Metabolomics, Lipidomics, and Kinetic Flux Profiling; Developing Tools for Monitoring the Physiology of Ecologically Relevant Microbial Communities.

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University of Tennessee, Knoxville

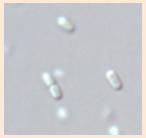
Departments of Chemistry and Microbiology
And the Biological and Small Molecule Mass Spectrometry Core

A Synergistic Platform for Chemical Biology

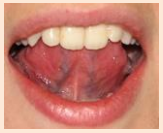
Using Chemical Tools to Probe Biological Diversity

Biological Systems

Bacterial Cell-Cell Signaling and Metabolism



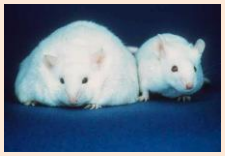
In the Lab



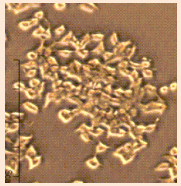
In Human Health

In the Environment

Mechanisms of Metabolic Disease



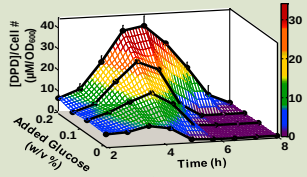
Obesity



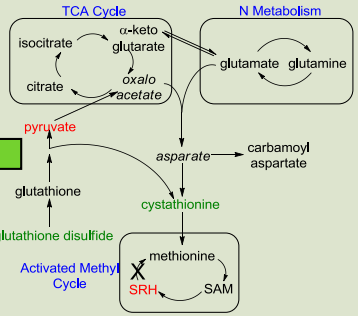
Diabetes

Bioanalytical Chemistry

Biological Insight



Measurement of Biological Signals

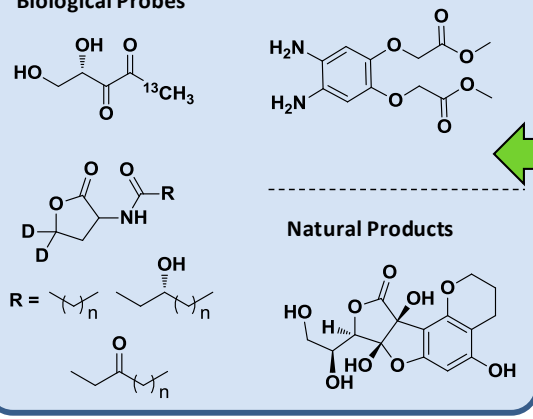


Analysis of Biochemical Pathways

Quantitation of Metabolism

Chemical Synthesis

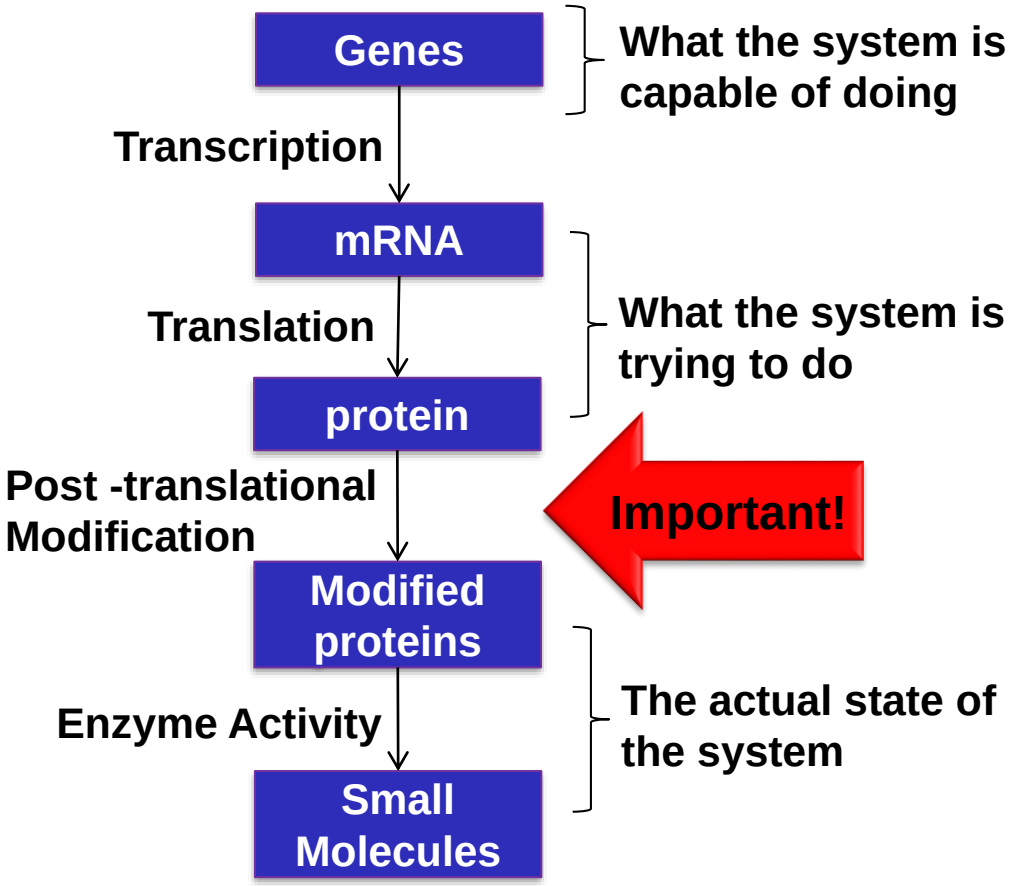
Biological Probes



Natural Products

Metabolomics

Why Metabolomics?



There are two broad classes of metabolomic experiments:

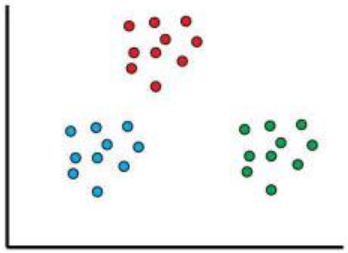
1) **Untargeted methods attempt to identify every component in the metabolome.**

2) **Targeted methods detect and quantitate a set of known compounds.**

The chemical diversity of the metabolites makes either type of analysis a challenging analytical problem.

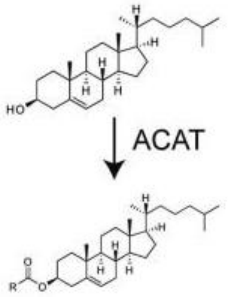
Rabinowitz, J.D., (2007), *Expert Review or Proteomics*, 187-198

Information Gained from Metabolomics



Systems Level

Metabolic Pathways



Individual Enzymes and Metabolites

Systems: Gross changes in metabolite clusters can be used to fingerprint conditions.

Pathways: Quantitative changes in a metabolite pathway can be used to understand the effects of perturbations.

Enzymes: Specific metabolite changes can be correlated to enzymes to study their function.

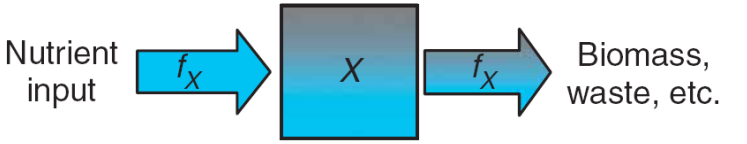
Vinayavekhin, N. *et al.* (2009), *ACS Chem. Bio.*, 91

Pool Sizes versus Flux

Metabolite Concentrations, or Pool Sizes, are not enough to fully understand metabolic function.

The rate at which metabolites flow through a pathway, or the Flux, of the systems is needed to gain a detailed view of metabolism.

Addition of Stable Isotopes to the media can be used to quantitate Flux.



With X^T at steady state,

$dX^U/dt = -f_X(X^U/X^T)$

The analytical solution is

$X^U/X^T = \exp(-f_X t/X^T)$

Setting $k_X = f_X/X^T$, we get

$X^U/X^T = \exp(-k_X t)$

Labeled form

Unlabeled form

Yuan, J. et al. (2008), *Nature Protocols*, 1328-1340

Metabolomics at UTK

Older Platform	Current Platform	Current Platform	Current Platform	Just Arrived!
HPLC-Triple Quadrupole	**UPLC-Orbitrap**	**UPLC-Orbitrap**	**GC or UPLC-Orbitrap**	**UPLC-Quadrupole-Orbitrap**
Capabilities: **~120 Known Water Soluble Compounds and Flux Analyses** **No Molecular Discovery** **2 h/sample**	**Capabilities:** **~150 Known Water Soluble Compounds and Flux Analyses** **~8,000 Spectral Features from Unknowns** **0.5 h/sample**	**Capabilities:** **~400 Known Lipid-Like Compounds and Flux Analyses** **~12,000 Spectral Features from Unknowns** **1.5 h/sample**	**Capabilities:** **Technology Development** **~10,000 Spectral Features from Unknowns** **Used for Analysis of the Extracellular Matrix or for Discovery of Compound of Intermediate Molecular Weight**	**Capabilities:** **Molecular Characterization, Structural Elucidation, Proteomics, and Glycomics**

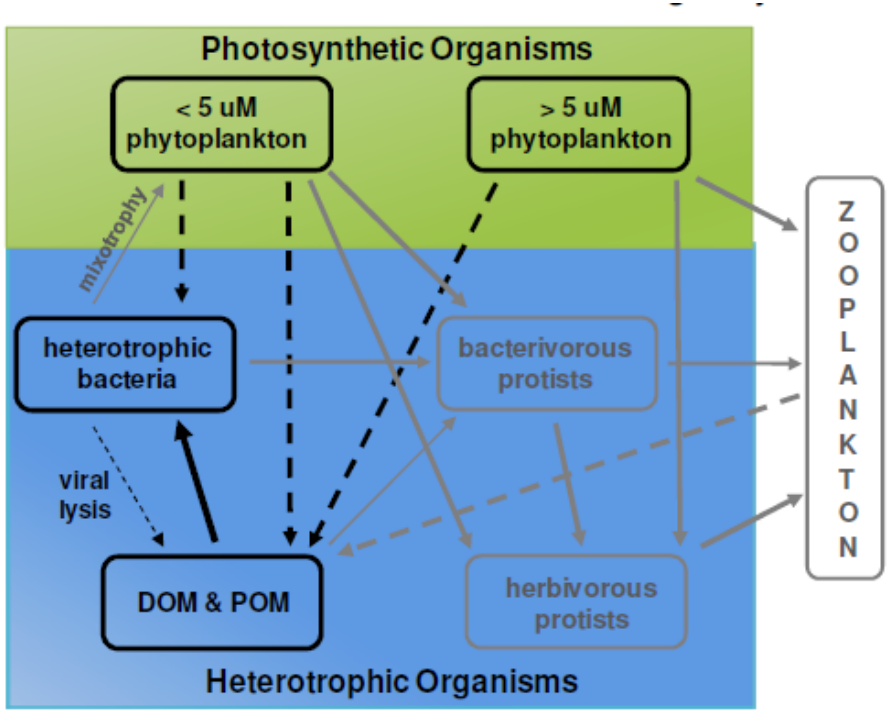
The Impact of Viral Lysis on Marine Microbial Populations

Viral lysis of bacteria causes release of dissolved organic matter.

Virus-derived organic matter is important in the recycling of carbon and nutrients.

Relatively little is known of the biochemical composition of viral lysates

Virus activity alters the metabolism of marine plankton, and this may influence the composition of organic matter released into the environment.



Wilhelm and Suttle. (1999), *BioScience*, 1999

Ankrah, N.Y., May, A.L., Middleton, J.L., et. al.
The ISME Journal 2014, 8(5), 1089-1100

Alterations in Host Metabolism were Expected

At the onset, we expected that viral infection would lead specific alterations in host metabolism that would benefit the phage based on work done in model systems.

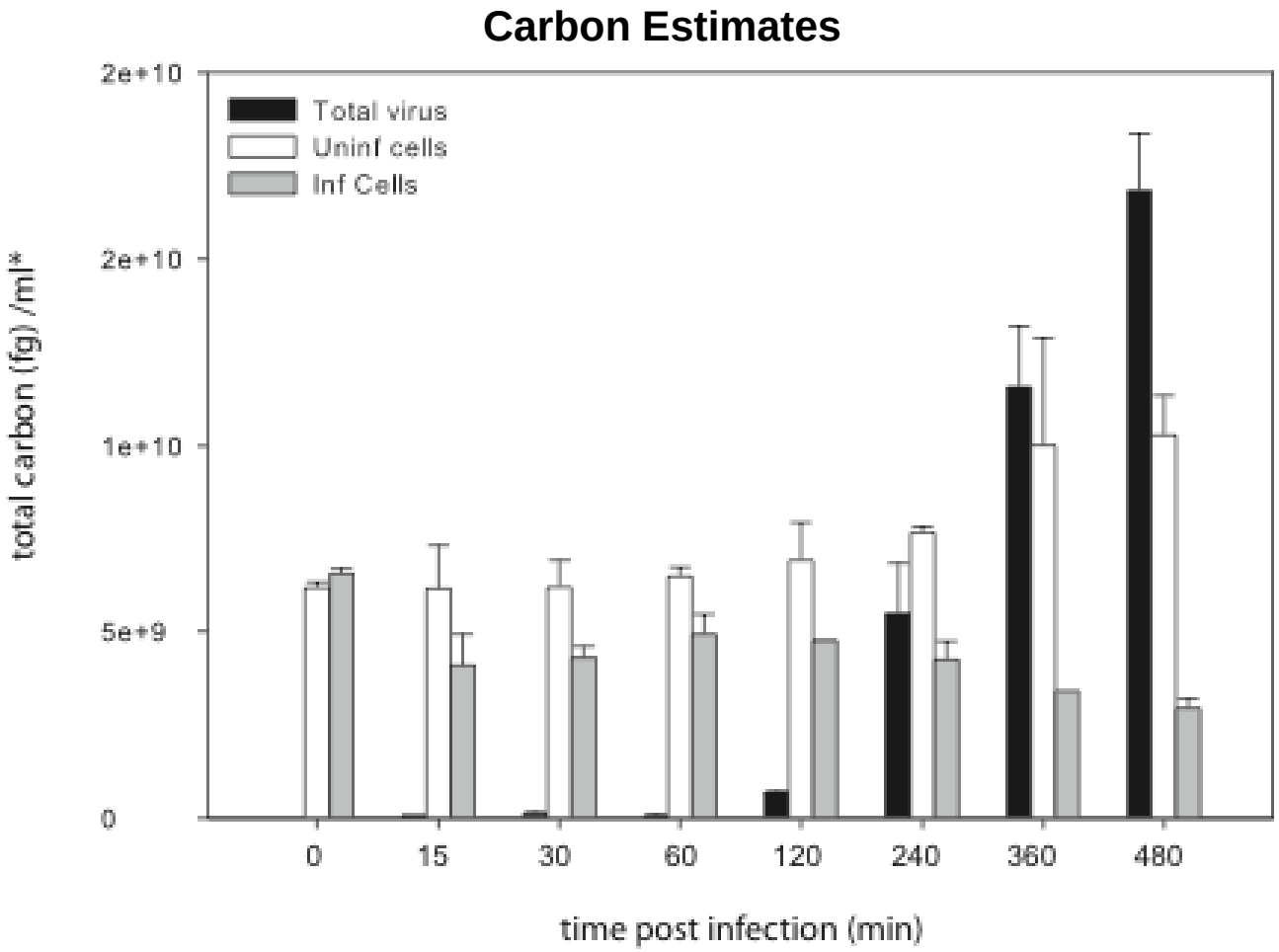
Both lysogenic and lytic coliphages initially promote similar alterations in host that halt host cell DNA synthesis, degrade host DNA, and assemble the machinery for viral production.

A number of viruses contain auxiliary metabolic genes (AMGs) to overcome rate limiting steps in host biosynthesis. (Breitbart, M. (2012), *Annual Review of Marine Science, 425)*

Host manipulation has been demonstrated in marine cyanophage which encode and express photosynthesis proteins homologous to those found in their hosts (Thompson LR, et al., (2011) *Proceedings of the National Academy of Sciences,* E757.)

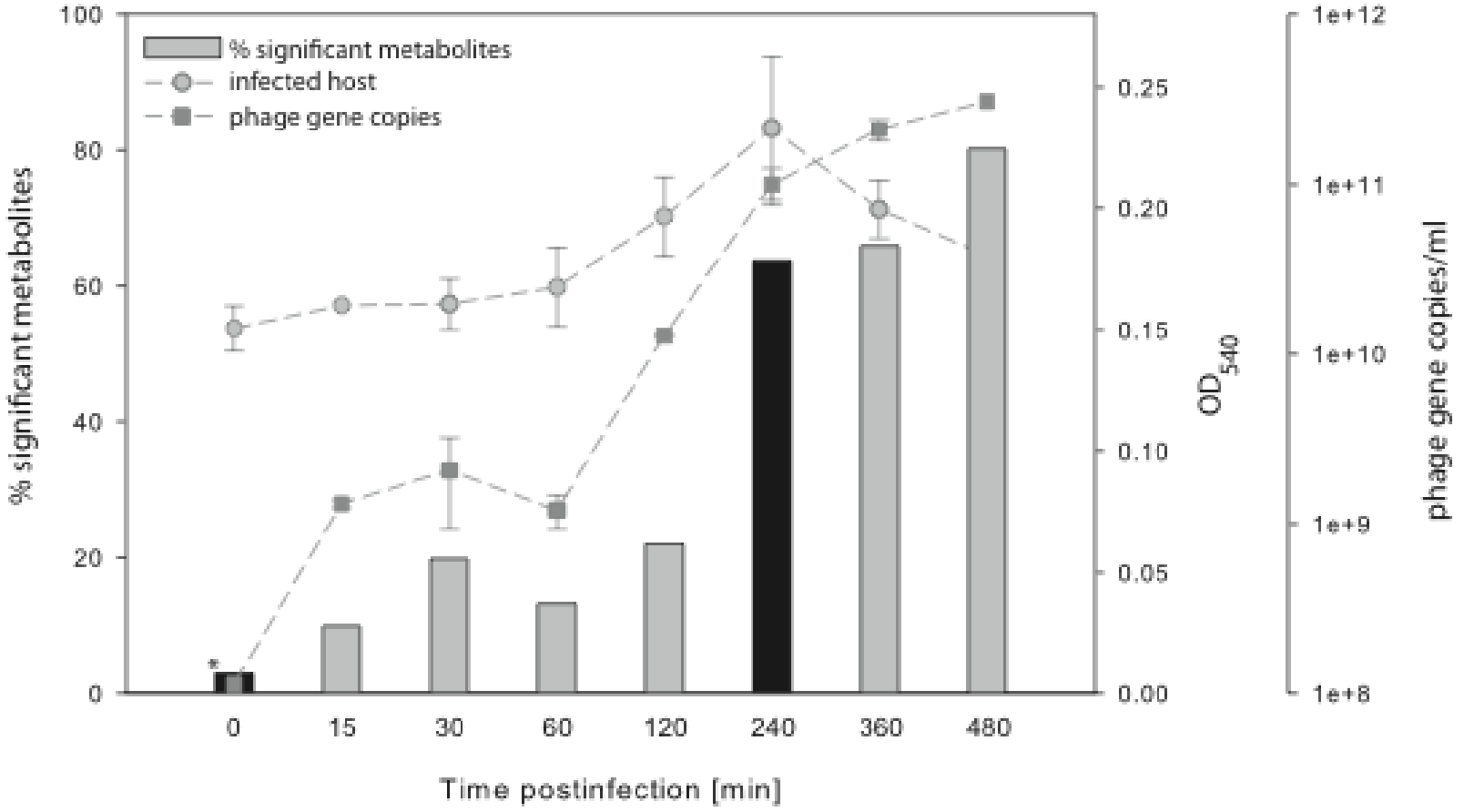
A few marine phage-host systems appear to sequester sufficient resources for nucleic acid synthesis entirely from degradation of host DNA. (Wikner et al., (2006), FEMS Microbiology Ecology, 1574)

Infection Alters the Distribution of Biomass for Roseobacter 2047 and Lytic Roseophage 2047B



But does it dramatically alter the concentration and composition of the small molecule metabolites released as DOM, and what are the effects on cellular physiology?

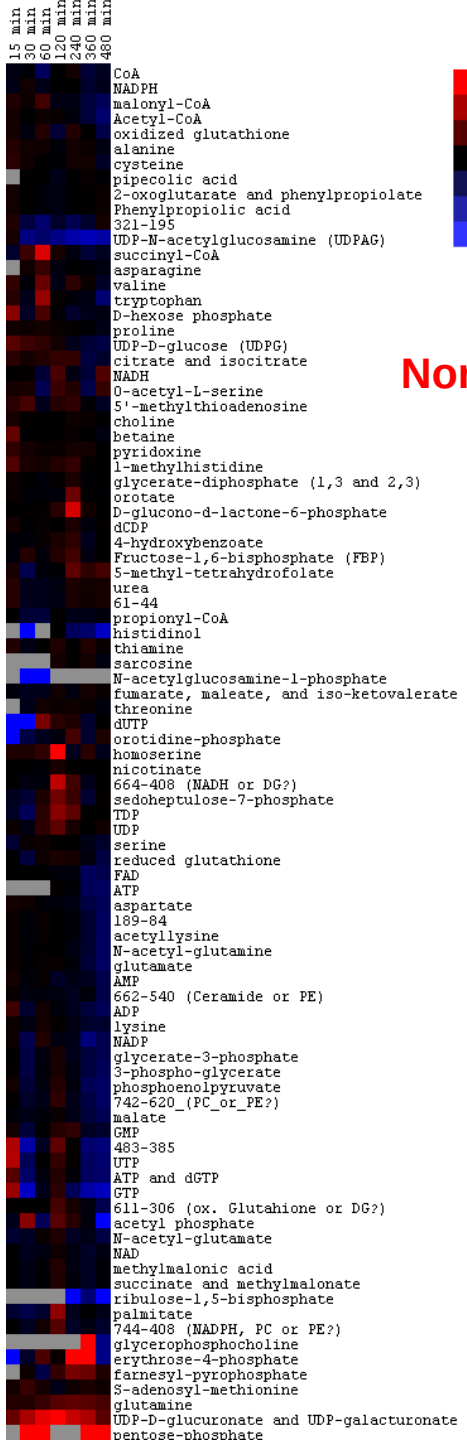
Metabolic Alterations Occur During the Lytic Phase of Infection



*t=0 phage infected was taken immediately following phage infection (~3 min until metabolism quenched)

Black bars represent ^{13}C-Acetate Addition for Flux Experiments
Circles represent 2047 Growth (OD_{540})
Squares represent Phage 47A+B (phage gene copies/mL)

Comparison of Intracellular Metabolites

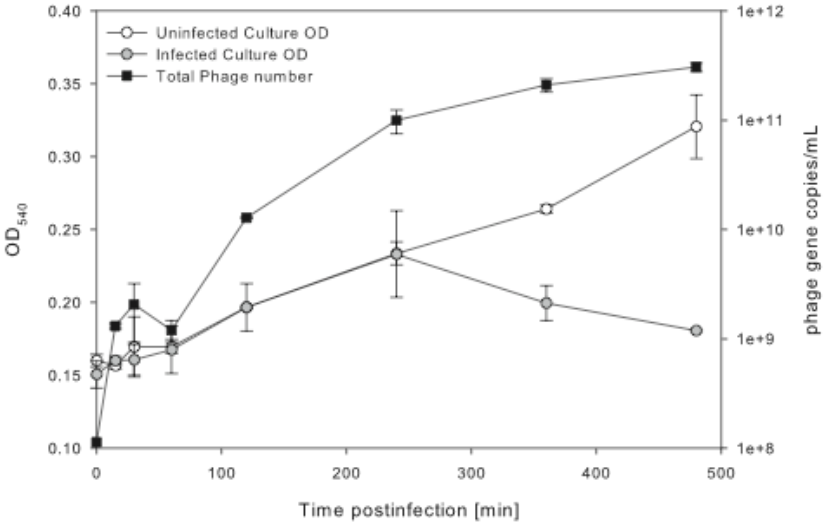


No Normalization

Normalized by Cell Count

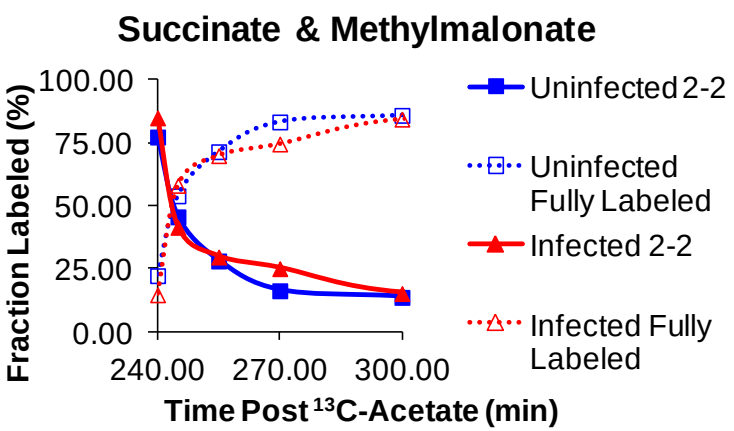
Viral infection led to an increase in intracellular metabolite concentrations.

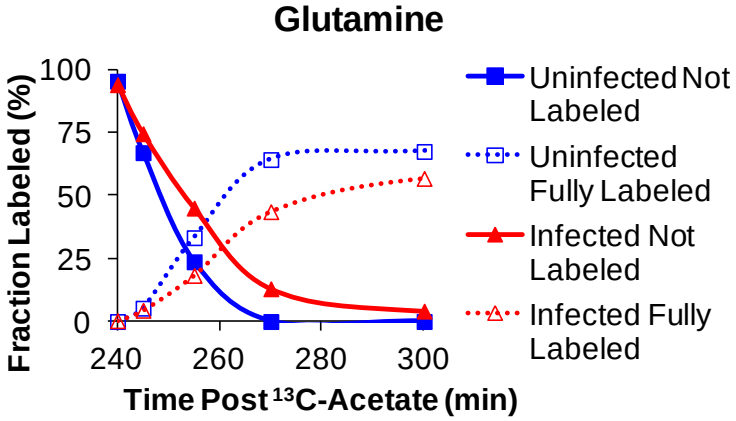
Change in Phage Number and ODs Postinfection

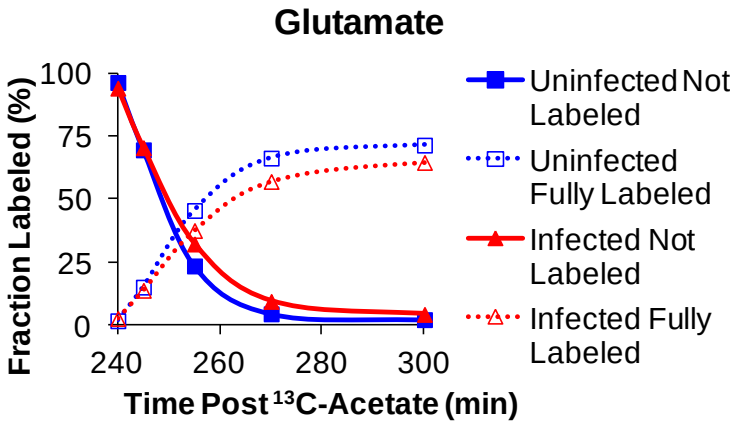


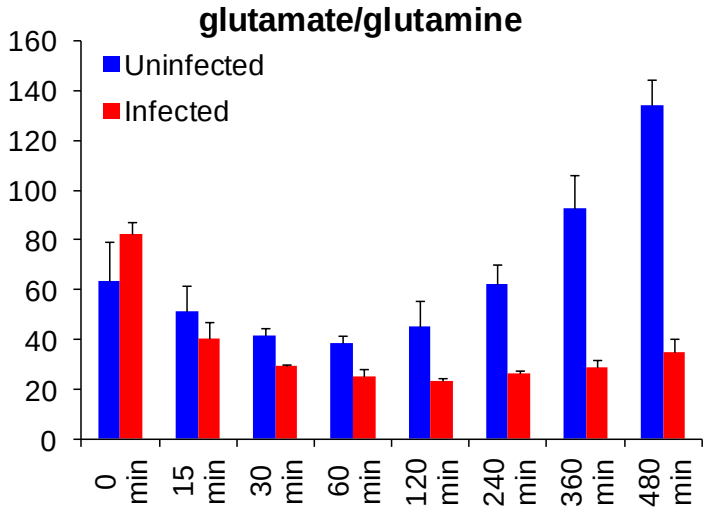
Carbon Flux and Conclusions

^{13}C-Flux Profiling revealed that the infected cells are very metabolically active during the lytic portion of the infection.









Released metabolites also seem to provide a plentiful supply of N to cells that are not lysed during infection.

Overview of Quorum Sensing

How do bacteria communicate?

Through small molecules

Why do bacteria communicate?

To protect themselves

To create communities

Many other reasons

What could be gained from understanding Bacterial Communication?

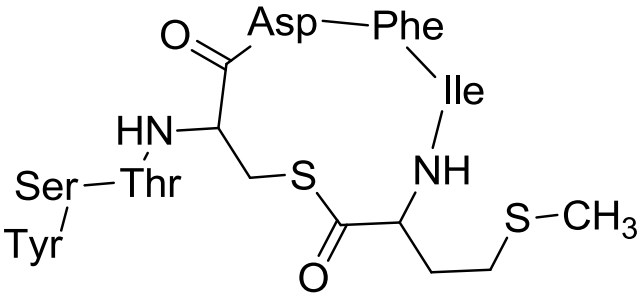
Methods for Biofilm Control

Discovery of Novel Therapeutics

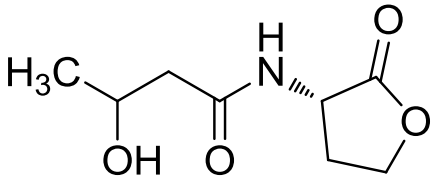
Bassler, B.L.; Camilli, A., (2006), *Science*, 113-116

Classes of Infochemical Molecules

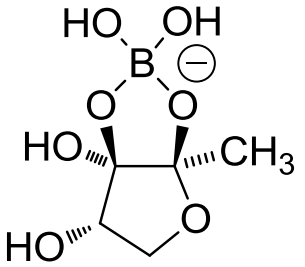
A variety of bacterial pheromones have been identified, and they differ in both structure and function.



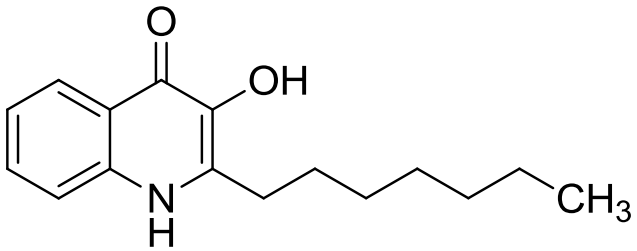
S. aureus AIP-I
derived from AgrD



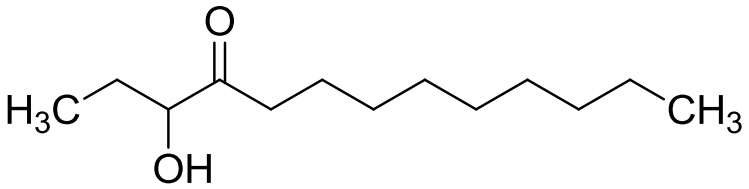
V. harveyi AI-1
3-hydroxy-C4-HSL



V. harveyi AI-2

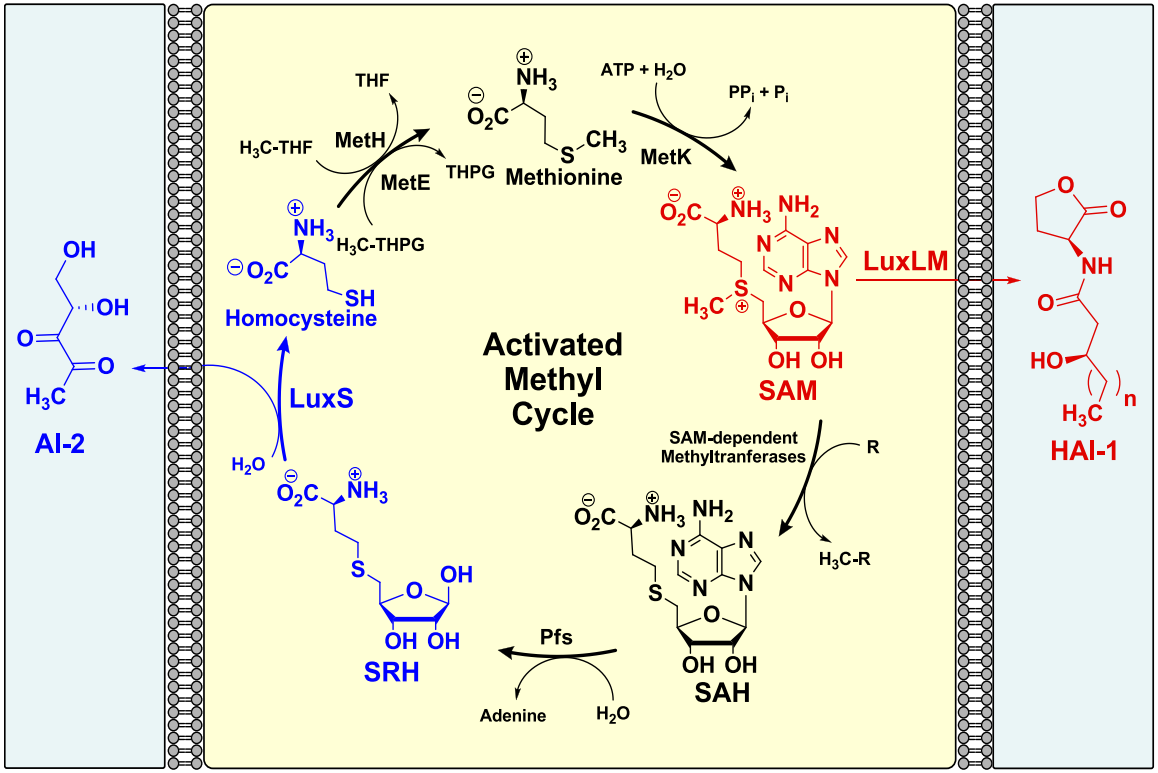


P. aeruginosa PQS



V. cholerae CAI-1

Pheromones are Linked to Metabolism



Quorum sensing links metabolism to extracellular signaling.

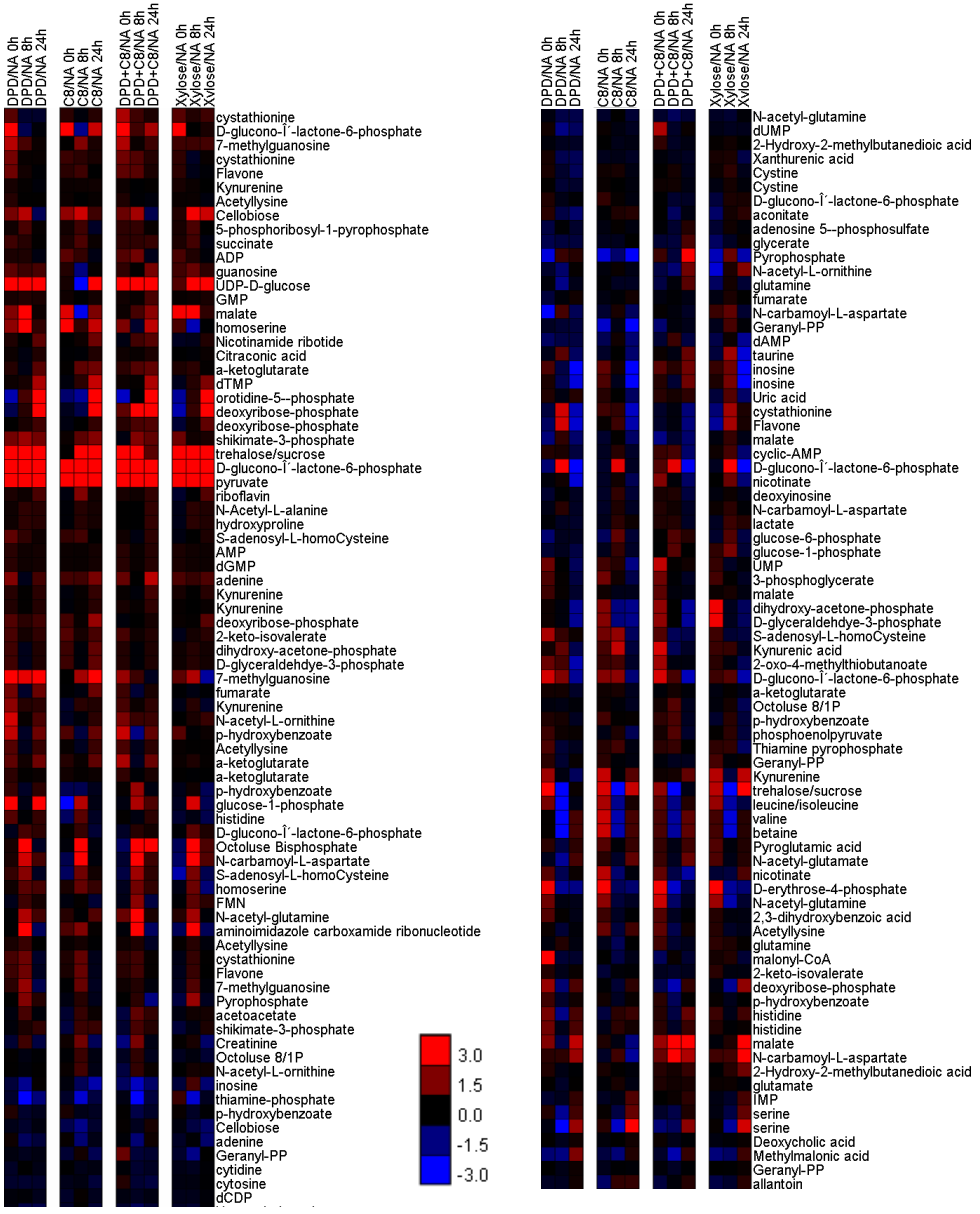
Autoinducer-2 is made during a critical methionine salvage step in the activated methyl cycle, and species that produce Autoinducer-1 have a further link to metabolism.

Pheromones and Metabolomics of Marine Snow in the Sargasso Sea

Experimental Outline	
Addition	**DPD, C8, DPD+C8, Xylose, NA**
Concentration	500 nM
Time points	0, 8, 24 h
Replicates	3

155 Features Match Known Metabolites

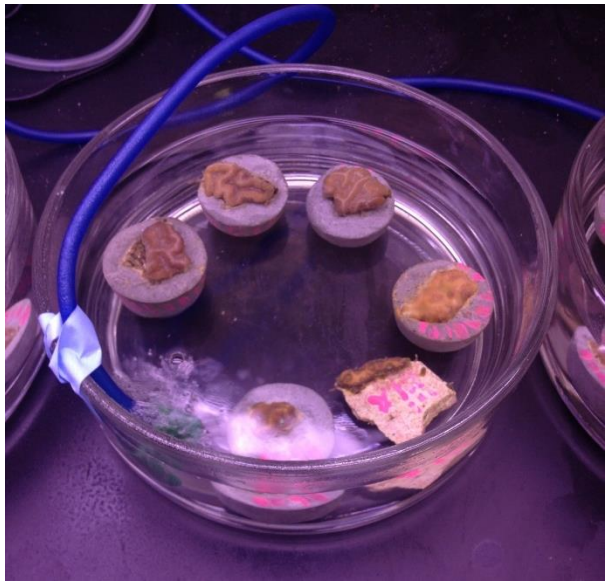
19,834 Total Spectral Features (many adducts from high salt concentrations, etc.)



Pheromones and Metabolism in Coral Black Band Disease (BBD)

Black Band Disease

***Diploria* Coral Fragments**



In collaboration with the Richardson Lab at FIU, a three part investigation to understand the 1) AHL production from disease associated bacteria, 2) metabolic fluxes in BBD, and 3) the impact of infochemicals on coral metabolism was performed.

Isolate Source		
BBD infected coral (BBD) (n=20)	SML of healthy coral (HSML) (n=2)	SML from healthy part of BBD infected coral (BSML) (n=1)
3OHC4	3OHC4	3OHC4
3OHC6	3OHC6	3OHC6
C5	3OC12	
3OHC5		
3OC5:1		
C6		
3OHC8		

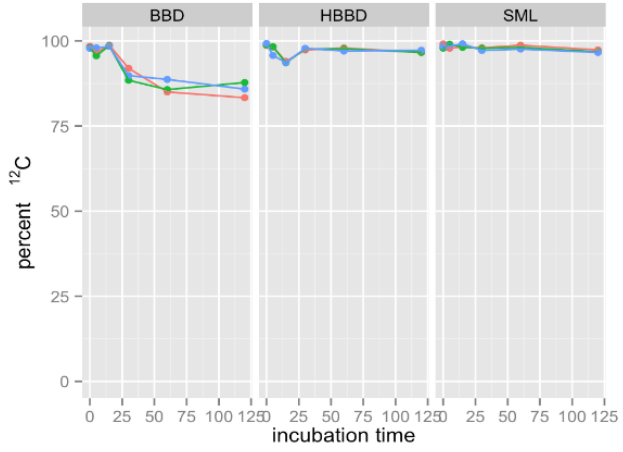
(155 bacterial isolates from BBD,
38 were from apparently healthy coral (HSML),
and 36 from the apparently healthy part of BBD infected coral (BSML) were tested for AHL production.)

Metabolic Fluxes in Coral Mucous

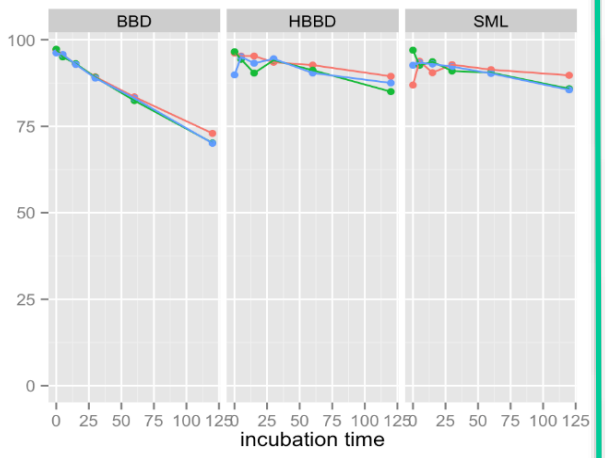
C flux measured after addition of U^{13}C-acetate or glucose (5mM labeled carbons)

N flux measured after addition of $^{13}NH_4CL$ (15mM)

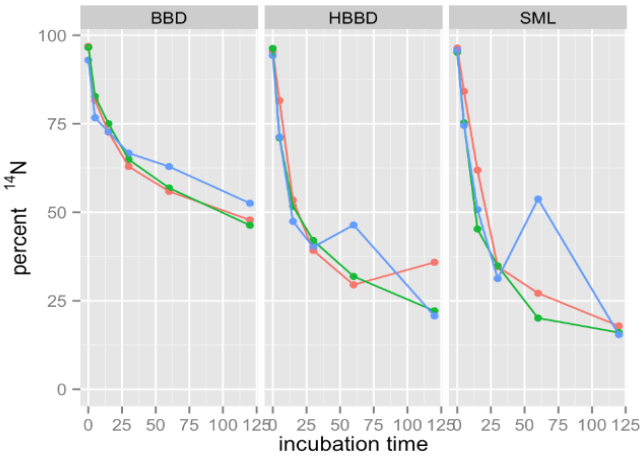
Aspartate



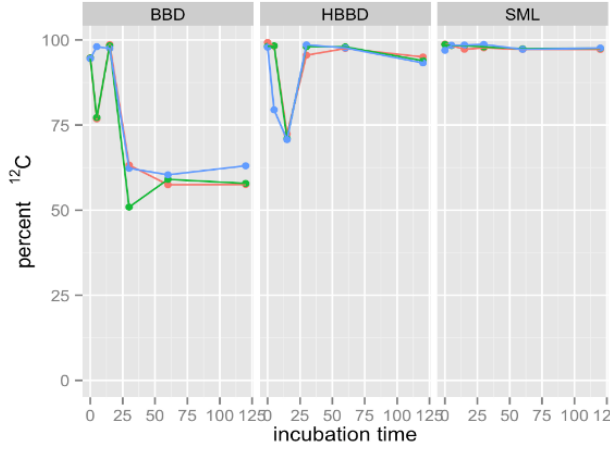
Glutamate



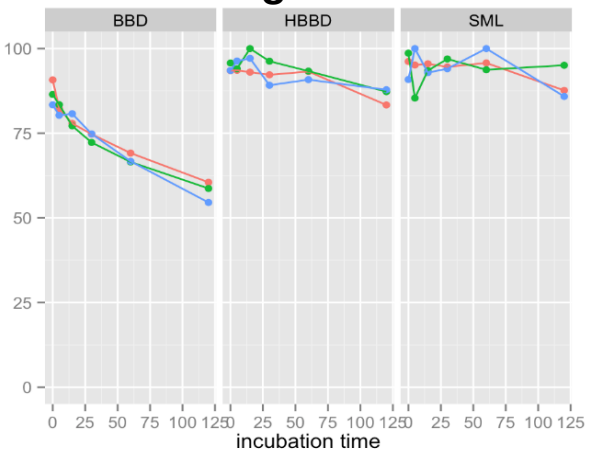
Glutamate



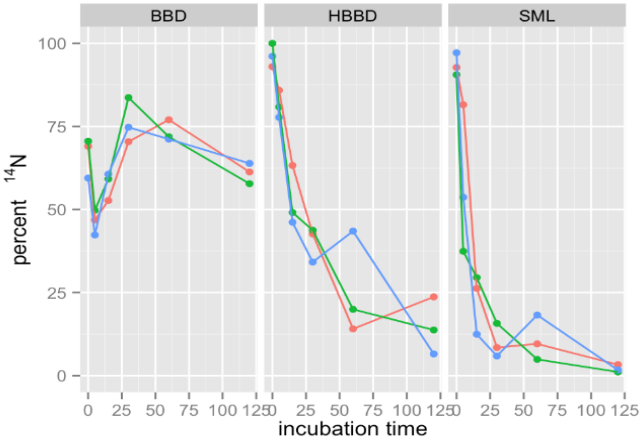
Succinate



UDP-D-glucuronate



Glutamine

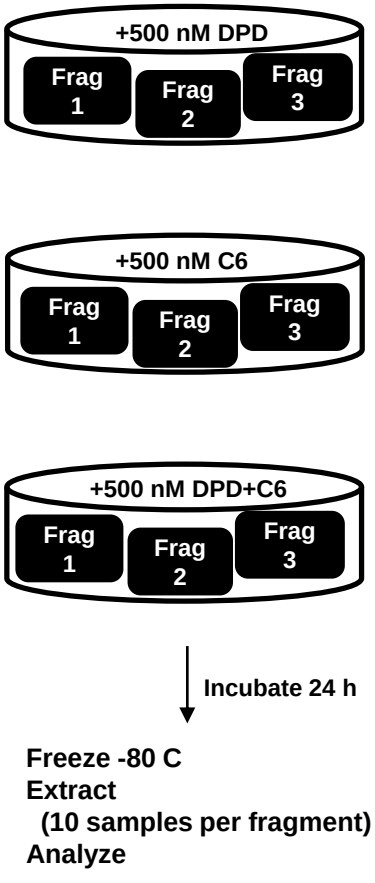


Metabolic fluxes of C and N differ for microorganisms in infected coral mucous, and N recycling from the host coral may promote growth

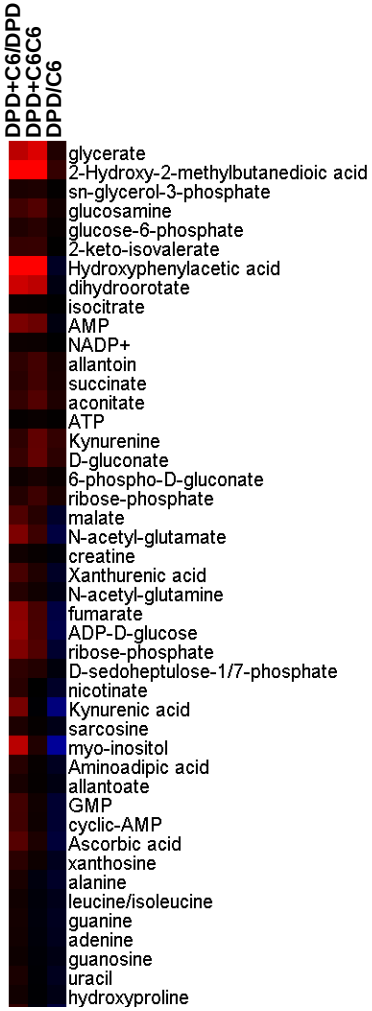
Pheromones Alter Metabolism in Healthy Corals

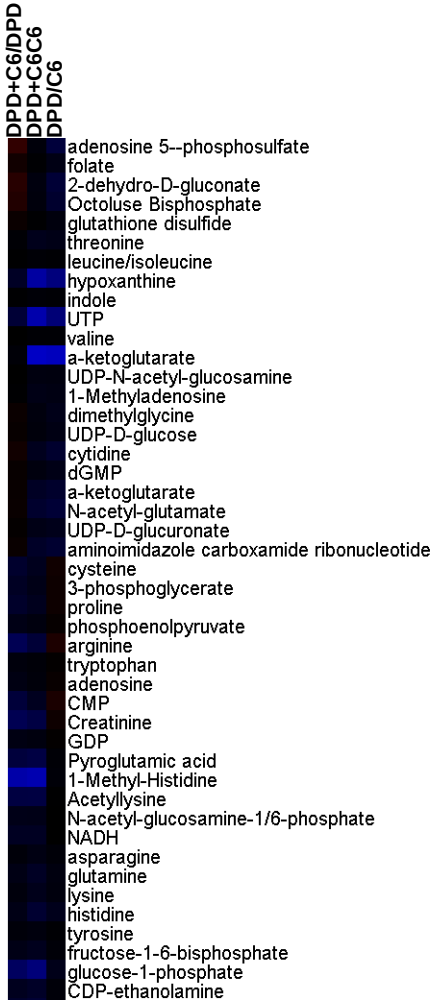
Coral Fragment Metabolomic Data

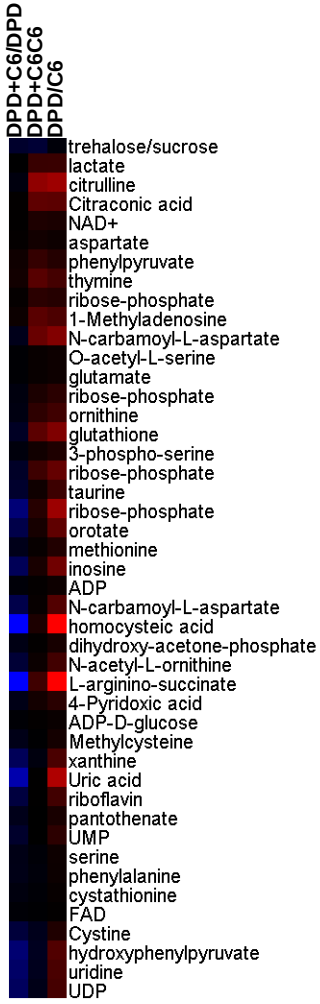
Experimental Setup:

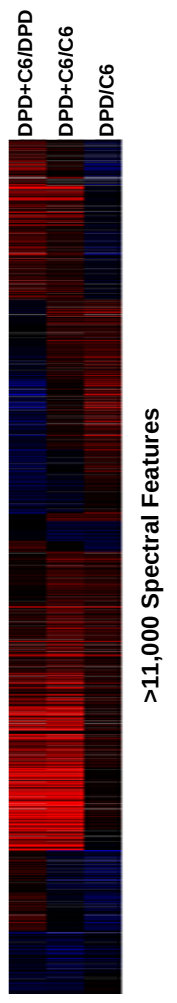


Freeze -80 C
Extract
(10 samples per fragment)
Analyze









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