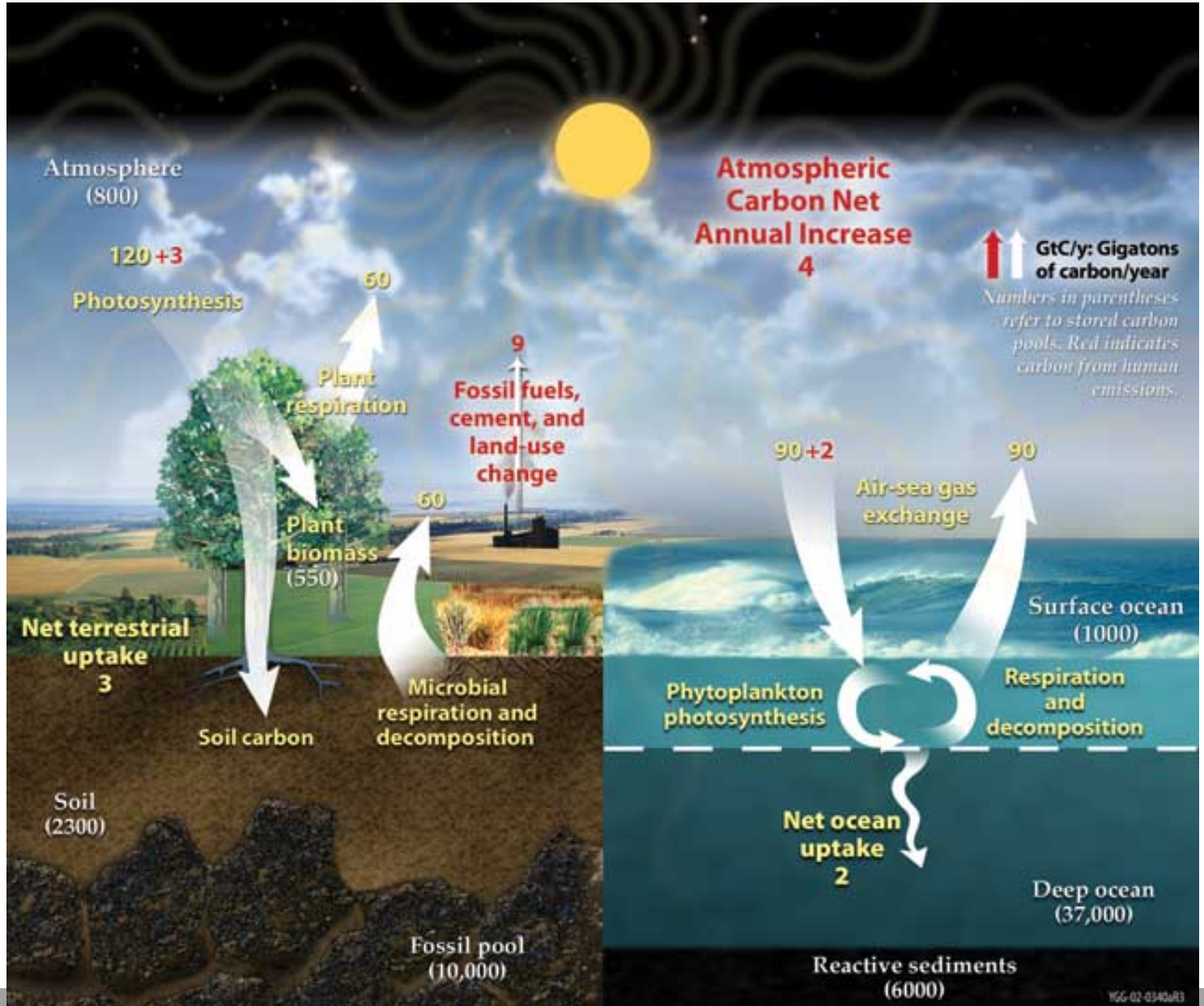
JGI JOINT GENOME INSTITUTE
UNITED STATES DEPARTMENT OF ENERGY

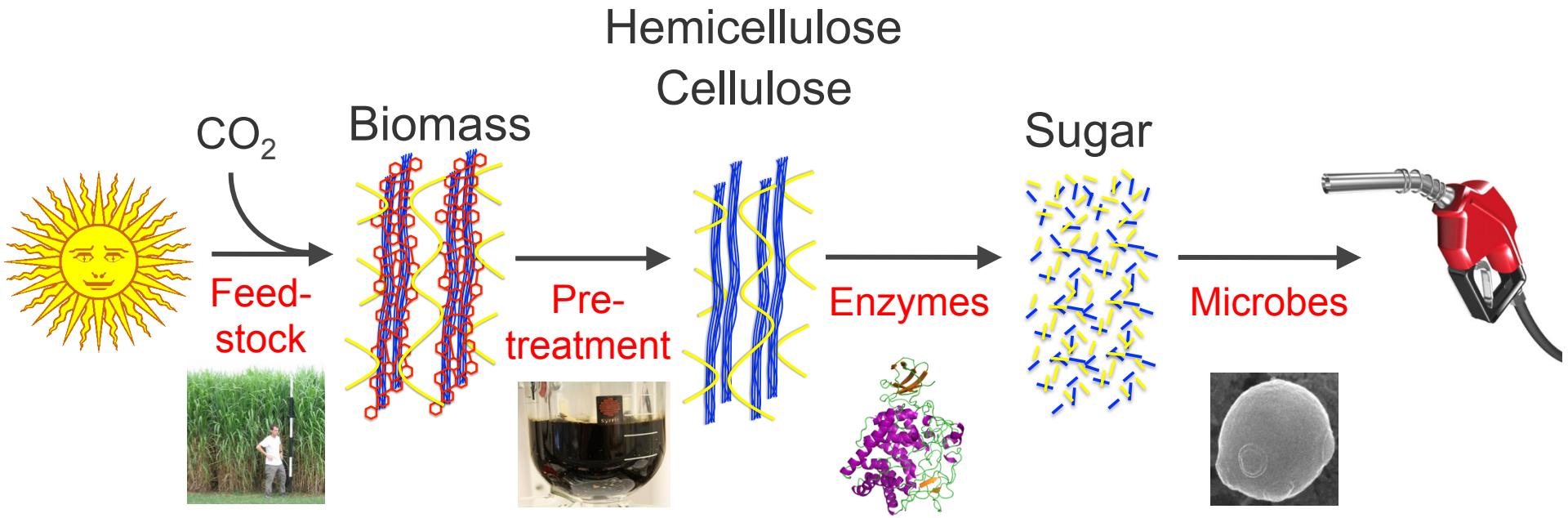
Metabolomic Technologies at JGI

JGI User Meeting
Trent Northen

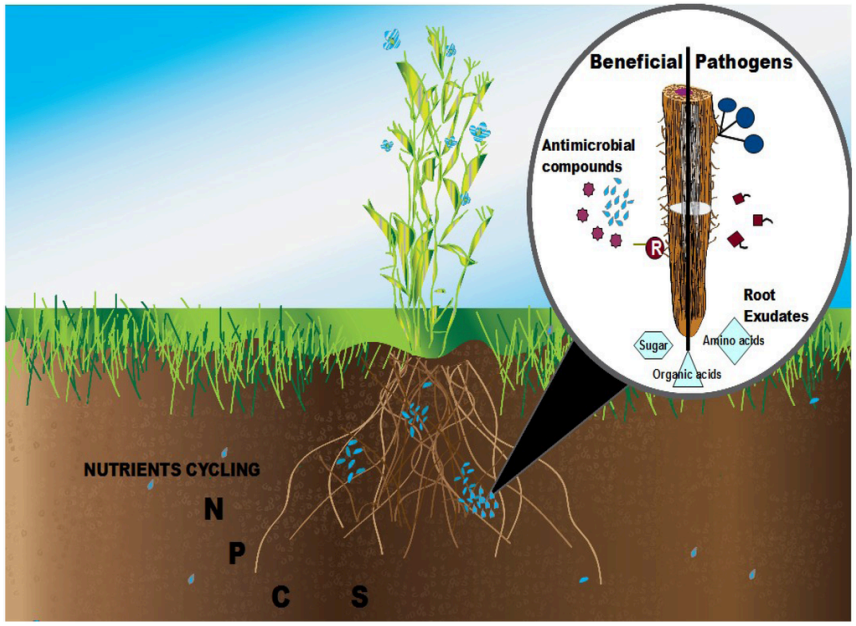
Motivation: Microbial metabolism is central to global carbon cycling

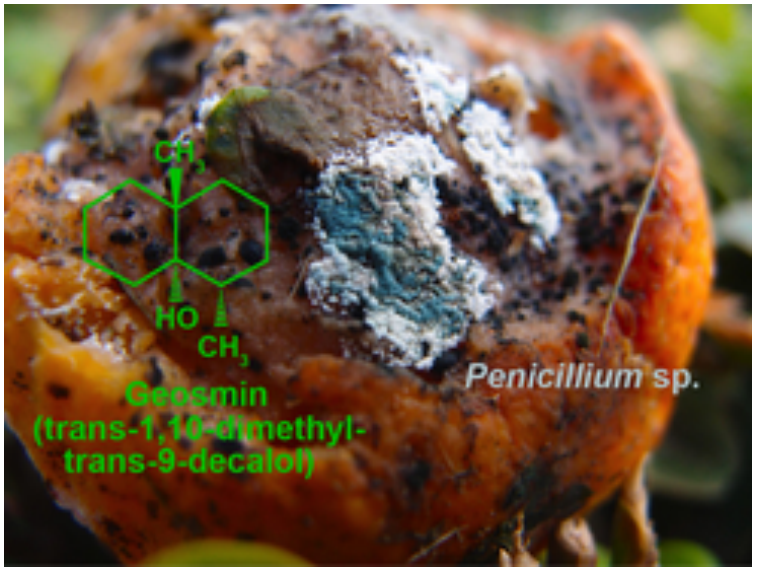


Motivation: Bioenergy and Bioproducts



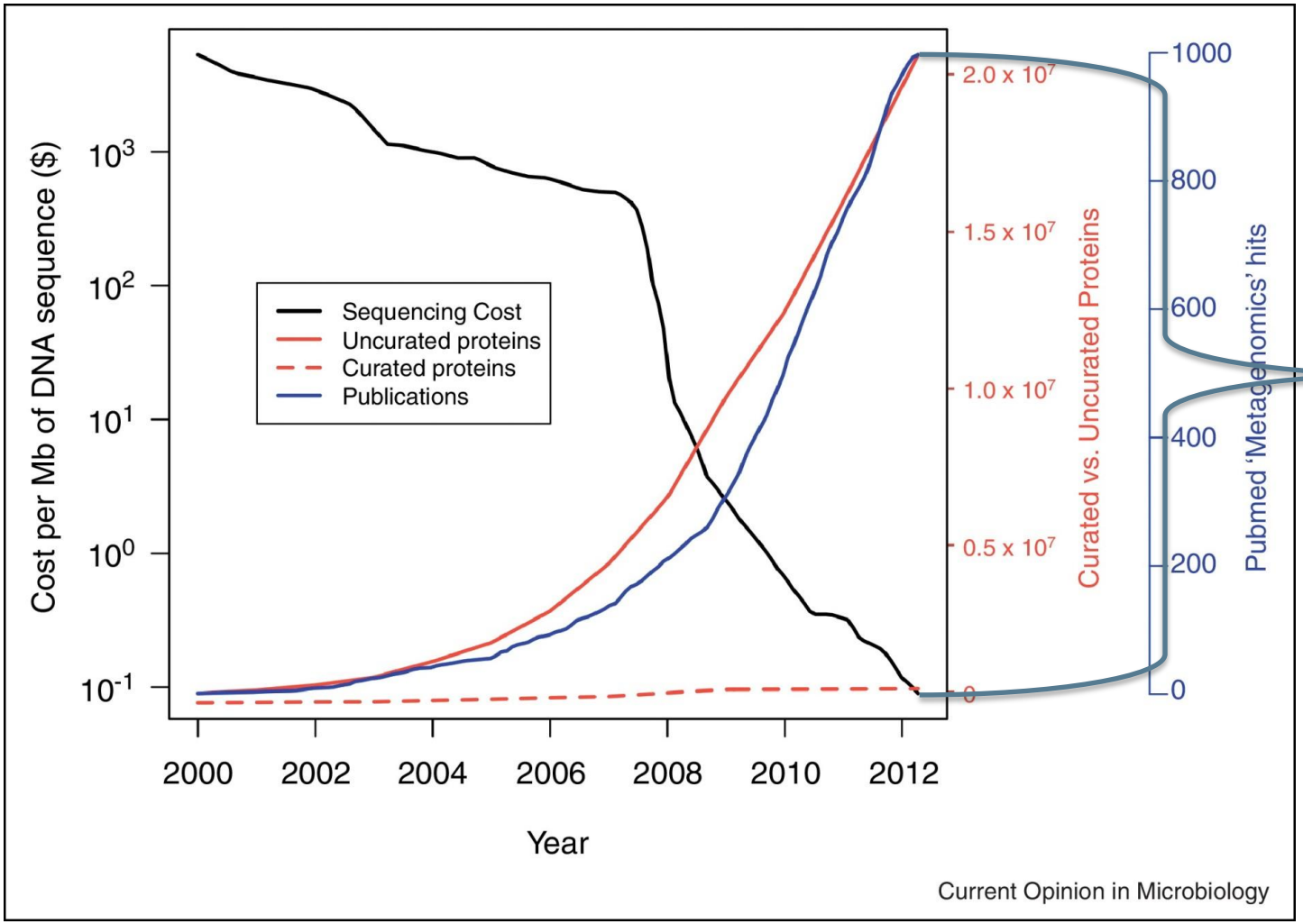
Motivation: understanding the diverse activities of microbial secondary metabolites





https://www.mpg.de/6656708/odour-activation-geosmin-fly

Need for rapid sequence-to-function technologies to complement sequencing

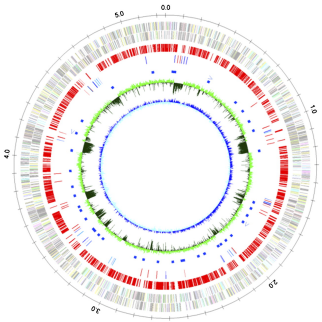


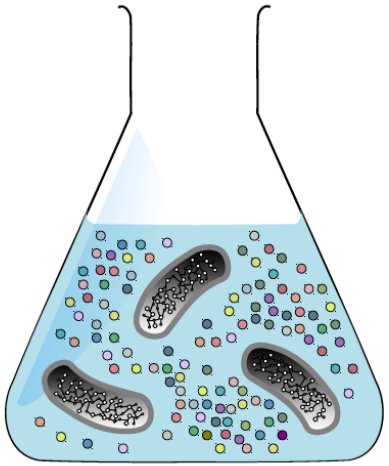
Huge need for functional data to complement sequencing

Temperton, Current Opinion in Microbiology, 2012

Overview of metabolomics

Sequence

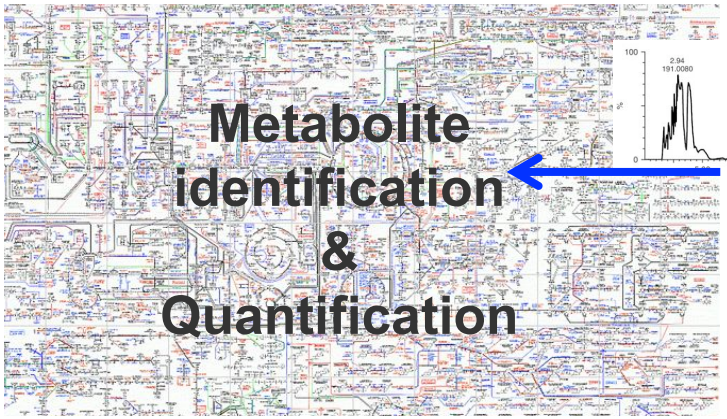


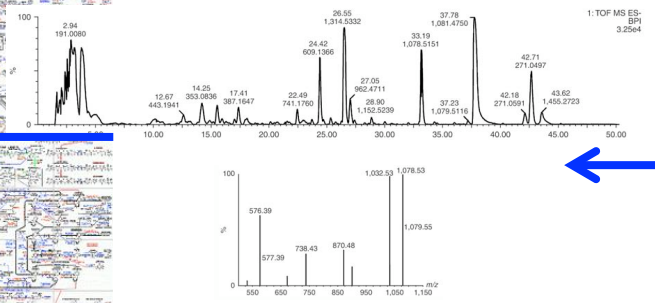


Extraction

HPLC

Function

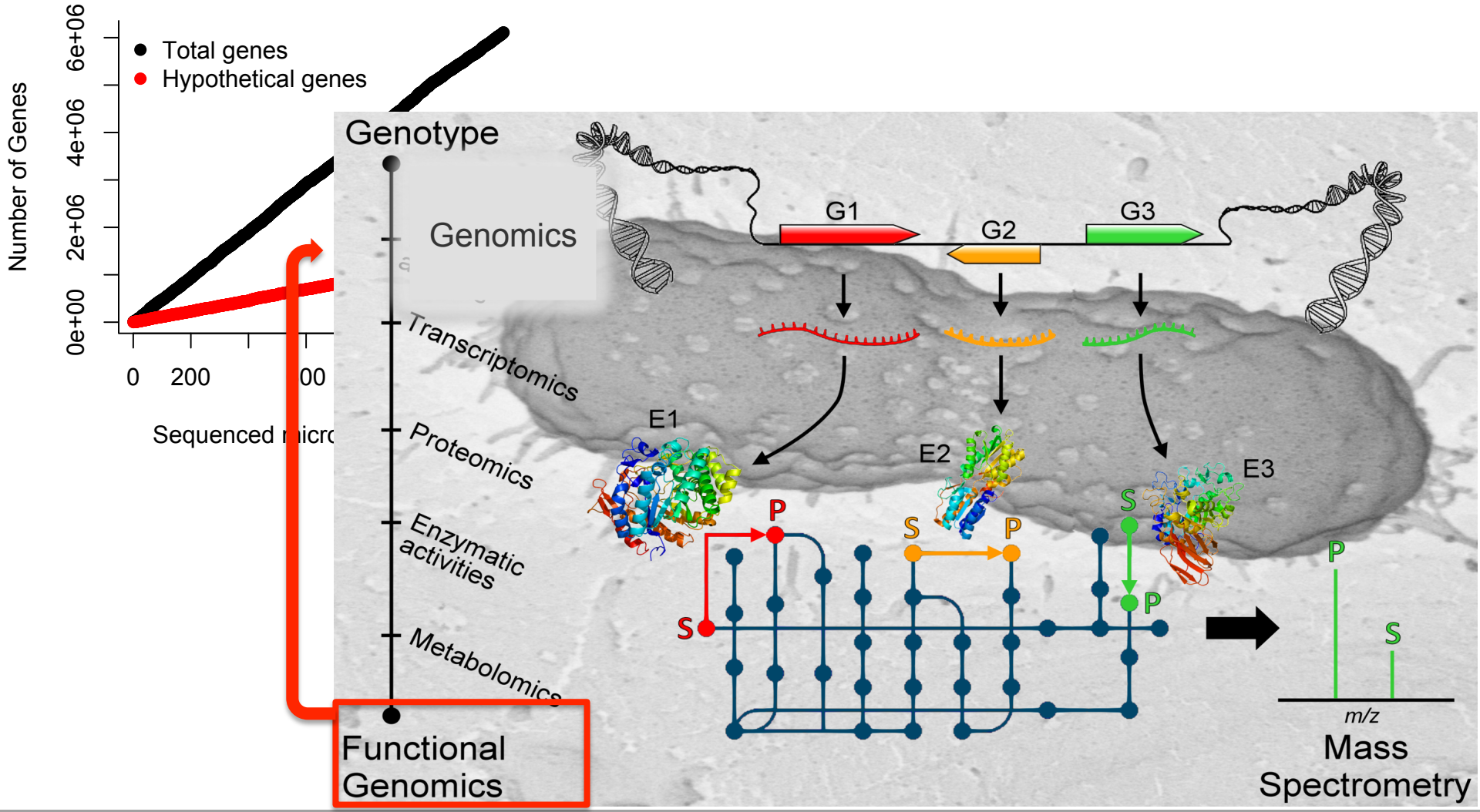




Mass Spectrometry

Metabolomics provides a functional complement genomics

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



Vision: JGI effectively integrates metabolomics with sequencing/ synthesis providing users new biological insights

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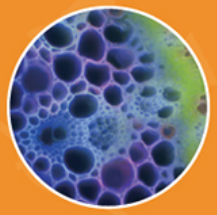
Mission Areas

Bioenergy



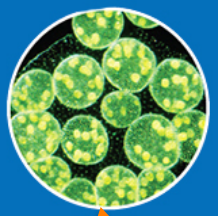
Carbon Cycling

Biogeochemistry

Programs

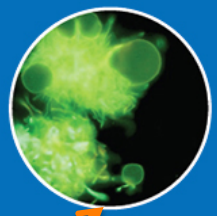
Metagenomes

Plants



Fungi

Microbes

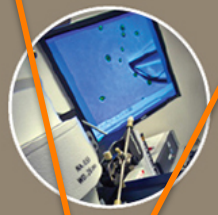


DNA Synthesis Science

Infrastructure

DNA Sequencing

- Illumina platforms: HiSeq, MiSeq, NextSeq
- PacBio
- Oxford Nanopore

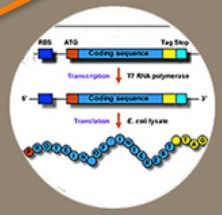


Advanced Genomic Technologies

- Sing[illegible]ell Seq
- Metabolomics
- Methylation
- TnSeq
- Epigenomics
- Transcriptomics

Computational Analysis

- Assembly/ Annotation/Analysis
- High-Performance Computing
- Big Data Integration & Analysis Systems



DNA Synthesis

- Design & Pathway Assembly
- Host Engineering

Experienced JGI Metabolomics Team

Trent Northen

Interest: metabolomics for microbial functional genomics and microbial community metabolism.

- PhD w/10 years metabolomics.
- Deeply connected to BER science missions through 8yrs involvement in BER science programs.
- TRNorthen@lbl.gov

Leslie Silva

Interest: microbial metabolomics

- PhD w/ 3 years metabolomics
- Key developer of JGI workflows.
- LPSilva@lbl.gov

Katherine Louie

Interest: microbial metabolomics

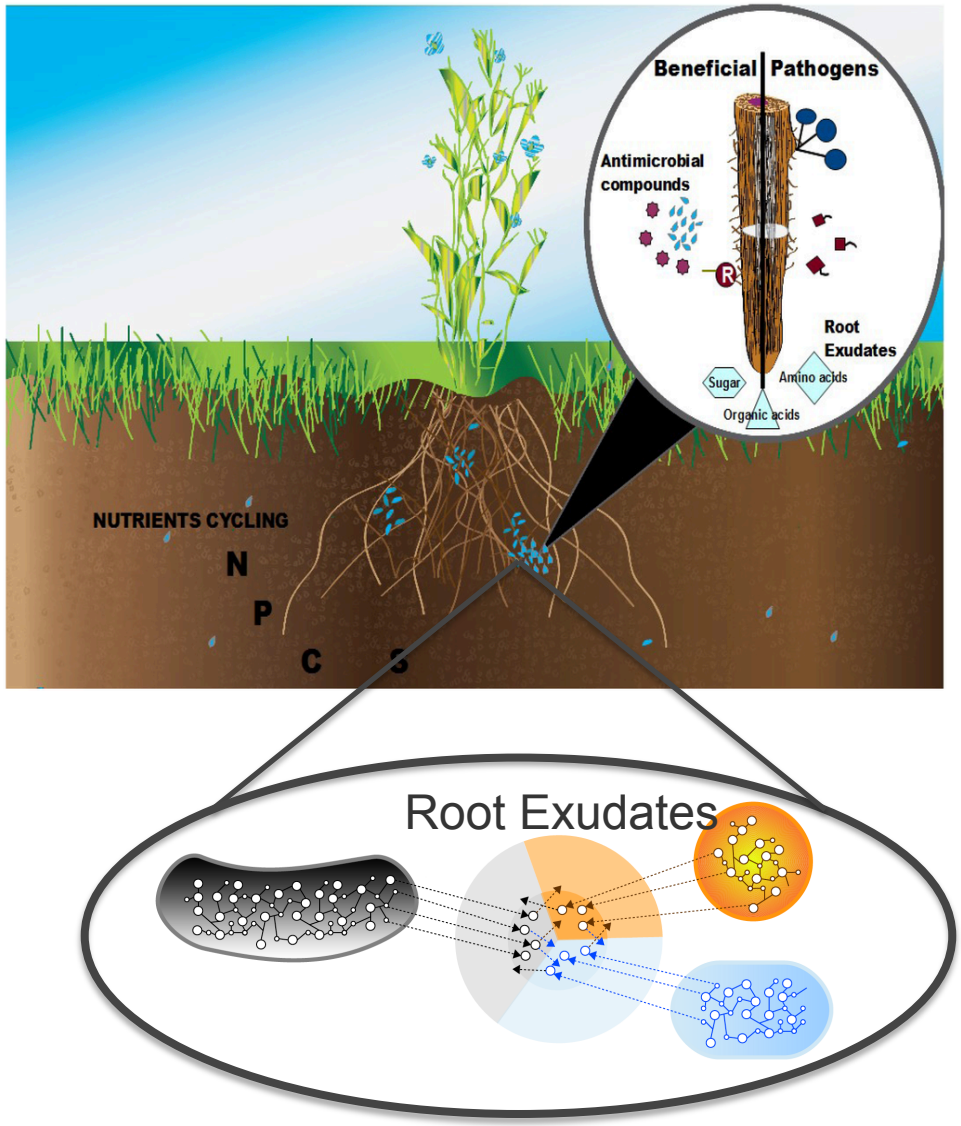
- PhD w/ 4 years of diverse metabolomics experimentation.
- Key developer of JGI workflows.
- KBLouie@lbl.gov

Ben Bowen

Research focus: metabolomics informatics

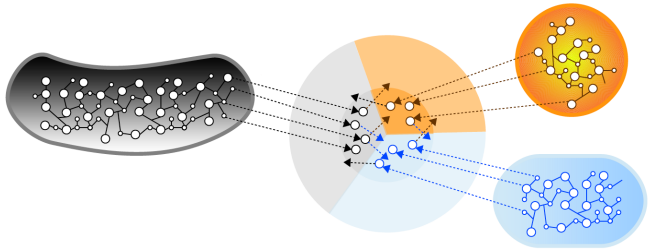
- PhD ~10 years mass spectrometry informatics
- BPBowen@lbl.gov

Initial products: analysis of primary and secondary metabolites from media and cells

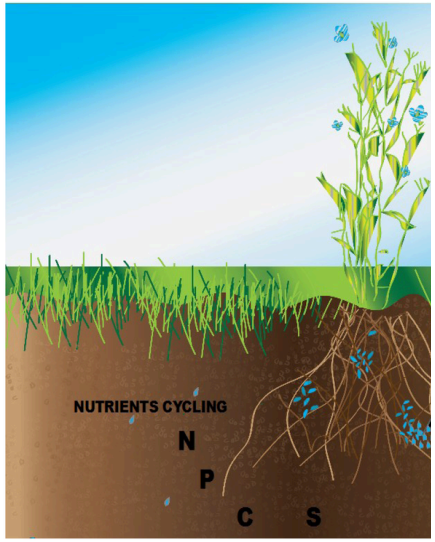


Initials metabolomics products

- **Exometabolomic analysis of polar metabolites:**
  - Improve gene annotations
  - Examine nutrient exchange and resource competition
  - Provide critical insights into microbial foodwebs and carbon cycling ultimately to predict carbon cycling to metagenomic data

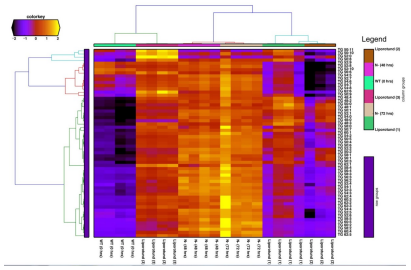


- **Secondary metabolite analysis**
  - Improve gene and pathway annotations
  - Improve our understanding of microbial communication
    - Plants
    - Microbes

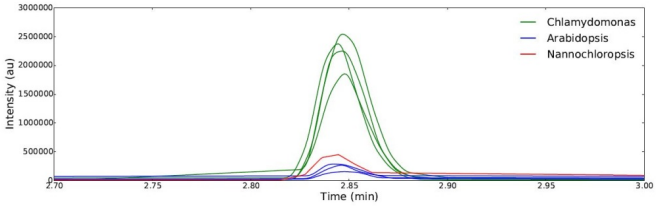


Early Success Stories—Sequencing + secondary metabolite analysis

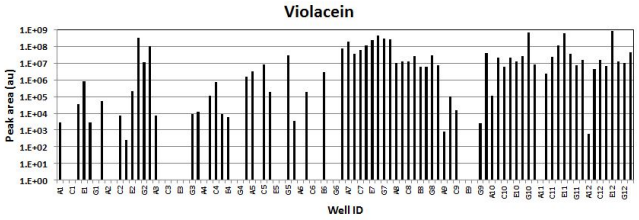
- **Integrated metabolomics with transcriptomic analysis and mutagenesis to determine that psr1 is a key regulator of lipid metabolism in *Chlamydomonas reinhardtii* (Yee et al, Nature Plant, 2015)**



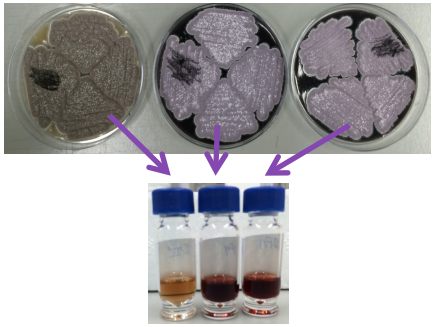
- **Integrated metabolomics with sequencing to verify accuracy of PacBio quantification of adenine methylation (*Yee et al, in review)***



- **Integrated metabolomics with DNA synthesis to optimize secondary metabolite (violacein) production (Sam Deutsch - JGI, Nathan Hillson - JBEI)**



- **Integrated metabolomics with DNA synthesis to support refactoring actinorhodin biosynthetic pathway (Sam Deutsch)**



Example: Metabolomics + DNA Synthesis Refactoring Actinorhodin pathway (21 genes)

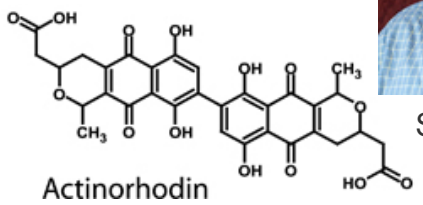
Internal LBNL PI By Nathan Hillson, Col's: Sam Deutch, Jeff Kim, Paramvir Dehal and Trent Northen

Sam Deutsch (JGI)

Nathan Hillson (JBEI)

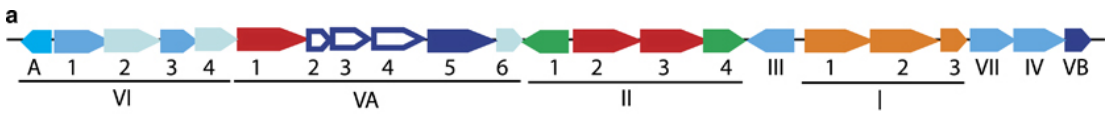
Challenge: Achieve predictable expression of a pathway by refactoring

- Representative of many biosynthetic clusters (size, complexity, GC content)
- Pathway well characterized (but never before refactored)

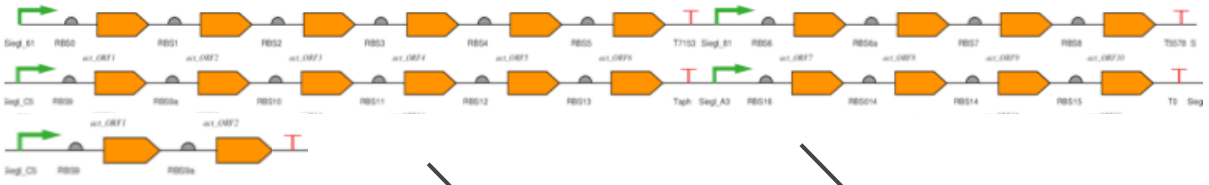


Actinorhodin

Native pathway – complex set of operons highly regulated under native conditions



Refactoring



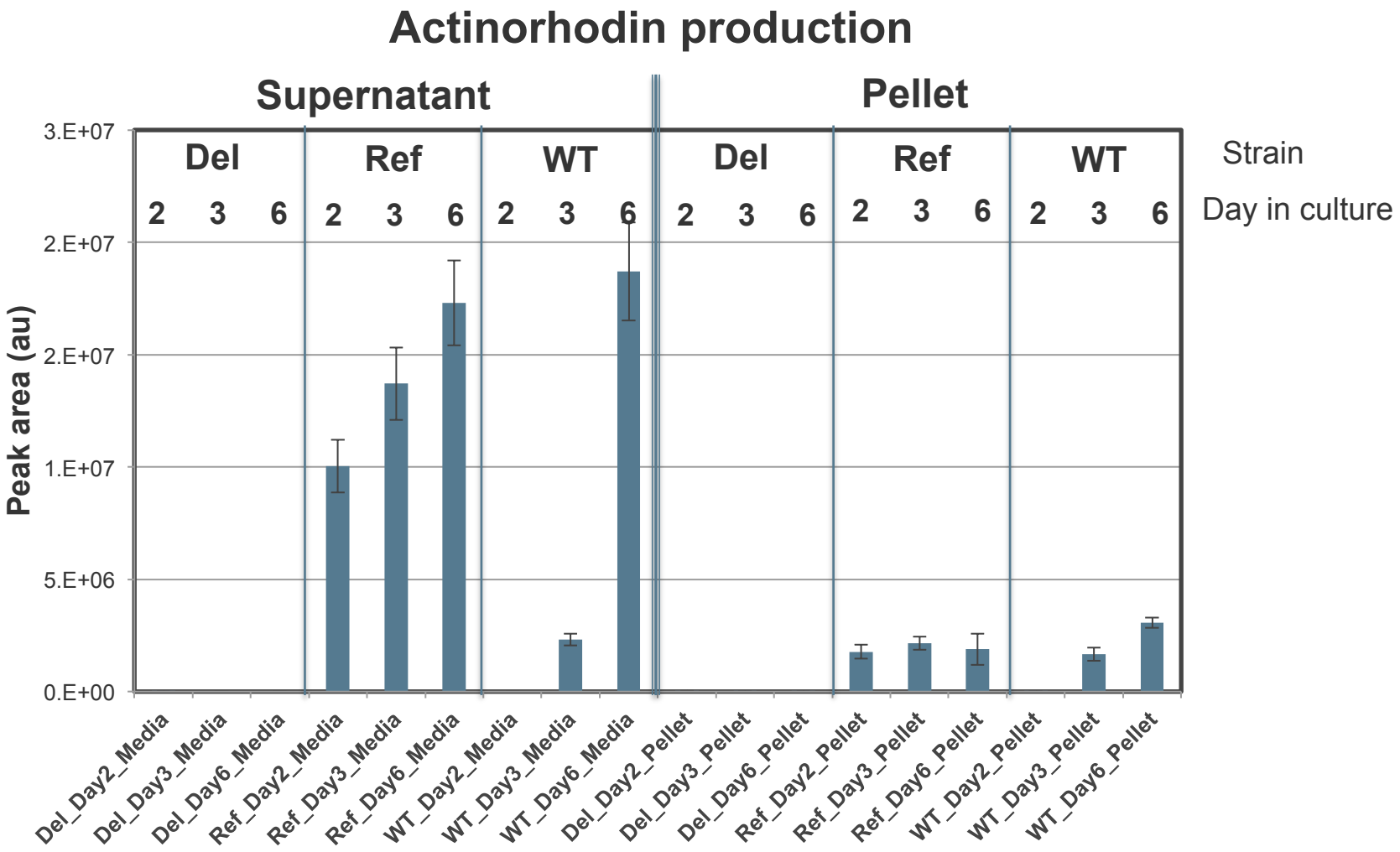
Redesigned to predictably achieve phenotypic outcome under laboratory conditions

Codon optimized genes: lower GC, remove repeats & secondary structure, preserve high translational potential

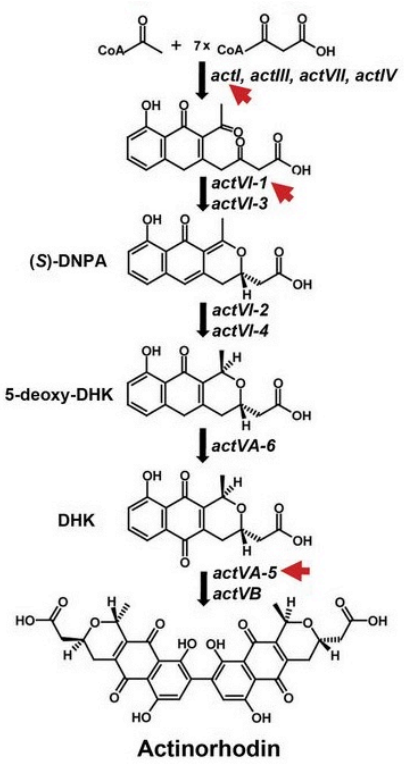
Previously validated constitutive promoters and terminators

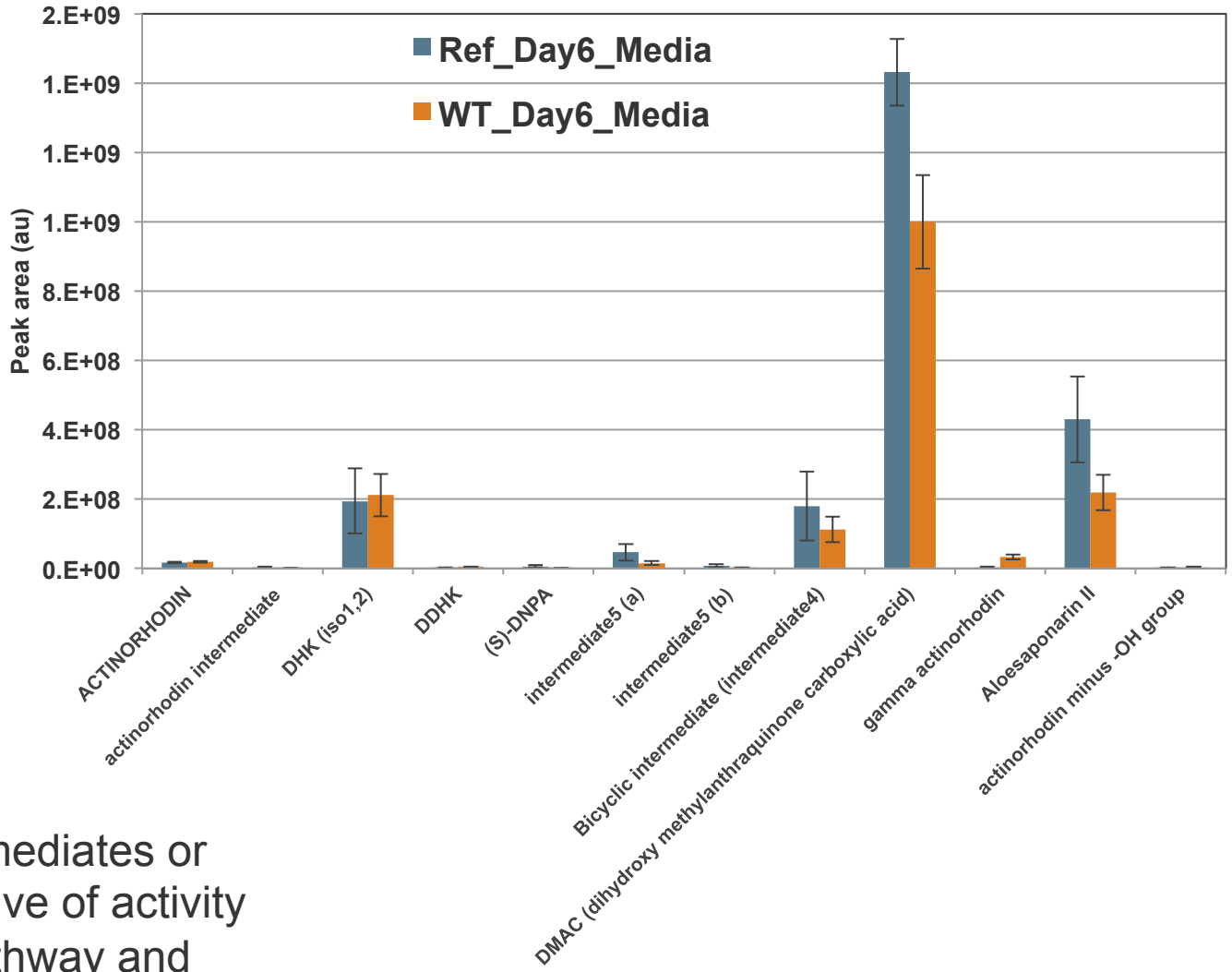
Calculated optimal RBSs specific for each rCDS

Pathway output is 100% of Wildtype; different timecourse



Accumulation of intermediates gives insight into pathway dynamics





Accumulation of intermediates or shunt products indicative of activity in different parts of pathway and between strains, etc

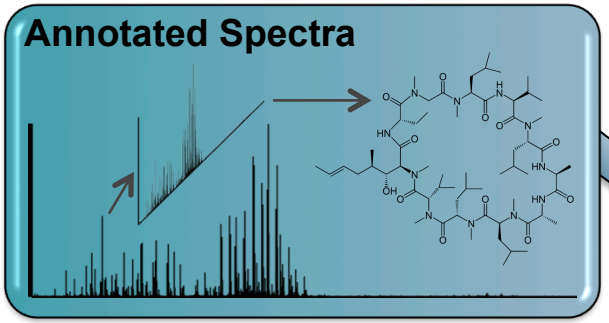
Metabolomics Bioinformatics to explore the biochemical space: MIDAS and Pactolus, chemical networks for integration with IMG

Ben Bowen

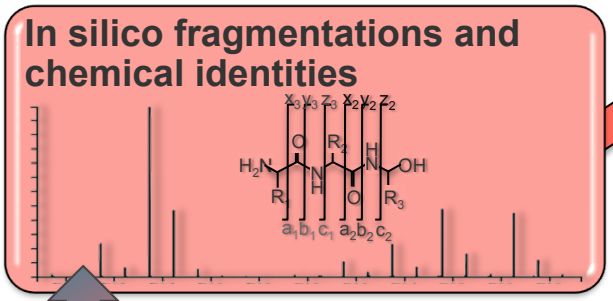
Linking genomics with metabolomics

- Compare and query *in silico* spectra with measured and theoretical spectra to algorithmically determine chemical identities.

Annotated Spectra

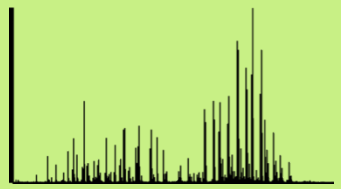


In silico fragmentations and chemical identities



Propagate chemical annotations within and across datasets

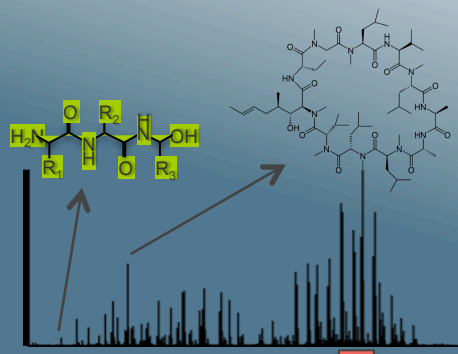
Raw spectra with unknown peaks

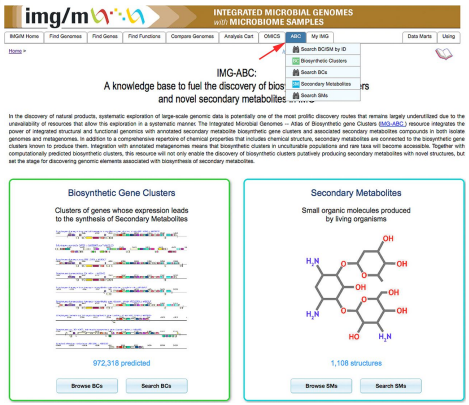


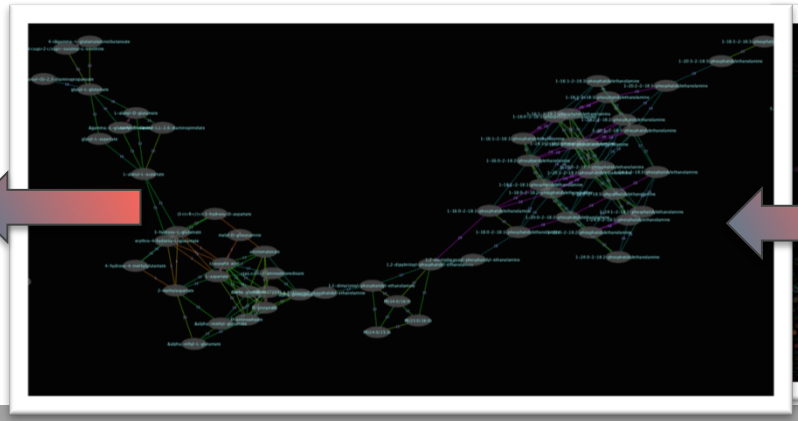
Integrate and match experimental and simulation spectra

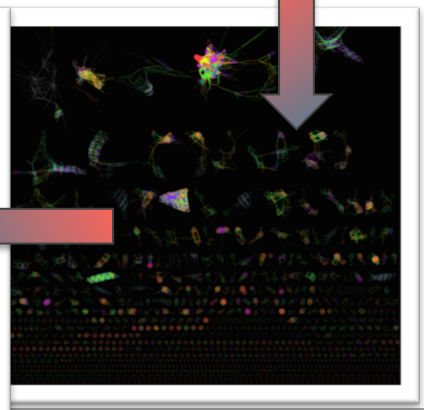
Raw spectra with:

- **Probabilistic chemical IDs**
- ***In silico* chemical IDs**

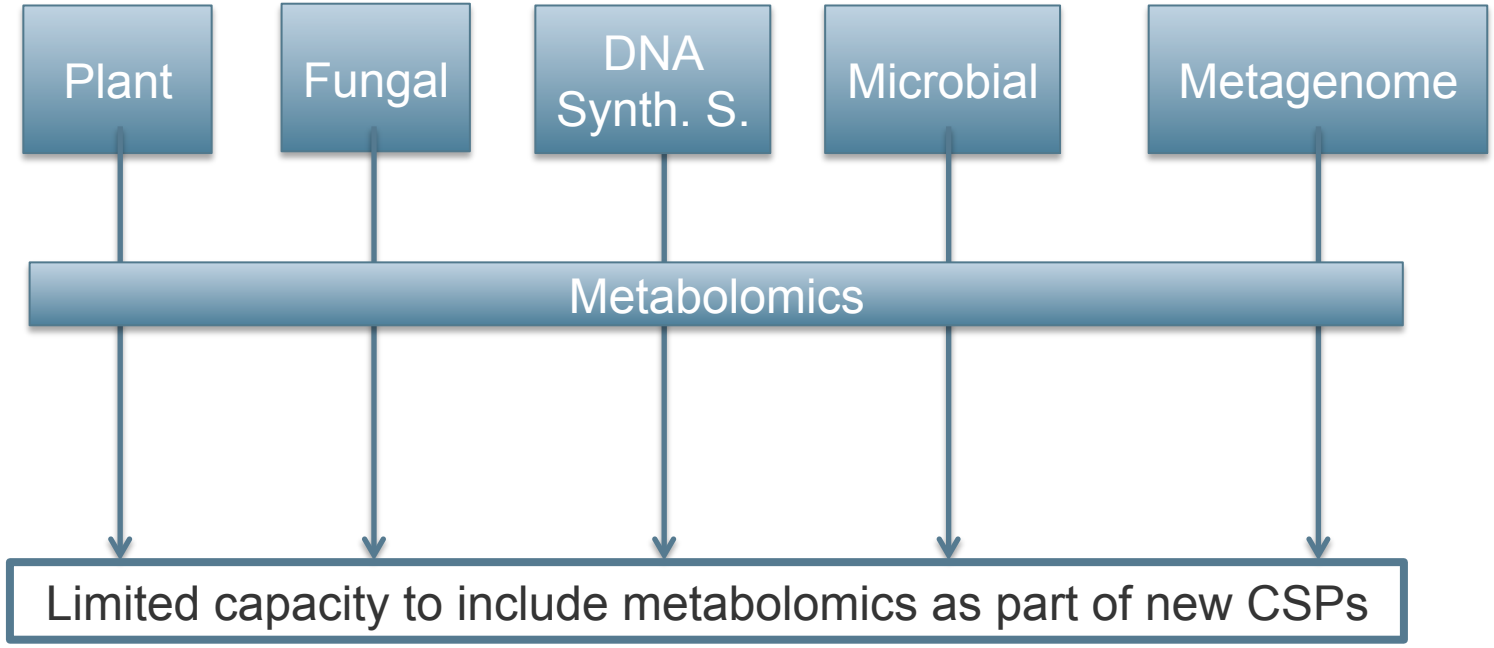








Consider including metabolomics as part of new CSPs



- ***Metabolomics should be in all cases tightly linked with sequencing and/or DNA synthesis***
- Typical metabolomics experiments are around 50-200 samples for polar metabolite analysis and 50-500 samples for secondary metabolite analysis.
- Larger requests will be considered on a case by case basis.

For questions about the appropriateness of projects, program specifics or application process, please contact Susannah Tringe: SGTringe@lbl.gov http://bit.ly/CSP-2017

For questions about metabolomics please contact Trent Northen: TRNorthen@lbl.gov