Environmental Proteomics: Potential, Challenges, and Accomplishments

Kenneth F. Reardon
Chemical and Biological Engineering
Cell and Molecular Biology Graduate Program
Colorado State University
Kenneth.Reardon@ColoState.Edu

What is “Environmental Proteomics”?

What is "Environmental Proteomics"?

- Proteomic analysis of microorganisms of interest to environmental scientists and engineers
  - Individual microorganisms
  - Microbial communities

Proteomics related to environmental science and engineering

<u>Research foci</u>

- Microbial physiology
- Microbial metabolism
- Effects of environmental factors (T, pH, chemicals)
- Interactions among species (ecology)

<u>Applications</u>

- Wastewater treatment
- Bioremediation
- Mechanisms of pollutant toxicity
- Biomarkers of pollution
- Climate change
- Understanding extreme environments

What is "Environmental Proteomics"?

- Proteomic analysis of microorganisms of interest to environmental scientists and engineers
  - Individual microorganisms
  - Microbial communities
- Why limit to microorganisms?
  - Plants
  - Plant-microbe interactions
  - Animals?

What is "Environmental Proteomics"?

- Proteomic analysis of microorganisms of interest to environmental scientists and engineers
- Proteomic analysis of microorganisms in their environment

NIH Roadmap FOR MEDICAL RESEARCH

Human Microbiome Project

OVERVIEW

Within the body of a healthy adult, microbial cells are estimated to outnumber human cells by a factor of ten to one. These communities, however, remain largely unstudied, leaving almost entirely unknown their influence upon human development, physiology, immunity, and nutrition. To take advantage of recent technological advances and to develop new ones, the NIH Roadmap has initiated the Human Microbiome Project (HMP) with the mission of generating resources enabling comprehensive characterization of the human microbiota and analysis of its role in human health and disease.

MINIREVIEW

Effects of polymicrobial communities on host immunity and response

Xiaoying Lu[1], Zoya Kurago[2] & Kim A. Brogden[1]

[1]Department of Periodontics, Dows Institute for Dental Research, College of Dentistry, University of Iowa, Iowa City, IA, USA; and [2]Department of Oral Pathology, Dows Institute for Dental Research, College of Dentistry, University of Iowa, Iowa City, IA, USA

Vol 444|21/28 December 2006|doi:10.1038/nature05414

nature

ARTICLES

An obesity-associated gut microbiome with increased capacity for energy harvest

Peter J. Turnbaugh[1], Ruth E. Ley[1], Michael A. Mahowald[1], Vincent Magrini[2], Elaine R. Mardis[1,2] & Jeffrey I. Gordon[1]

The worldwide obesity epidemic is stimulating efforts to identify host and environmental factors that affect energy balance. Comparisons of the distal gut microbiota of genetically obese mice and their lean littermates, as well as those of obese and lean human volunteers have revealed that obesity is associated with changes in the relative abundance of the two dominant bacterial divisions, the Bacteroidetes and the Firmicutes. Here we demonstrate through metagenomic and biochemical analyses that these changes affect the metabolic potential of the mouse gut microbiota. Our results indicate that the obese microbiome has an increased capacity to harvest energy from the diet. Furthermore, this trait is transmissible: colonization of germ-free mice with an 'obese microbiota' results in a significantly greater increase in total body fat than colonization with a 'lean microbiota'. These results identify the gut microbiota as an additional contributing factor to the pathophysiology of obesity.

18 DECEMBER 2009 VOL 326 SCIENCE

Bacterial Community Variation in Human Body Habitats Across Space and Time

Elizabeth K. Costello,[1] Christian L. Lauber,[2] Micah Hamady,[3] Noah Fierer,[2,4] Jeffrey I. Gordon,[5] Rob Knight[1,6]*

Elucidating the biogeography of bacterial communities on the human body is critical for establishing healthy baselines from which to detect differences associated with diseases. To obtain an integrated view of the spatial and temporal distribution of the human microbiota, we surveyed bacteria from up to 27 sites in seven to nine healthy adults on four occasions. We found that community composition was determined primarily by body habitat. Within habitats, interpersonal variability was high, whereas individuals exhibited minimal temporal variability. Several skin locations harbored more diverse communities than the gut and mouth, and skin locations differed in their community assembly patterns. These results indicate that our microbiota, although personalized, varies systematically across body habitats and time; such trends may ultimately reveal how microbiome changes cause or prevent disease.

Proteomics related to biomedical "environment"

Research foci

- Physiology
- Metabolism
- Effects of environmental factors (T, pH, chemicals)
- Interactions among species (ecology)

Applications

- Disease (polymicrobial)
- Health related to metabolism
- Microbiome-host interactions
- Drug metabolism (efficacy)

Proteomics related to biomedical "environment"

Research foci

- Physiology
- Metabolism
- Effects of environmental factors (T, pH, chemicals)
- Interactions among species (ecology)

Applications

- Disease (polymicrobial)
- Health related to metabolism
- Microbiome-host interactions
- Drug metabolism (efficacy)

The same list as before!

Differences between “normal” proteomics and environmental proteomics

Differences between “normal” proteomics and environmental proteomics

Sanity??

Differences between "normal" proteomics and environmental proteomics

Research foci

- Physiology
- Metabolism
- Effects of environmental factors (T, pH, chemicals)
- Interactions among species (ecology)

\+ The physical and chemical nature of the organism's environment

Differences between "normal" proteomics and environmental proteomics

Research foci

- Physiology
- Metabolism
- Effects of environmental factors (T, pH, chemicals)
- Interactions among species (ecology)

\+ The physical and chemical nature of the organism's environment

Proteomic analysis of organisms in complex biological, chemical, and physical environments

What do we require of the tools to study these systems?

- Cultivation-independent
- Capable of system-wide analysis
  - (we don't know what we don't know)
- Capable of providing information on function

$\Rightarrow$ -omics methods

Genomics, transcriptomics, and proteomics

Pacific Science Center
http://www.exhibits.pacsci.org/insects/buttermoth.html

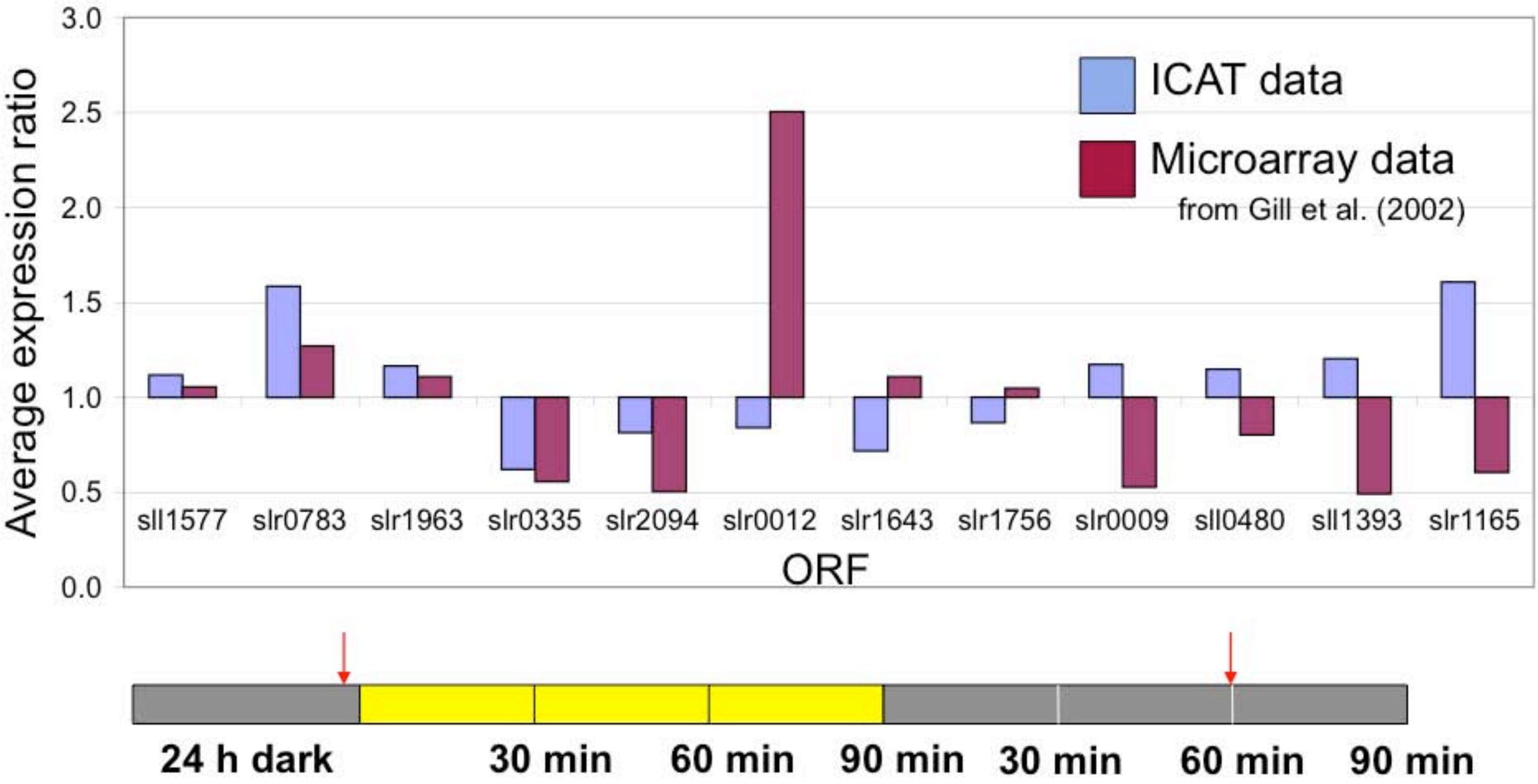
Transcriptomics

- ~~Requires sequenced genome~~
- ~~Requires printed chip~~
- *Can assay all genes or a selected subset*
- Knowledge of gene expression NOT always correlated to activity (e.g., PTMs)

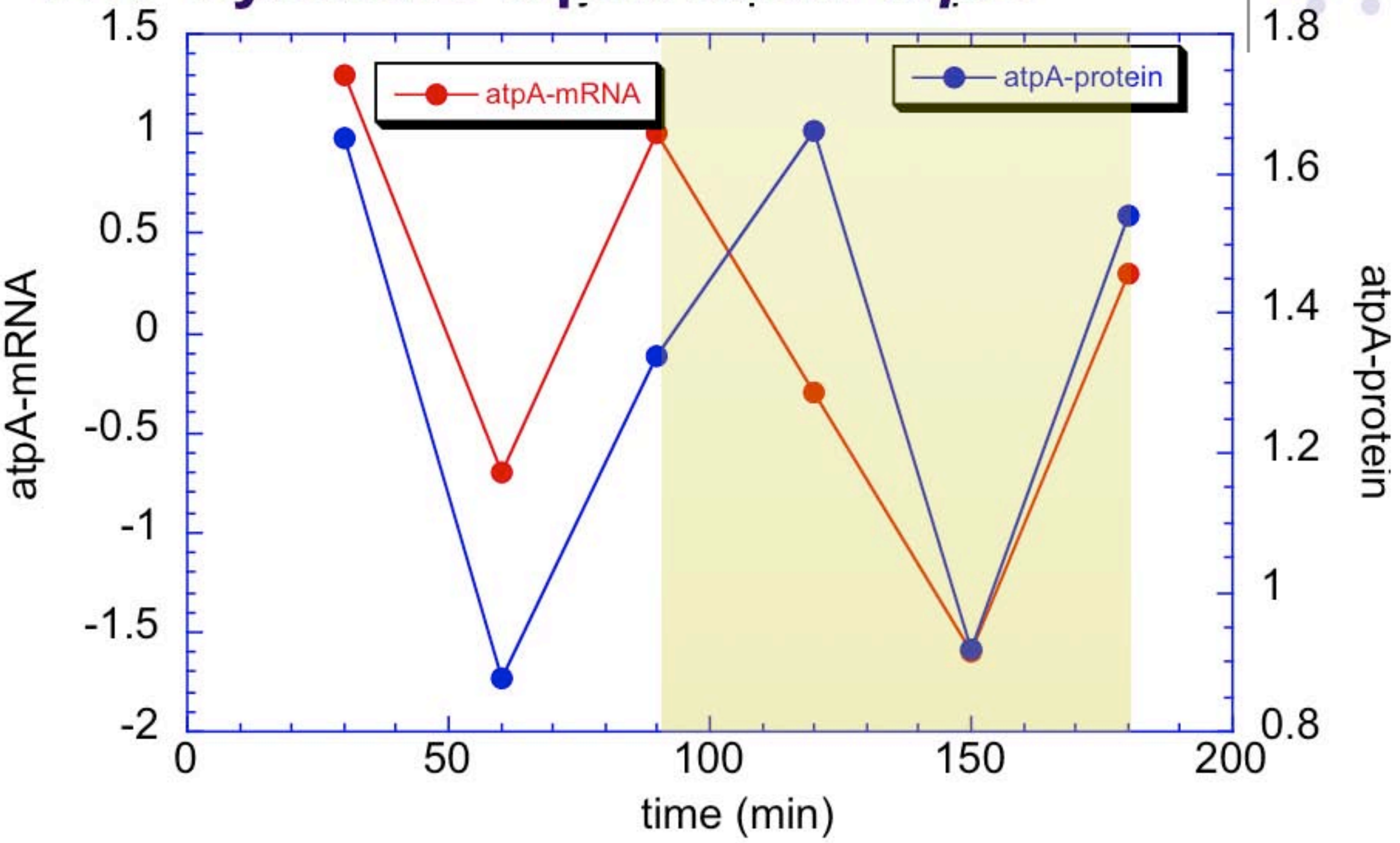
Proteomics

- Sequenced genome helpful but not required
- Discovery tool
- 500-2000 proteins
- Problems with low abundance proteins
- *Can study PTMs*
- *Detect regulation at higher level*
- *Actual, not potential, expression*

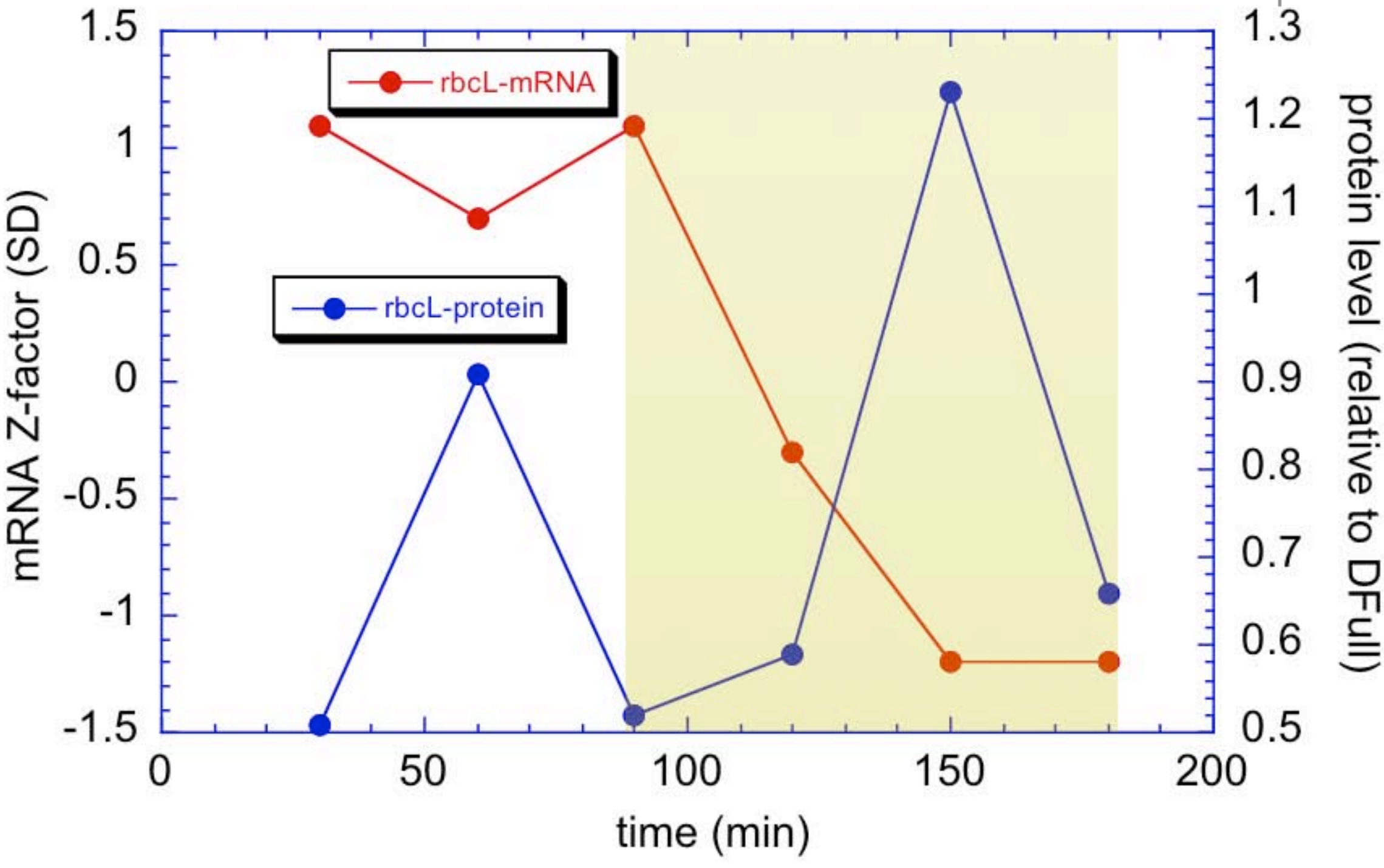
Protein (ICAT) vs. mRNA (microarray): D60/DFull



ATP synthase alpha chain *atpA*



ribulose bisphosphate carboxylase large subunit *rbcL*



Proteomic analysis of microbial communities

Proteomic assessment of interactions in a binary bacterial culture

Carla Lacerda, PhD

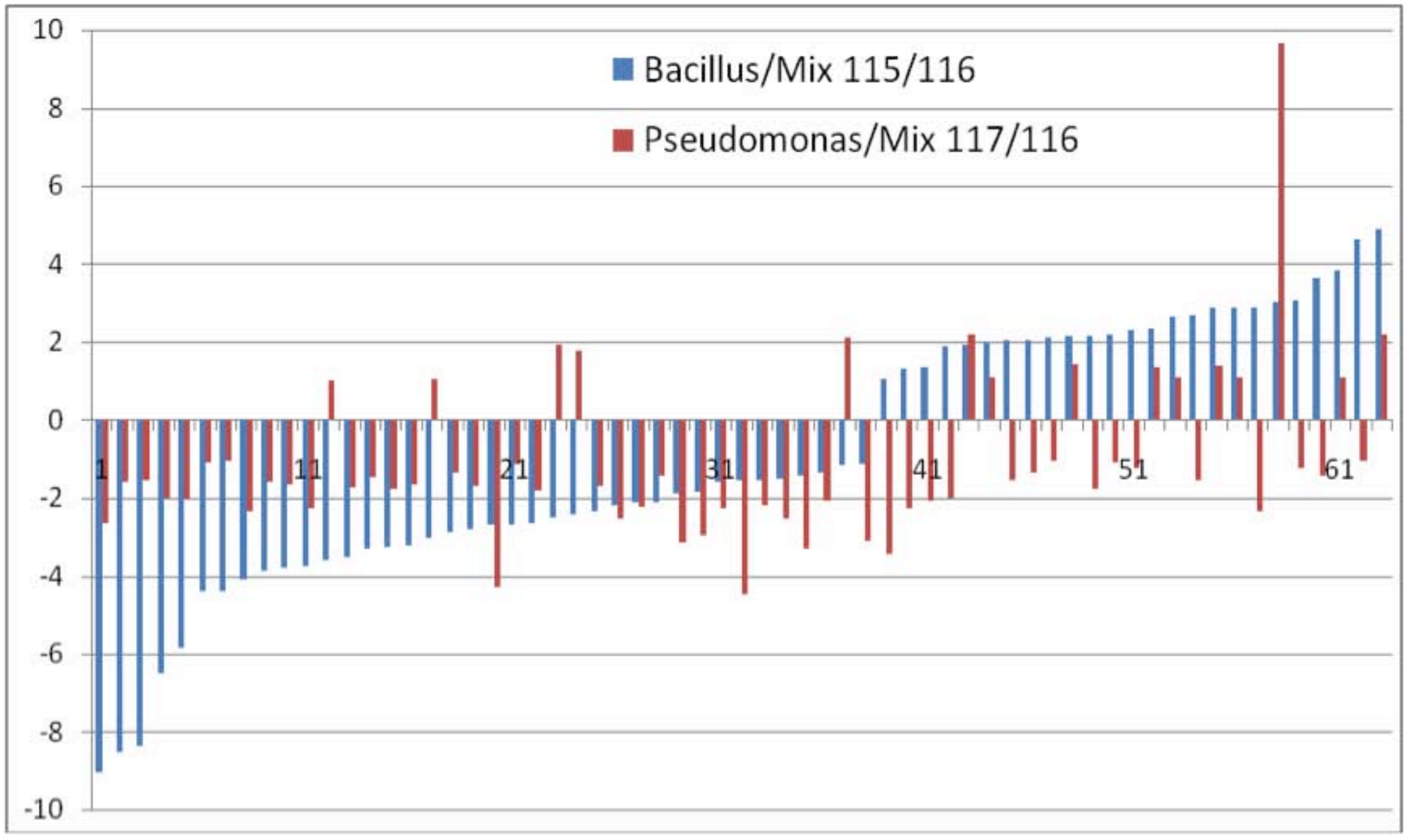
Microbial ecology

- Descriptive terminology
  - Mutualism
  - Synergism
  - Antagonism
- Mechanism?

Proteomics to probe microbial interactions

- Binary culture
  - *Bacillus atrophaeus* (*B. subtilis* DSM675) (G+)
  - *Pseudomonas putida* KT2440 (G-)
- Flasks of tryptic soy broth
  - *B. atrophaeus*
  - *P. putida*
  - 50:50 mixture (number concentration)
- Analysis
  - iTRAQ, ESI-Q/TOF
  - Mascot with NCIBnr bacterial database; Gene Ontologies

Simple community proteome changes



Simple community: observations of interactions

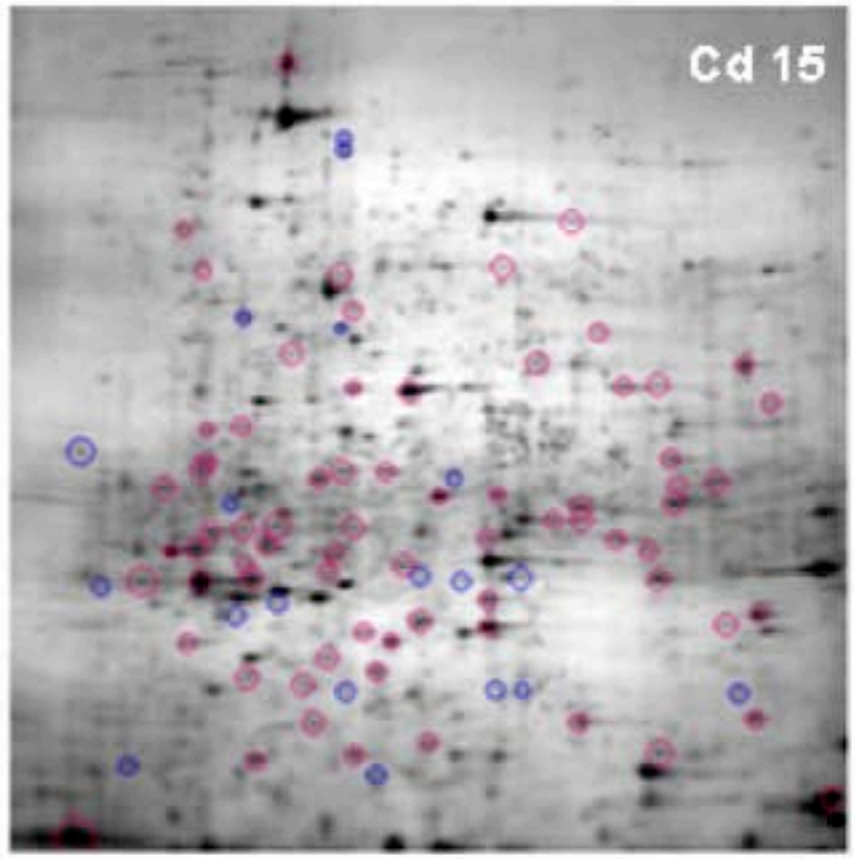
- Unique evidence of modulation of protein expression due to growth in co-culture
  - Complementary up- and down-regulation
  - Almost 2/3 of proteins of interest were directly involved in cellular metabolism
  - Co-cultures over-expresses antioxidant proteins

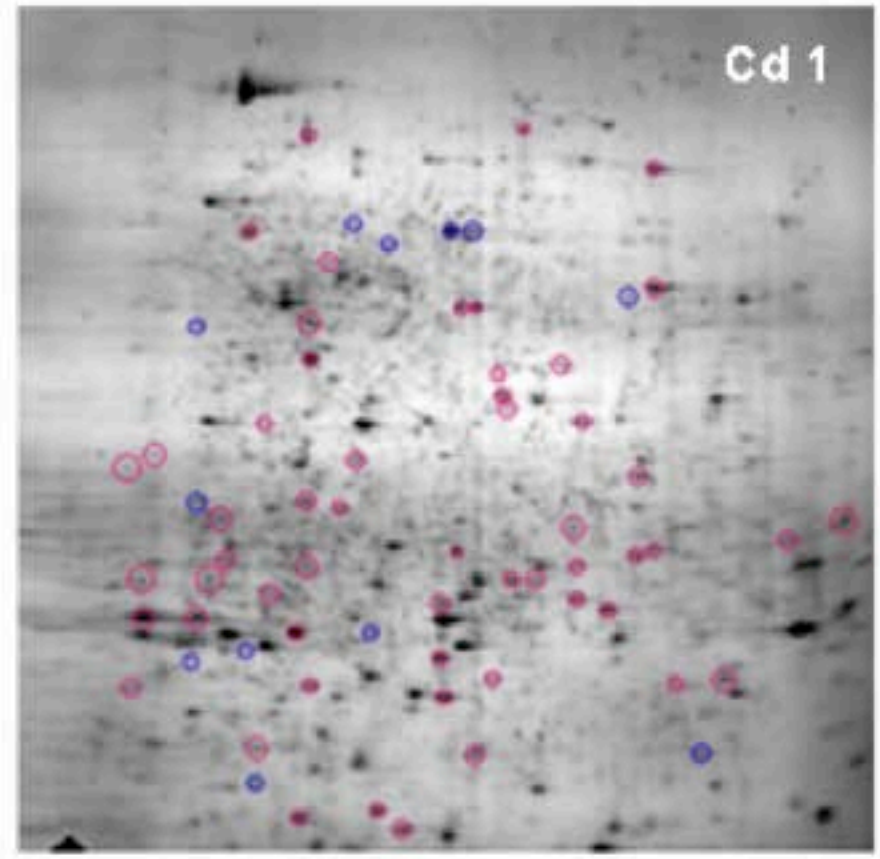
Metaproteomic analysis of the response of a microbial community to cadmium

Carla Lacerda, PhD

Cadmium shock experiment

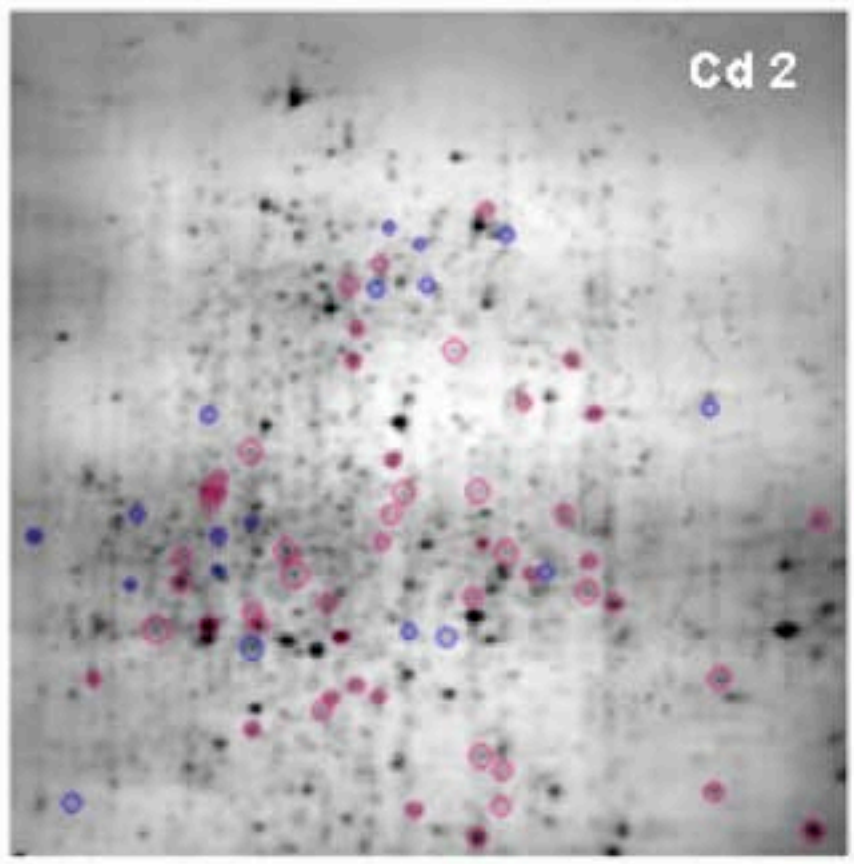
- Grow microbial community 20 h in bioreactor
- Add inhibitory level of cadmium
- Harvest at different time points
  - 0.25, 1, 2, and 3 hours
- Untreated control cultures

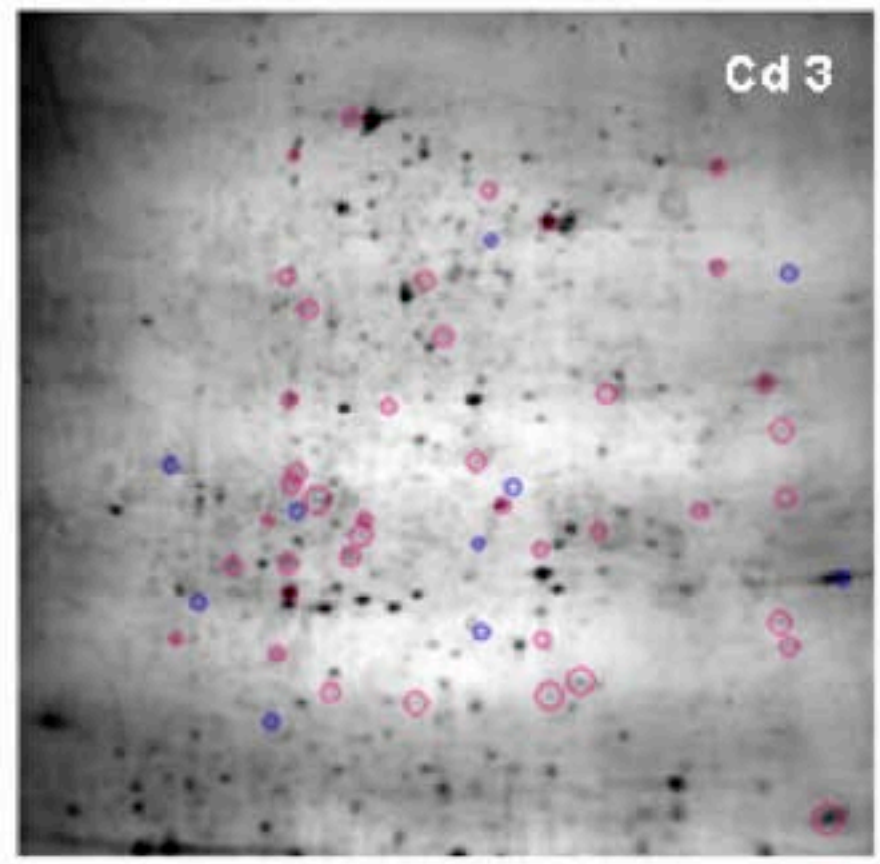




Pink: ↑

Blue: ↓



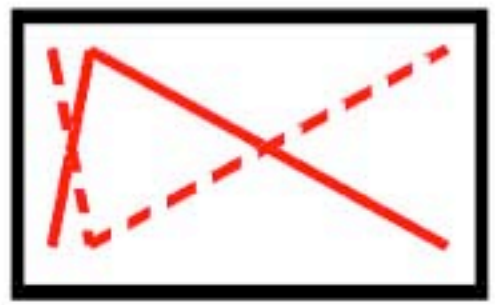
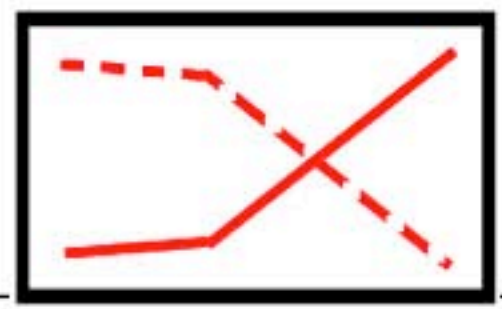
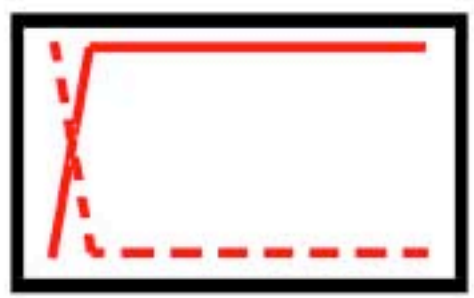


Summary of differentially expressed proteins*

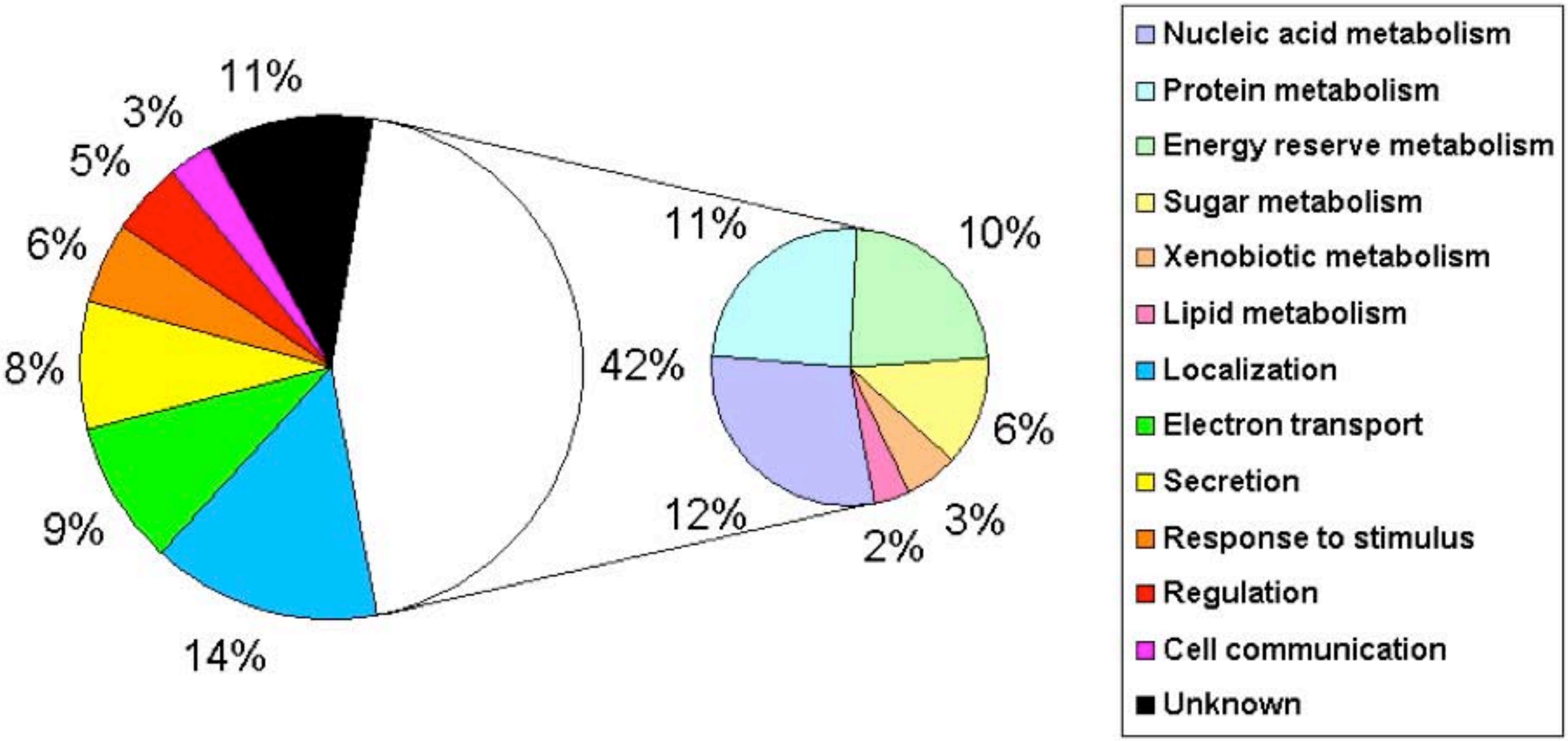
Time point	Down-regulated	Up-regulated
15 min	2%	17%
1 h	6%	10%
2 h	1%	8%
3 h	6%	7%

* ≥3x change

Complex community response: trends

Temporal response pattern		Proteins in trend	Protein functions
Short-term only (resistance)		30%	Metabolism Electron Transport Cell communication Regulation
Long-term only (adaptation)		11%	Secretion Localization Response to stimuli
Short- and long-term (tolerance)		50%	All classes

Complex community response: functional classification



(158 total, 109 unique proteins)

Challenges

Challenges of metaproteomics

- Unsequenced genomes (becoming less of a problem?)
- Species richness – "a ton of soil may contain 4×10^6 taxa" (Curtis et al., 2002)
- Metaproteomics vs. pure culture studies
  - If 50 species with 3000 ORFs per species and 75% expression → 10^5 proteins
  - 1 mg of protein distributed among 50x more proteins than pure culture
  - Representation of sequence heterogeneity

Challenges for environmental proteomics

- Experimental
  - Representative sampling
  - Sample preparation
  - Sample analysis (10^{many} different proteins?)
  - …
- Computational/Bioinformatics
  - Matching peptides to proteins (do we always need the metagenome?)
  - Function
  - …

Challenges for environmental proteomics

- Experimental
  - Representative sampling
  - Sample preparation
  - Sample analysis (10^{many} different proteins?)
  - …
- Computational/Bioinformatics
  - Matching peptides to proteins (do we always need the metagenome?)
  - Function
  - …

Tomorrow's presentations!

A few goals for our field?

- Improved methods for (representative) protein extraction from complex matrices

A few goals for our field?

- Improved methods for (representative) protein extraction from complex matrices
- Move beyond protein lists and pie charts

A few goals for our field?

- Improved methods for (representative) protein extraction from complex matrices
- Move beyond protein lists and pie charts
  - Function
  - Mechanism of action

A few goals for our field?

- Improved methods for (representative) protein extraction from complex matrices
- Move beyond protein lists and pie charts
- Link metaproteomic data with other –omics data and quantitative community composition data

A few goals for our field?

- Improved methods for (representative) protein extraction from complex matrices
- Move beyond protein lists and pie charts
- Link metaproteomic data with other –omics data and quantitative community composition data
- $N_{research\ reports} > N_{review\ articles}$

Acknowledgements

- Carla Lacerda
- Funding:
  - NSF BES-0329514
  - NSF IPY-0733074
  - EPSRC
- Technical assistance
  - Rick Reisdorph and Dr. Nichole Reisdorph (NJH)
  - Phillip C. Wright (U. Sheffield)
  - Kelvin H. Lee and Leila Choe (U. Delaware)