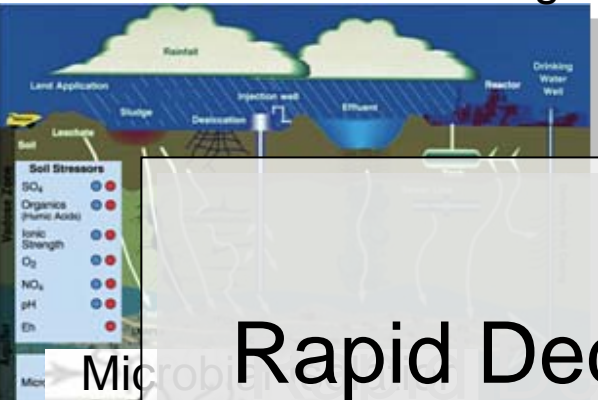# The Virtual Institute of Microbial Stress and Survival

Environmental Monitoring
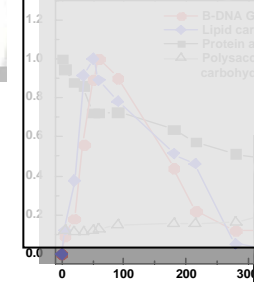
Pathway Models
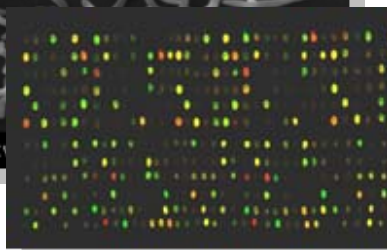
Pathway Inference

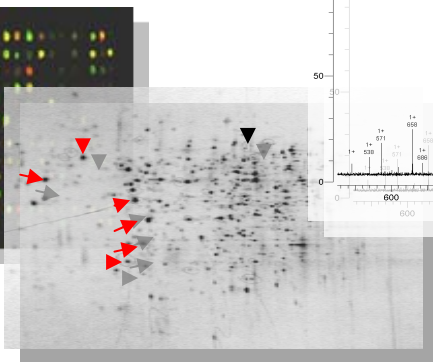## Rapid Deduction of Stress Response Pathways in Metal and Radionuclide Reducing Bacteria

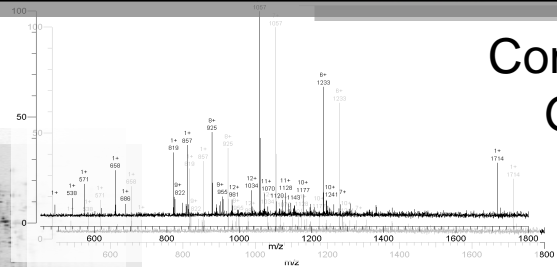sFTIR
In situ physiology

DNA Microarrays

Proteomics

Metabolomics

Comparative Genomics

Adam Arkin, (UCB/LBNL, Co-Director and Core Team Co-Leader)

Terry C. Hazen (LBNL, Co-Director and Core Team Leader)

Jay Keasling (UCB/LBNL, Core Team Co-Leader)

Eric Alm (LBNL, Core Team Co-Leader)

Inna Dubchak (JGI/LBNL)

Matthew Fields (Miami University)

Hoi-Ying Holman (LBNL)

Martin Keller (Diversa Inc.)

Aindrila Mukhopadhyay (LBNL, Core Team Co-Leader)

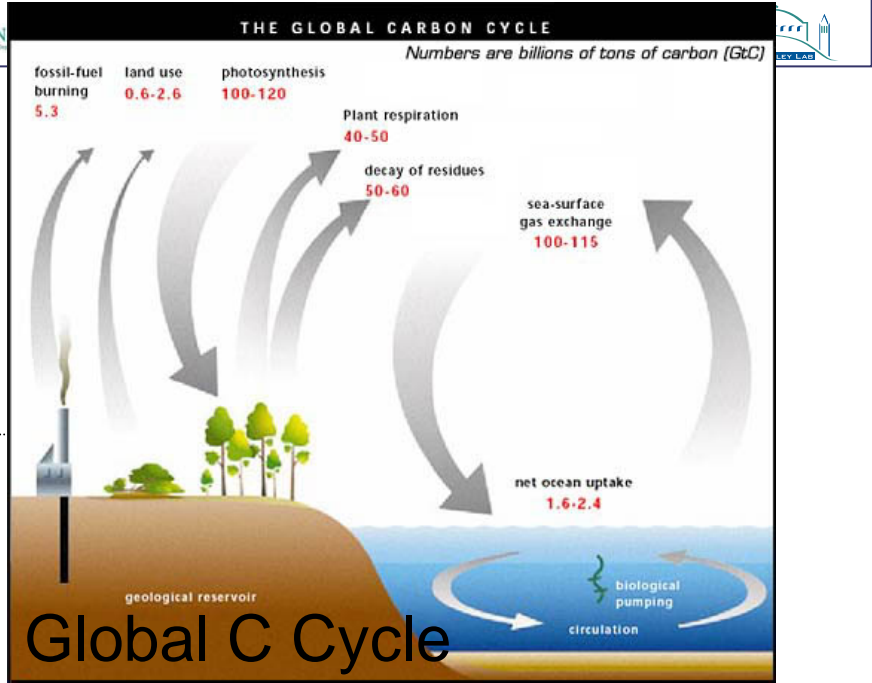**David Stahl (University of Washington)**
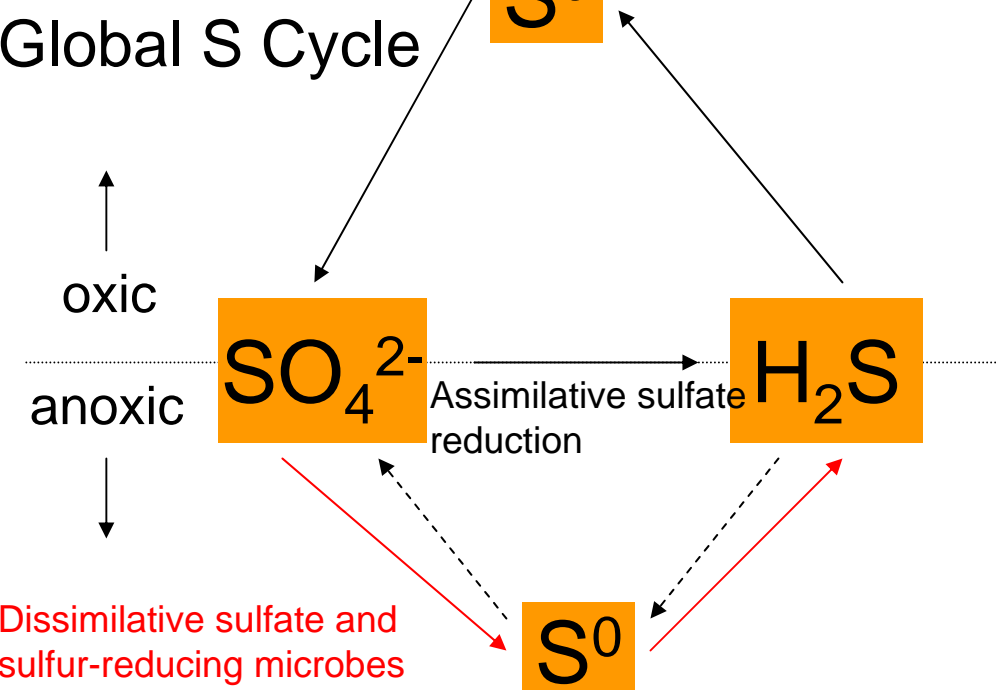
Judy Wall (University of Missouri)

Jizhong Zhou (ORNL/University of Oklahoma)

and many others

Why Sulfate Reducing Bacteria

Global S Cycle

S^0

oxic

anoxic

SO_4^{2-} H_2S

Assimilative sulfate reduction

Dissimilative sulfate and sulfur-reducing microbes

S^0

Global C Cycle

Souring of oil reservoirs
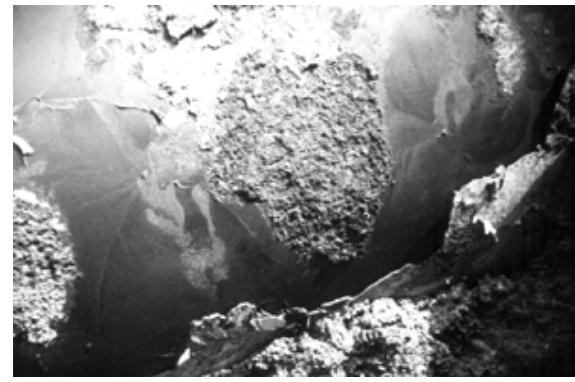
Microbial-induced corrosion

- History and metabolism of DvH

- Environmental and industrial importance of sulfate reducers

- Genomic content interest
  - Contentious model of energy metabolism
  - Spectacularly rich signal transduction complement.
  - More on all of this later.

- Adaptability of cells to their environment

- Efficiency of metabolism within a cell and across its community

- Plasticity and Evolution of Genomes

- Network Inference and Interpretation of Functional Genomics

Organization of Effort

Sample Collection, Characterization and Culturing

Molecular Interaction and Perturbation

Physiological Monitoring

Policy development and testing

Protocol Delivery

Homologous Pathway Collection

Knowledge Storage

Data Analysis

Model Building

Comparative Genomics

Conceptual Modeling

Applied Environmental Microbiology Core
LBNL, ORNL, U. Wash., UMC

Functional Genomics Core
LBNL, SNL, U. Wash., Diversa

Computational Core
LBNL, TIGR, U. Wash., UMC

Organization of Effort
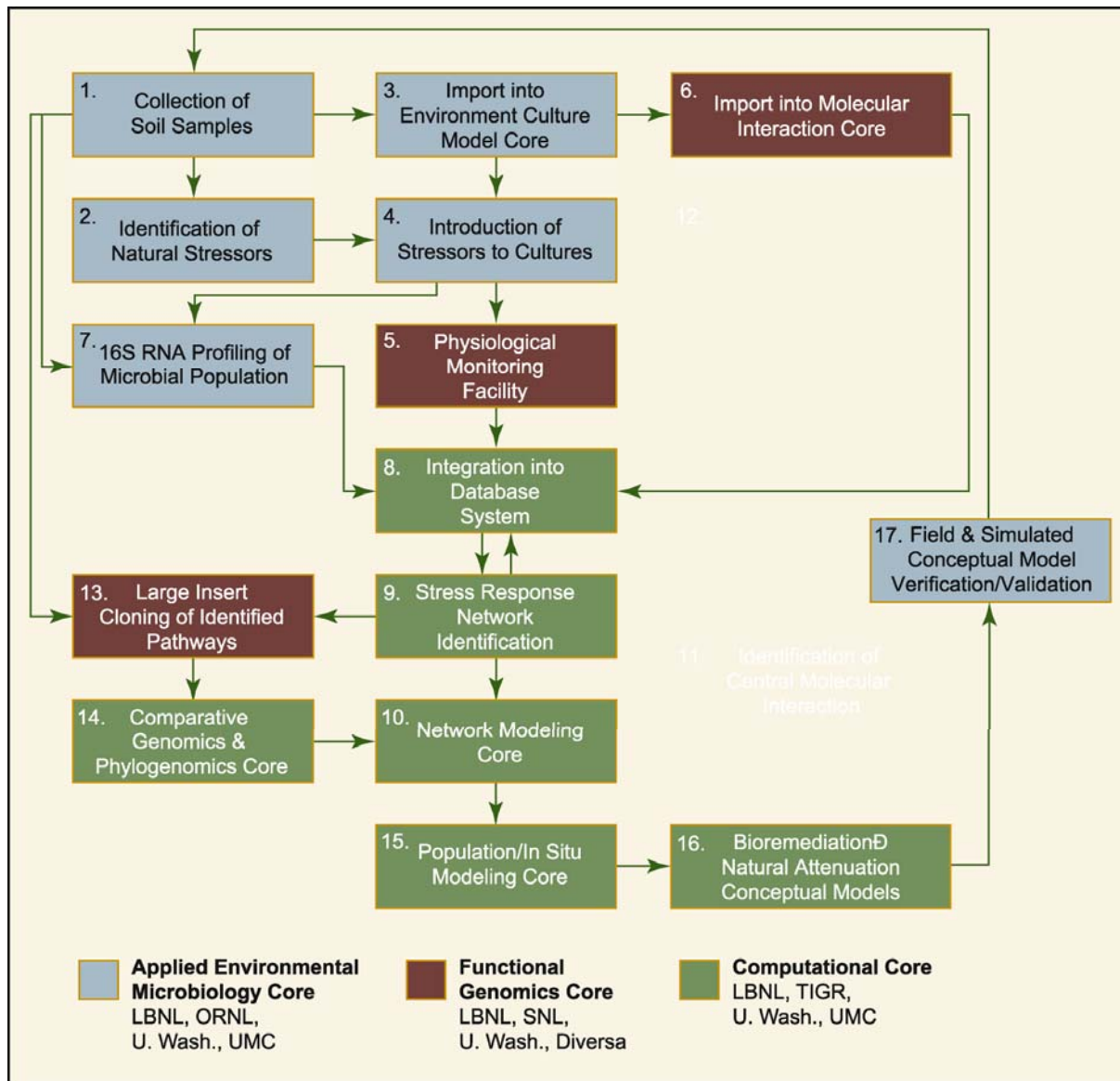
Organization of project
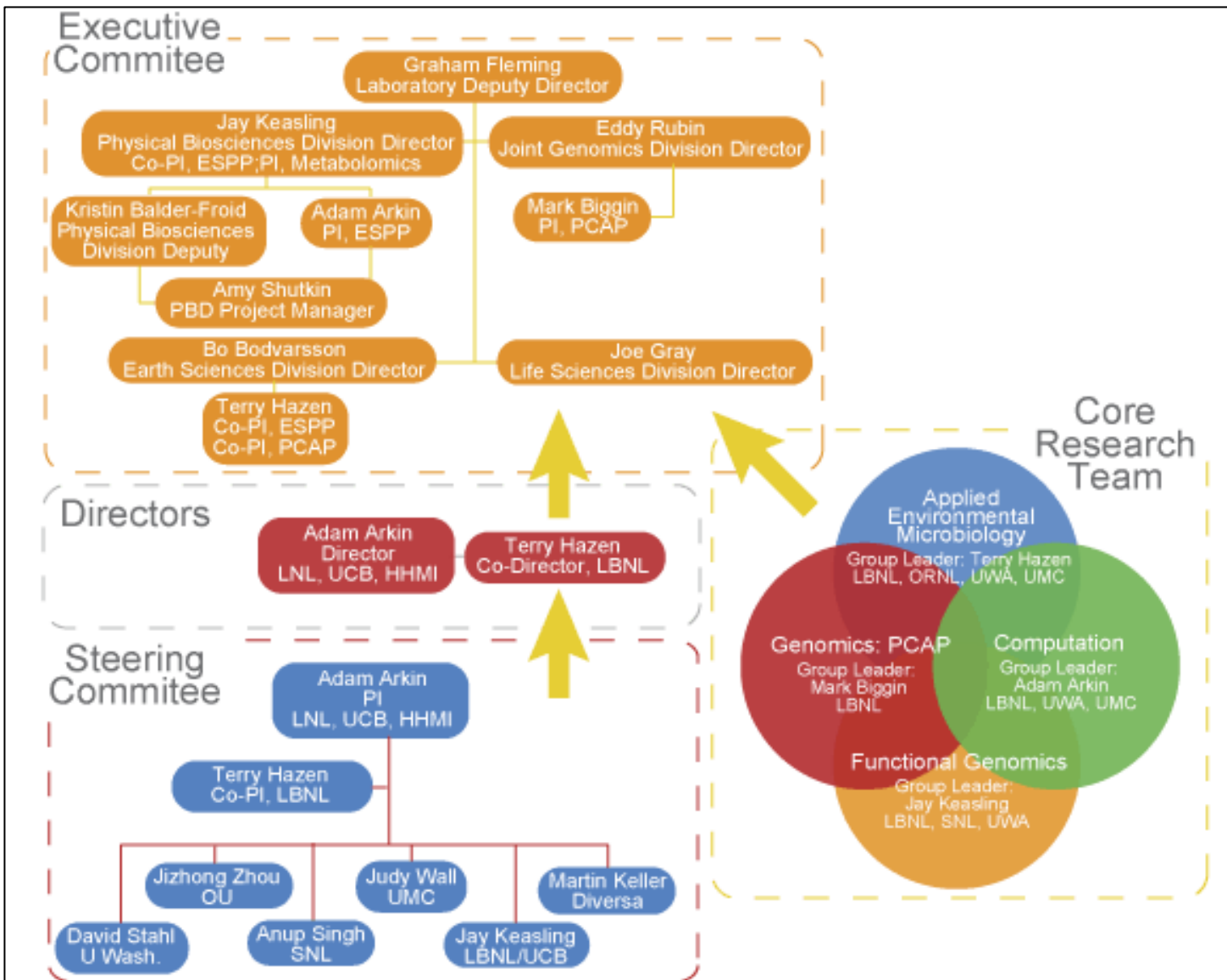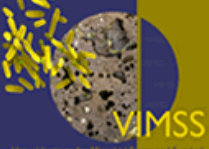
- Identify stressors in the field that impact the survival and efficiency of metal reducing microbes.

- Identify the impact of these stressors on our key organism: *Desulfovibrio vulgaris.*

- Infer the key stress response pathways in this organism and how they are brought online and interplay under different stress conditions.

- Demonstrate how these stress conditions impact metal reduction.

- Understand niche adaptations by comparison to other metal-reducing environmental microbes: *Shewanella oneidensis* and *Geobacter metallireducens.*

- Use knowledge to construct conceptual models for how these organisms survive in the environment and use these models to demonstrate an optimization of the metal reduction process.

Highlights from project

- VIMSS has created a flexible microbial physiological pipeline comprising biomass characterization, cellular imaging, transcriptomics, proteomics, and metabolomics, that can rapidly upgraded and repurposed for new projects.

- This pipeline lays the foundation for integration of functional genomic and physiological data and facilitates a synergetic approach to physiological characterization of microorganisms more efficiently than one lab could alone.

- VIMSS now houses 4 new GTL projects, a NASA Astrobiology Project, a DHS green genes project, LDRD on Microbial Fuel cells, and 2 NABIR projects - with >12 laboratories all over the country - a true virtual institute

- In an example from these collaborations, VIMSS has aided in the annotation of a metagenome from a deep subsurface gold mine. A nearly clonal population of a new speccies of sulfate reducer was discovered with new classes transposons and a number of unique regulatory and gene features

Home Page

Projects & Research

ESPP

Metabolomics

PCAP

MAGGIE

IPTAI

Greengenes

Hanford Cr

Projects & Research

ESPP
Environmental Stress Pathway Project
Adam Arkin, co-Principal Investigator (LBNL, Physical Biosciences Division) and
Terry Hazen, co-Principal Investigator (LBNL, Earth Sciences Division)

formerly known as Rapid Deduction of Stress Response Pathways in Metal/Radionuclide Reducing Bacteria

>> More about ESPP
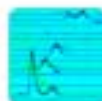
Metabolomics
New Technologies for Metabolomics
Jay Keasling, Principal Investigator (Physical Biosciences Division)

>> More about Metabolomics

PCAP
Protein Complex Analysis Project
Mark Biggin, Principal Investigator (Life Sciences Division)

>> More about PCAP

MAGGIE
Molecular Assemblies, Genes, and Genomics Integrated Efficiently
John Tainer, Principal Investigator (Life Sciences Division)

>> More about MAGGIE

IPTAI
Indiana Princeton Tennessee Astrobiology Initiative

>> More about IPTAI

Greengenes
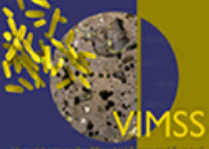16S rRNA gene database and workbench compatible with ARB

>> More about Greengenes

HCB
Hanford Chromium Bioremediation
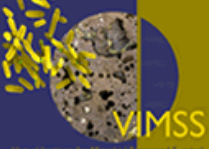
>> More about Handford Cr

Highlights from project

- The AEMC has a reliable, robust and repeatable procedure for production of extremophile biomass production under a variety of stressors.

- The AEMC has pioneered phenotype microarray procedures for extremophiles and anaerobes.

- The AEMC has pioneered the use of Synchrotron FTIR Microscopy to confrm stress response conditions and pick time points for biomass sampling under such stress.

- AEMC has identified Dv at DOE contaminated sites and has explored correlation of Desulfovibrio growth with metal reduction.

- In collaboration with NABIR projects on Uranium contamination, AEMC has begun to link natural stimulation studies to community measures of bacterial populations including Dv.

- AEMC has characterized contaminated environments to choose stressors for laboratory work.

- AEMC identified functional changes in bacterial systems under different stress conditions, nutrients, and niche, at FRC sites.

- AEMC has pioneered development of dual culture Dv and Mm in obligate syntrophic growth for studying this tiny community.

- AEMC has developed a flux-balance model for the syntrophic pair which explains many growth conditions and identifies the ech hydrogenase as dominant in proton/hydrogen pumping.

- The AEMC has isolated a number of subspecies of Dv including a phage deficient strain (DP4) that are now undergoing sequencing. They will also be tested phenotypically and in the co-culture to understand the variability of regulation in Dv.

- The AEMC has developed new techniques for metagenomics library construction and sequence from soil samples.

- The FGC has developed efficient transposon and well-defined knockout genetics for Dv that can be routinely used to discover genes important for fitness and to test predictions from the functional genomic data.

- The FGC has developed high-efficiency affinity tags for exogenous and endogenous pull-down experiments to find molecular complexes. These support both ESPP and PCAP.

- The FGC developed Differential In-Gel Electrophoresis for identification of post-translational and redox state of proteins.

- The FGC is able to compare and contrast three different industry standard proteomics techniques to each other and to functional genomic data for the first time. This allows new measures quality and consistency and the discovery of new biology.

- The FGC has a reliable and robust transcriptomics facility that has has now been applied to at least three environmental microbes, an Archaea and strains variants of Dv.

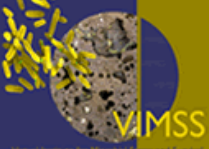- The FGC has is developing large scale metabolomics and fluxomics capabilties that are coming online now.
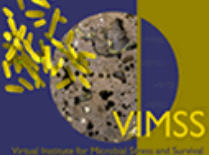
- The CC has developed a community comparative microbial genomics/physiology database and workbench called MicrobesOnline that is now being used for annotation of new organisms, functional genomic analysis, and understanding microbial regulatory networks.

- The CC has developed a number of statistical analysis tools for improving and comparing results from the VIMSS pipeline.

- The CC has developed methods for predicting operons and regulons in uncharacterized organisms and has exploited these results to understand operon evolution, better analyze microarray data, and detect cis-regulatory sequences.

- The CC has used MicrobesOnline in collaboration with Michael Gelfand and his RegTransDB to infer global regulatory networks such as Fur and Per and metabolic pathways in Dv and across organisms. MicrobesOnline and RegTransDB have been linked for advanced cis-regulatory site prediction.

- The CC has discovered that delta proteobacteria like Dv and Geobacter have an abnormally high abundance of signaling components that arise due to linear specific expansion unline model organisms like B. subtilis and E. coli which seem to acquire new histidine kinases by HGT. The AEMC has targetted a number of the unique HPK families for enriching signalling molecules in the AEMC environmental libraries.

- The CC has used comparative analysis across diverse sulfate reducers to discover a set of signature genes that are found in bacteria and Archaea with this phenotype. Coregulation of the signature genes was discovered in the VIMSS microarray compendium. A new thio-relay has been proposed based on this signature and the prediction is being followed up by deletion, protein complex studies, and by redox DIGE.
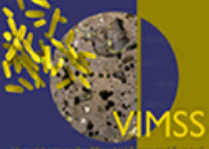
- Analysis of the VIMSS compendium and the Dv genome reveals that there is no generalized stress response with analogy to that found in *E. coli* or *B. subtilis*. Instead, up-regulating of the inferred fur regulon seems to be a common motif in Dv stress response. The lack of a general stress response might represent the control the complex signaling system has of Dv physiology.

- The *fur, per, zur* regulons seem to have a unique pattern of regulation and there seems to be so siderophores for metal scavenging to be controlled by these regulons.

- Dv demonstrates a unique nitrate response which may be important at the nitrate/U contaminated DOE sites. Full pipeline analysis of over ten stressors have demonstrated a stress response system in Dv very distinct from the model organisms and sometimes demonstrates combinations of *E. coli*-like and *B. subtilis*-like responses.
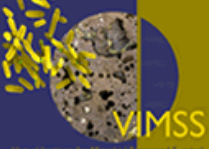
- While a great deal has been learned from the large scale analysis of stress response for environmental microbes much remains to be learned about how most effectively to use the pipeline.

- Coordination and management of a project of this scale in maintaining human resources, keeping scientific focus and prioritizing leads, managing data and developing technology is a dizzying and expensive enterprise.

- We are just learning what it takes to coordinate so many data types across a number of organisms linked to environmental information.

- Overview of the three cores

- Science stories from each of the investigators by theme showing team work

- A question and answer period

- And a debrief

Charge to the committee

- Evaluate our progress in light of what you've learned.

- Give specific advice to the PIs about technical issues of approaches.

- Suggest what would be the highest impact way we could use our infrastructure in the last year or so of operation.

- Comment on the prospects for follow-on research and projects

- Write up a summary of your findings.