MONTANA STATE UNIVERSITY
- Department of Microbiology
- Center for Biofilm Engineering

Responses of *Desulfovibrio vulgaris* to Physiological Constraints Relevant to Bioremediation in the Field

- Field Occurrence
- *Desulfovibrio vulgaris* responses to field relevant conditions

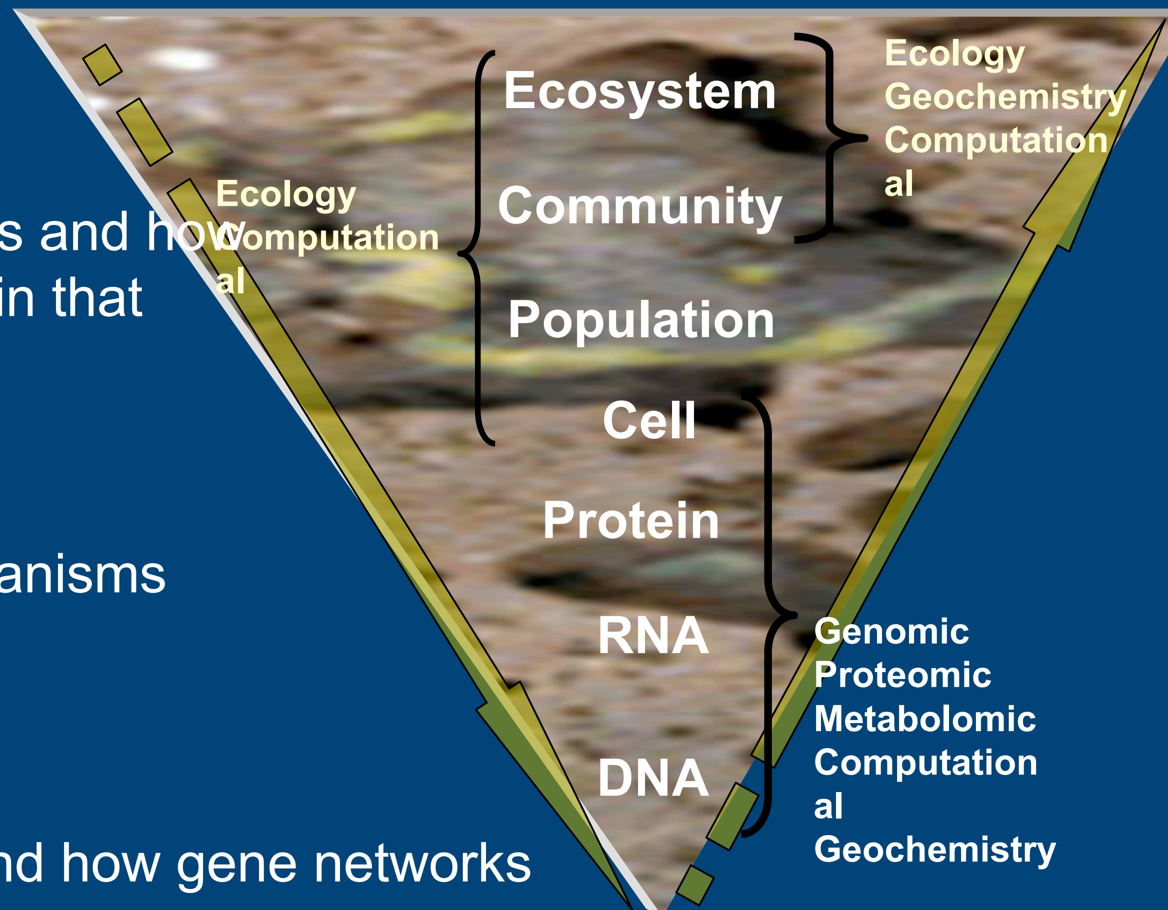
Matthew W. Fields

VIMSS Virtual Institute for Microbial Stress and Survival

Genes to Genomes to Function

Ecosystem

Identify key factors (i.e., stresses) that drive community structure and composition and impact the survival and efficacy of metal-reducers



Community

How do communities respond to stress and how do the populations of interest interact in that community

Populations

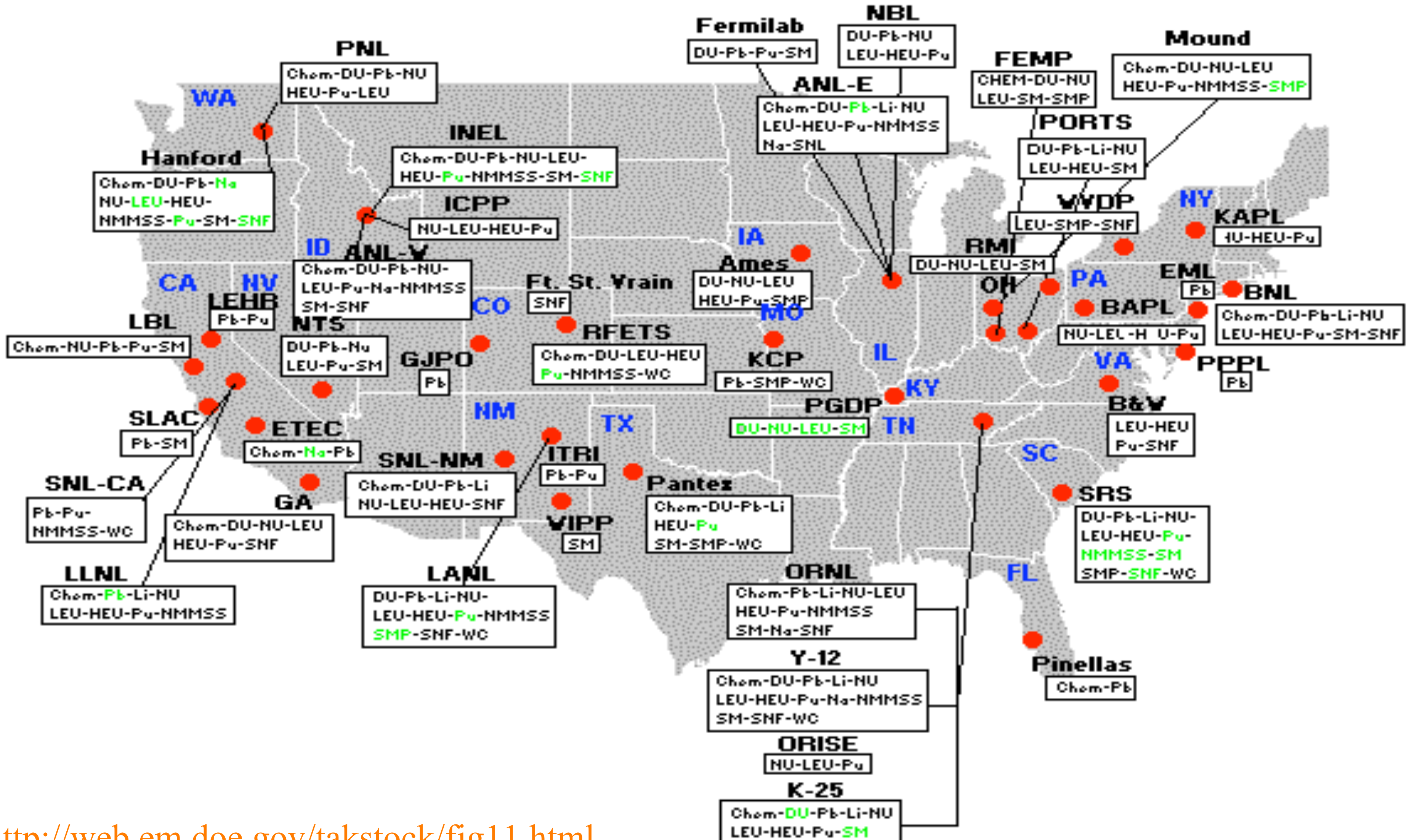
Determine the impact of stress on organisms

(*Desulfovibrio vulgaris)*

Cell

Infer key stress response pathways and how gene networks interplay under different stress conditions to optimize biochemistry

DOE Materials (Legacy Inventory)



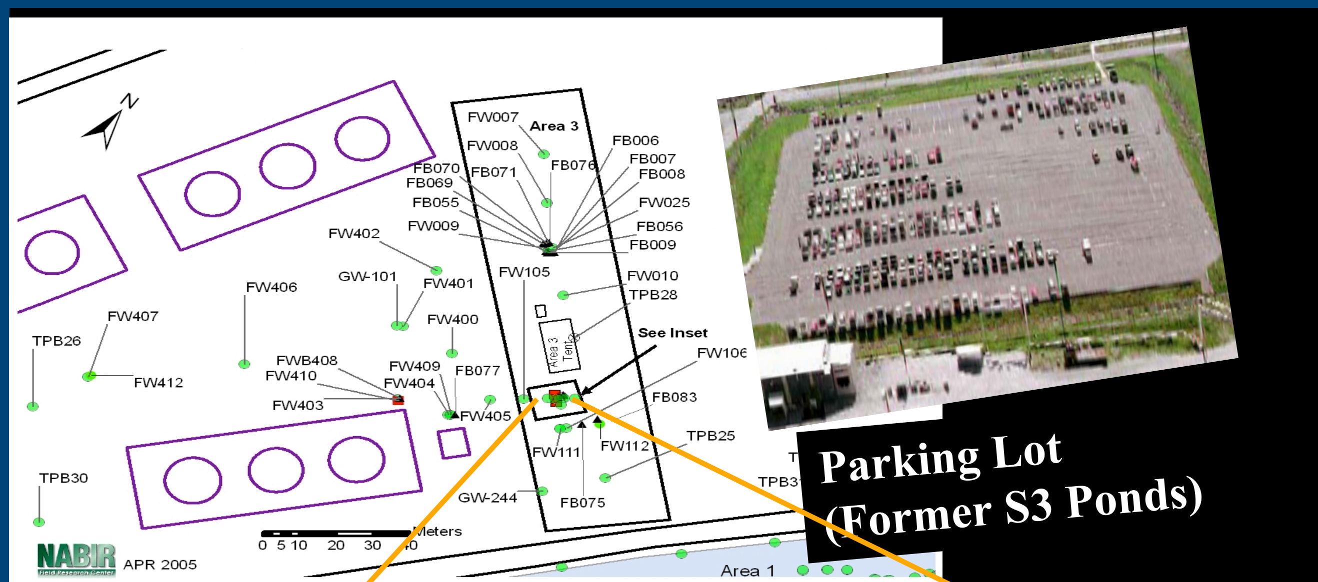
http://web.em.doe.gov/takstock/fig11.html

Bioremediation of U(VI) Contaminated Sediments *in situ* at the FRC

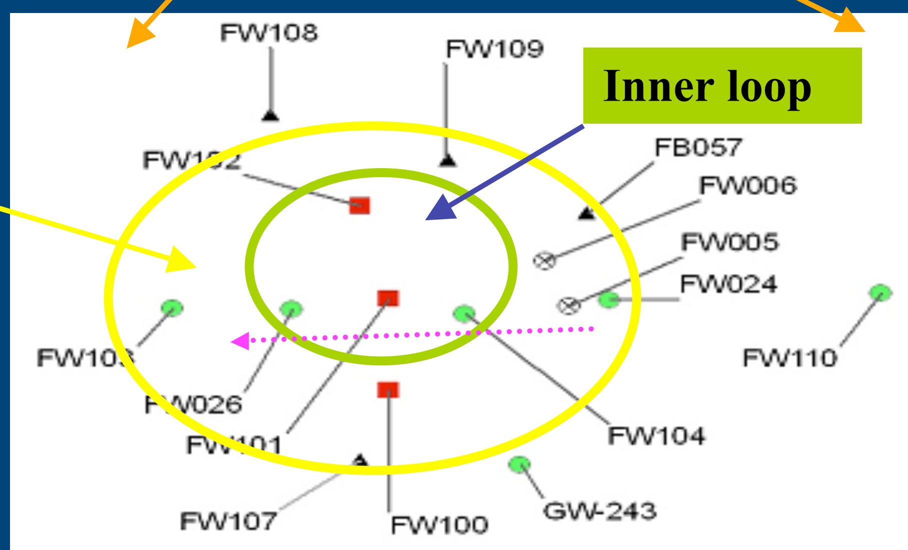
Outer loop
Injection:FW024
Extraction: FW103

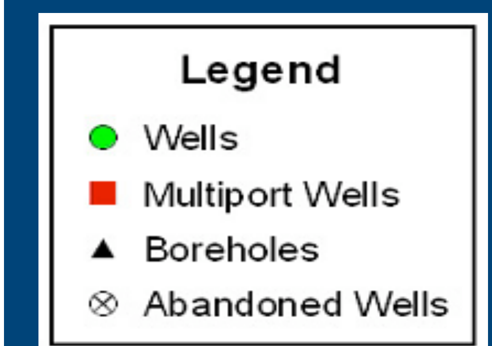
Inner loop
Injection: FW104
Extraction: FW026

Monitoring wells
FW101-2 (-40 ft)
FW101-3 (-35 ft)
FW102-2 (-40 ft)
FW102-3 (-35 ft)



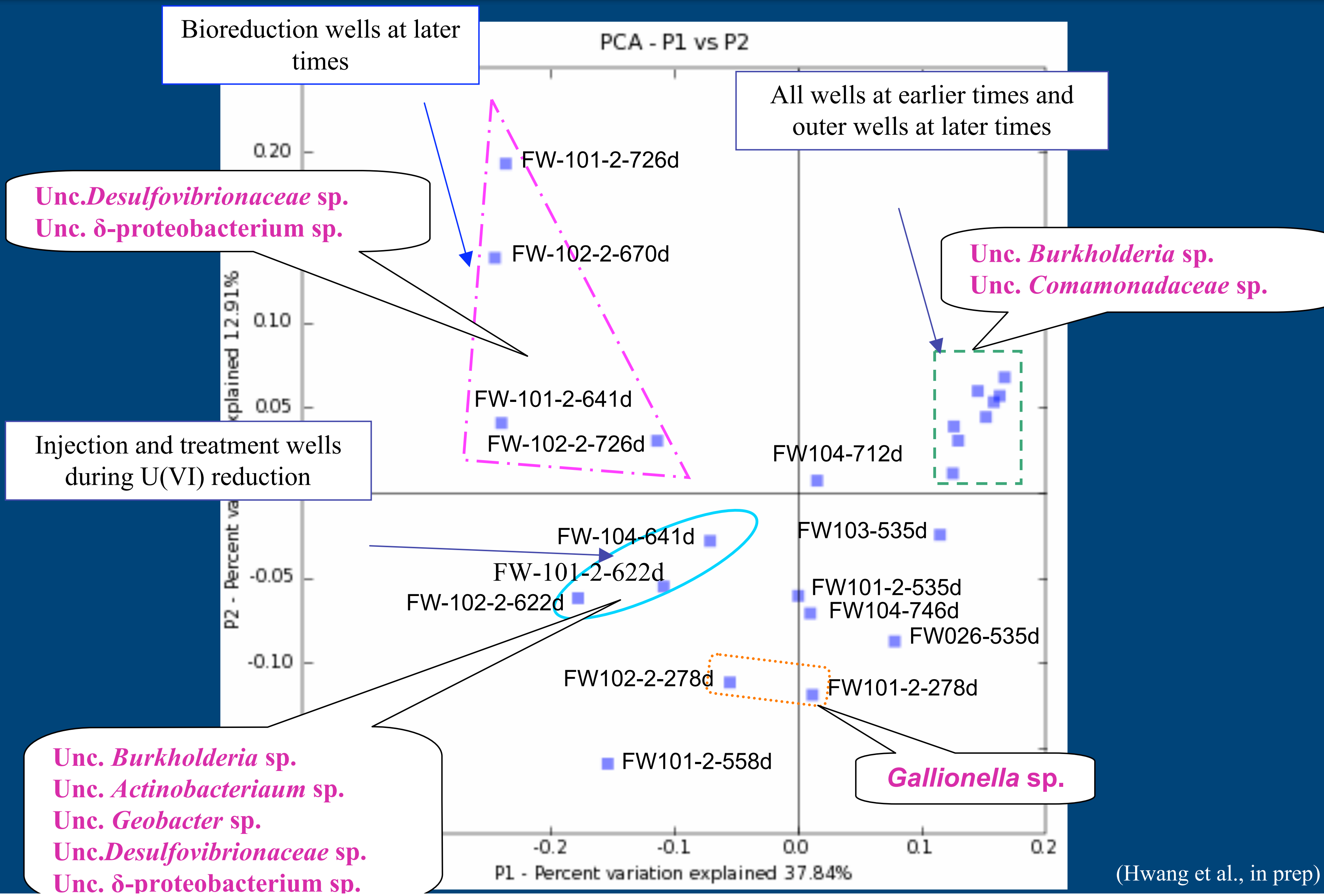
Outer loop





(Stanford/ORNL)

cipal Coordinate Analysis of Temporal and Spatial Population Cha

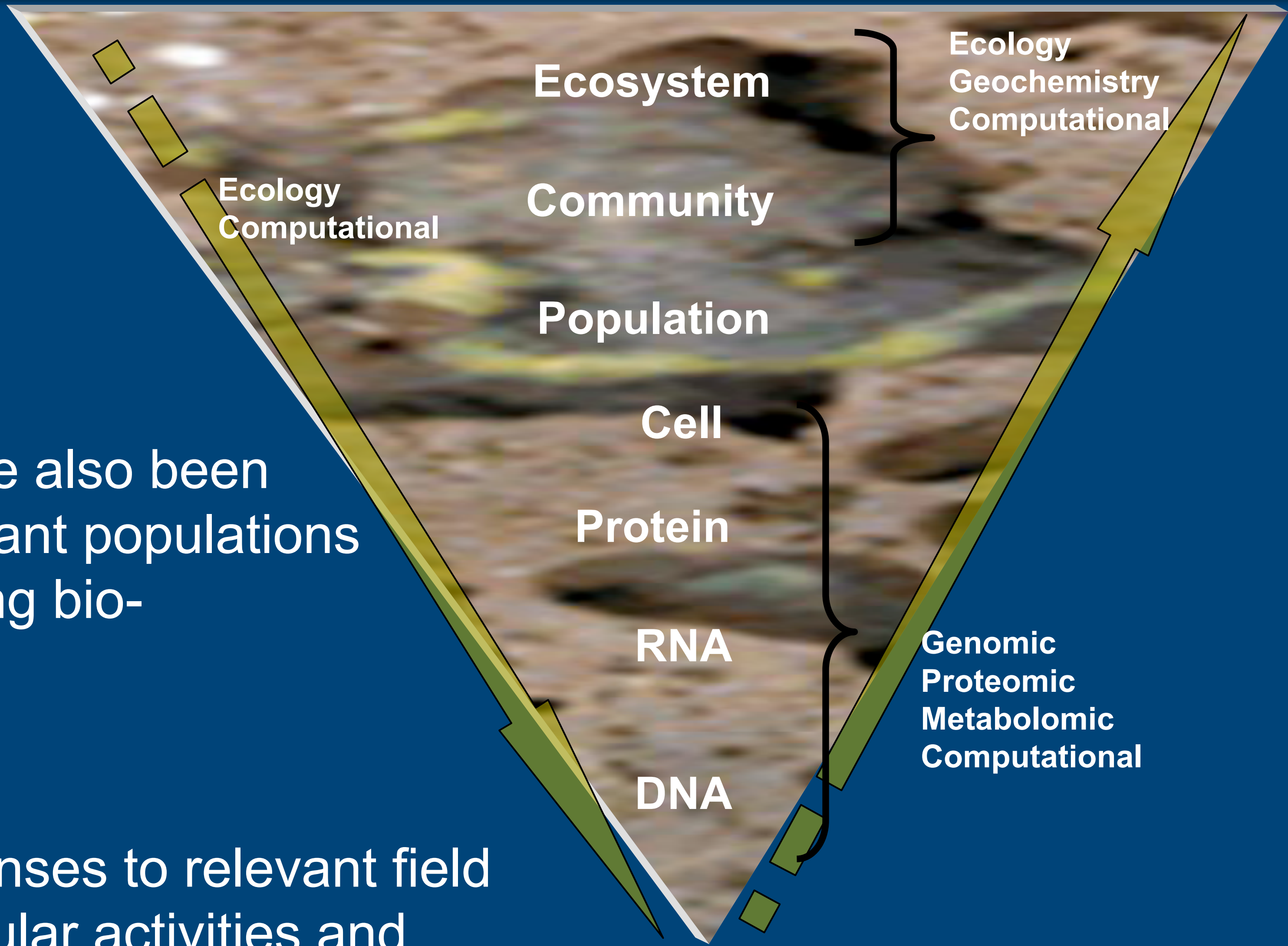


(Hwang et al., in prep)

Genomics to Elucidate Field Relevant Responses

Desulfovibrio are present at elevated numbers at the FRC during bio-stimulation

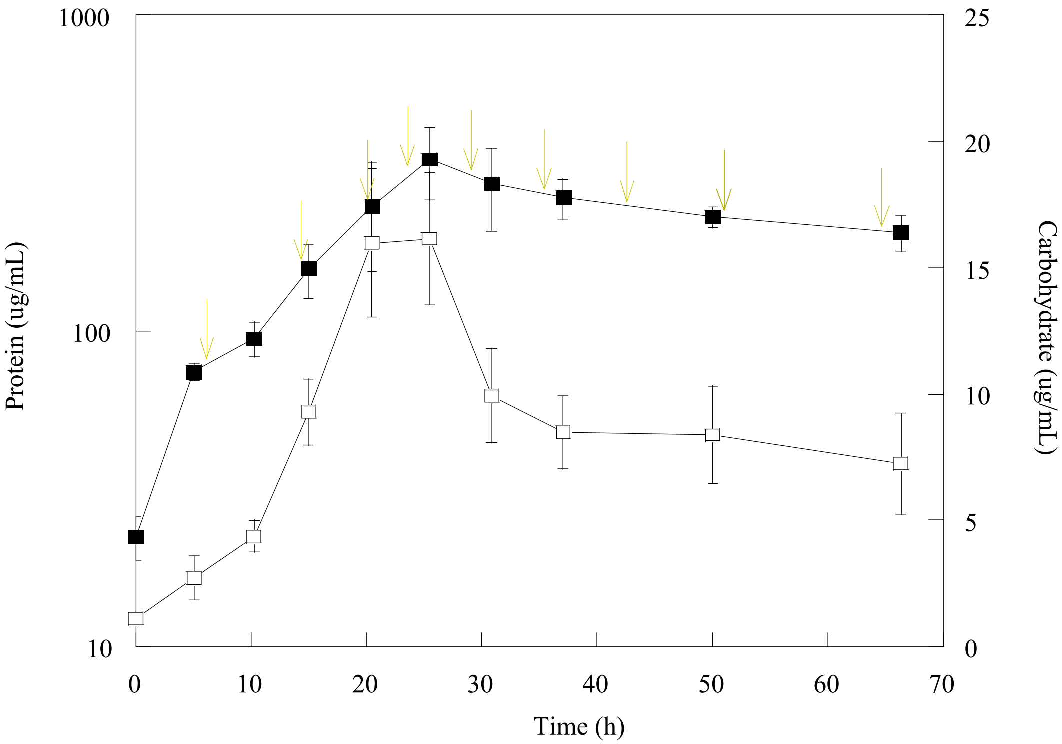
Desulfovibrio spp. have also been observed as predominant populations at Hanford 100-D during bio-stimulation (*Hazen et al.*)



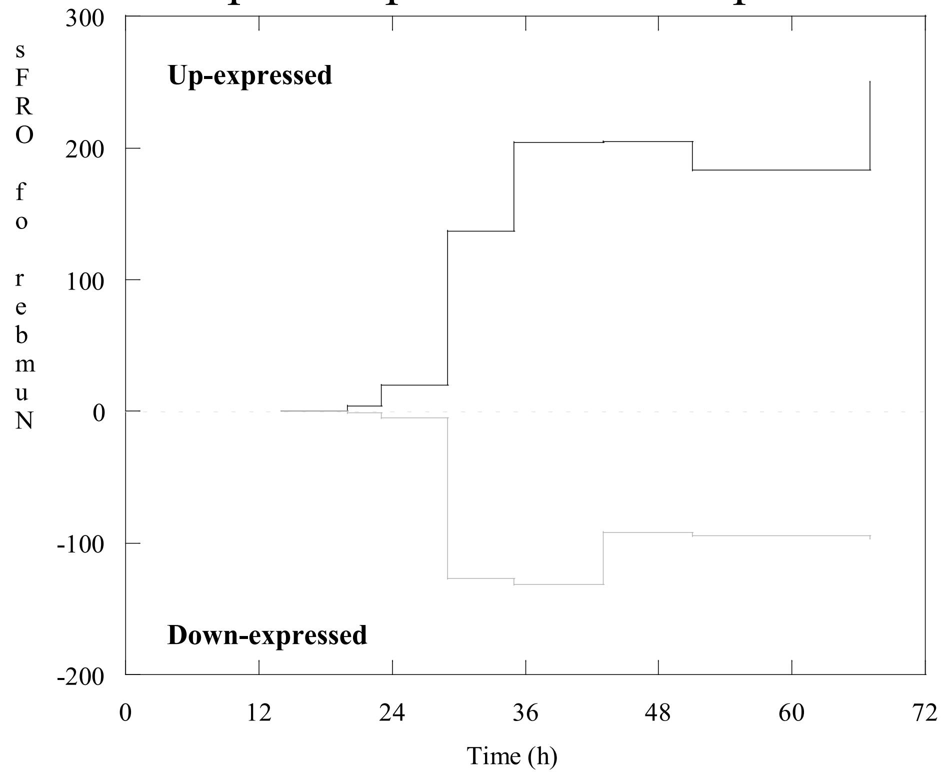
How do cellular responses to relevant field conditions impact cellular activities and survival?

Temporal Transcriptomics of Electron Donor Depletion

Cell Protein and Carbohydrate

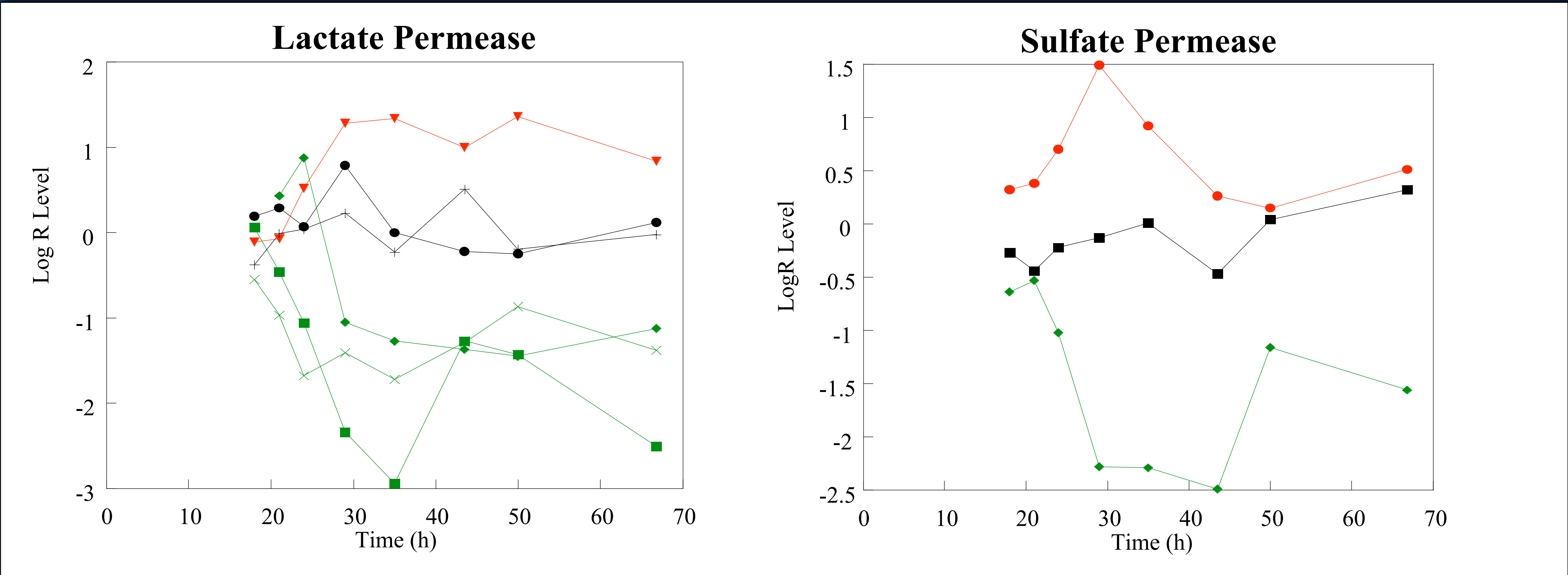


Temporal Up- and Down-Expression



(Clark et al., 2006)

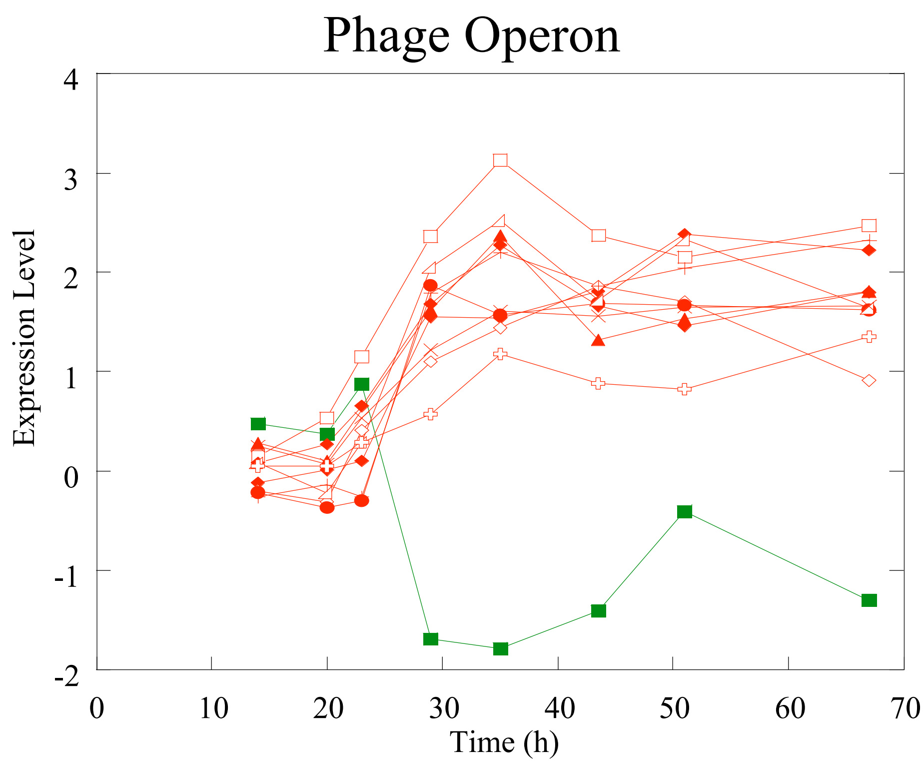
Lactate and Sulfate Permeases Displayed Different Trends of Expression

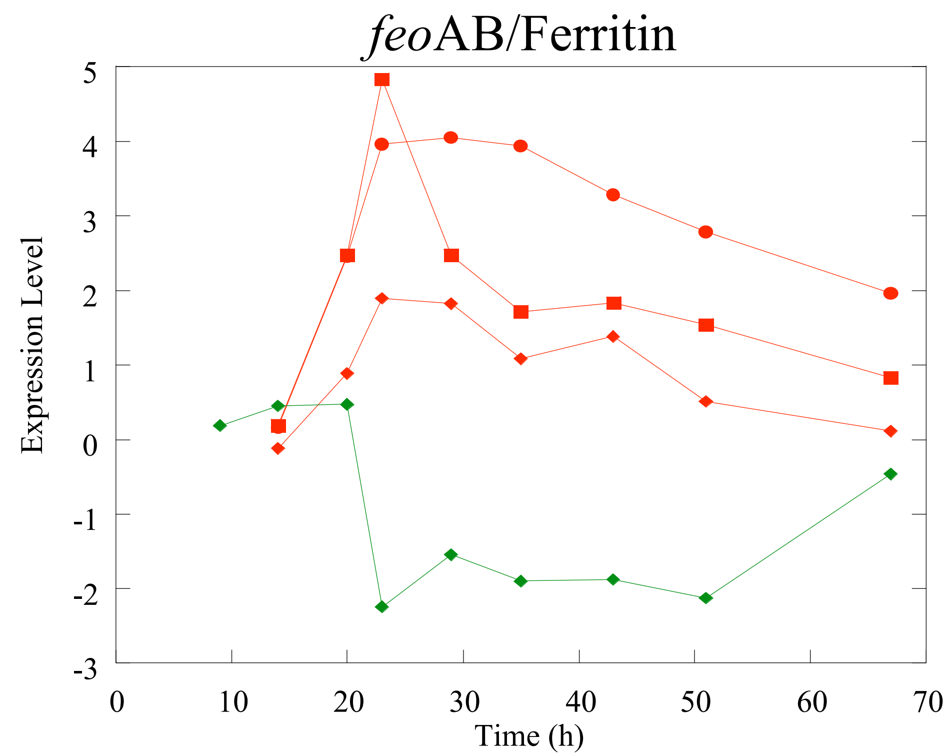


- Results suggested that different permeases were used with respect to changing nutrient levels
- An alternative explanation could be growth-rate dependent regulation
- Three presumptive LDH genes did not show significant changes

(Clark et al., 2006)

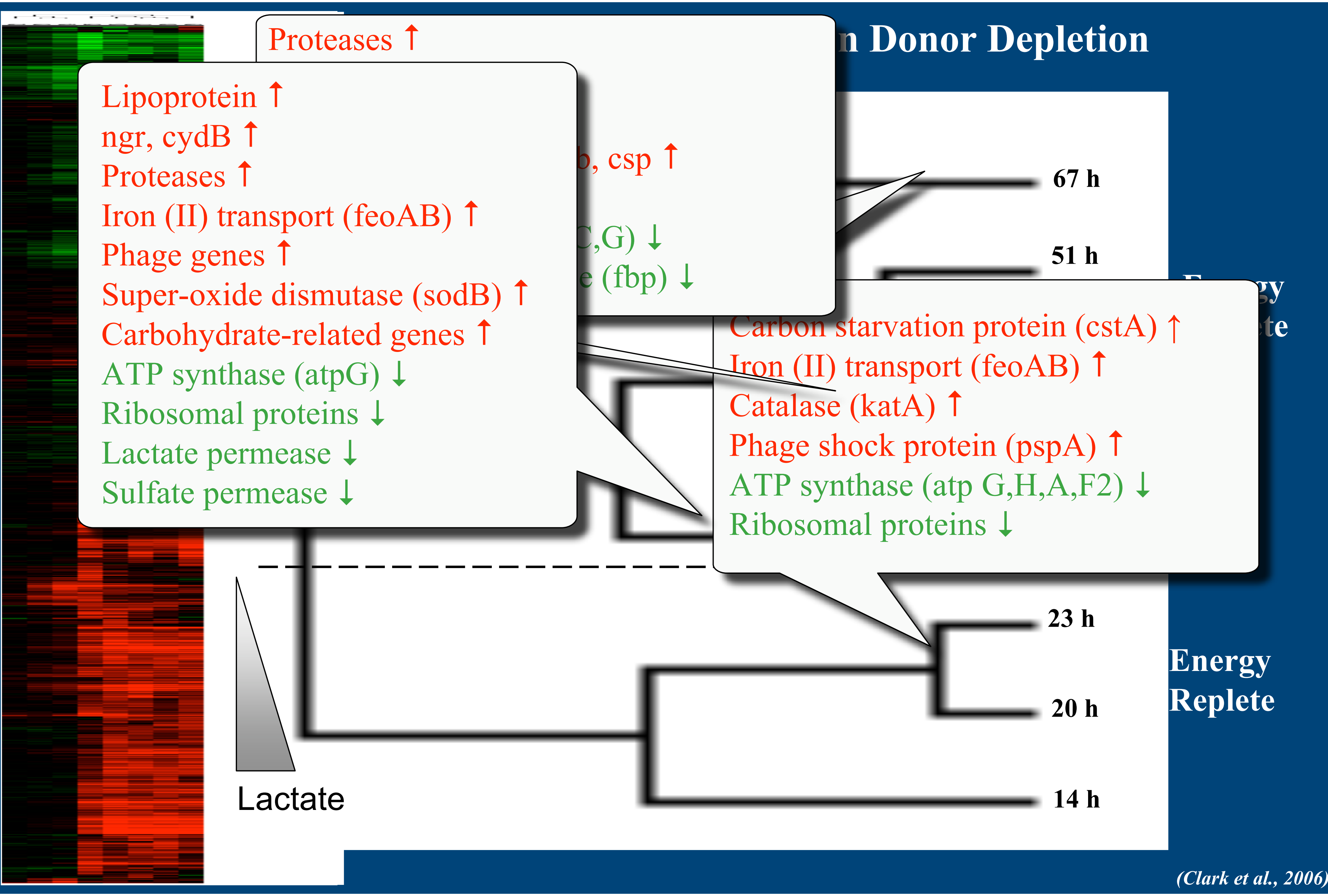
Major Changers as Electron Donor was Depleted





- Almost all phage-related genes were up-expressed into and during stationary-phase
- A possible *feo* system was up-expressed and a ferritin was down-expressed

(Clark et al., 2006)



n Donor Depletion
Proteases ↑
b, csp ↑
C,G) ↓
e (fbp) ↓
Lipoprotein ↑
ngr, cydB ↑
Proteases ↑
Iron (II) transport (feoAB) ↑
Phage genes ↑
Super-oxide dismutase (sodB) ↑
Carbohydrate-related genes ↑
ATP synthase (atpG) ↓
Ribosomal proteins ↓
Lactate permease ↓
Sulfate permease ↓
Carbon starvation protein (cstA) ↑
Iron (II) transport (feoAB) ↑
Catalase (katA) ↑
Phage shock protein (pspA) ↑
ATP synthase (atp G,H,A,F2) ↓
Ribosomal proteins ↓
67 h
51 h
23 h
20 h
14 h
Energy
Replete
Lactate
(Clark et al., 2006)

Some Conclusions from e^- Donor Depletion

In addition to expected changes (e.g., energy conversion, protein turnover, translation, transcription, and DNA replication/repair)

Genes related to : *phage*
carbohydrate flux
outer envelop
iron homeostasis

played a major role in the cellular response to nutrient deprivation under the tested growth conditions

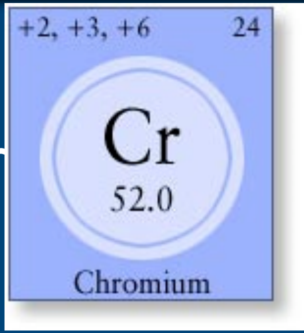
*rpo*S – universal stasis transcriptional factor ?

The results indicated that a subset of approximately 110 genes were uniquely up-expressed as the cells transitioned to stationary-phase (14 on the megaplasmid).*

(Clark et al., 2006)

Cr(VI) Responses in *D. vulgaris*

Cr is the third most common pollutant at hazardous waste sites and the second most common inorganic contaminant after Pb



D. vulgaris requires H_2S, hydrogenases and cytochrome *c3* for the reduction of Cr(VI) (Chardin et al., 2002)

Cr(III) can be detected on the cell surface and in the periplasm (Goulhen et al., 2005)

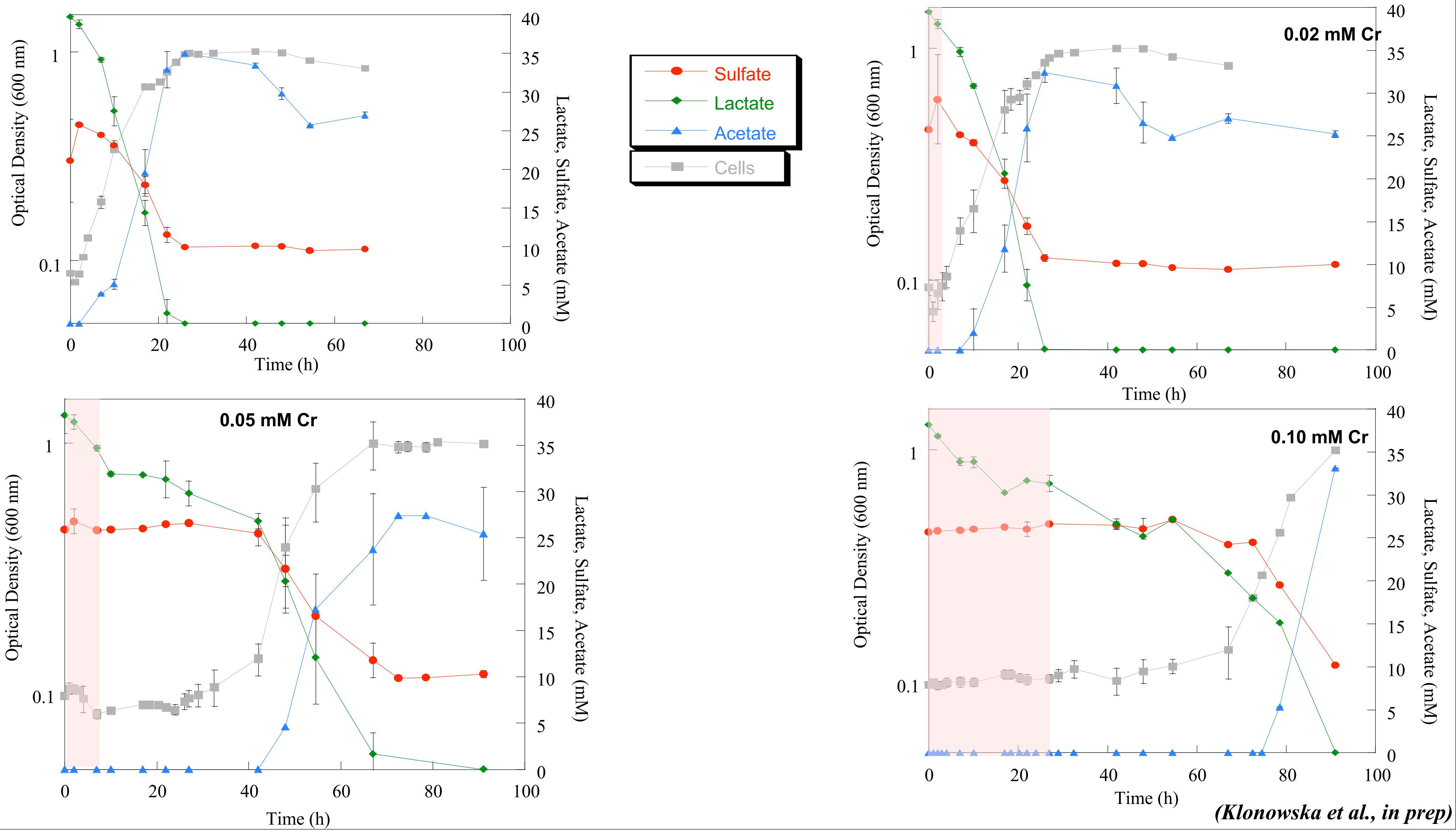
Energy production without growth in the presence of Cr(VI) (Chardin et al., 2002)

(acetate, sulfate, growth-??)

(re-establish E_h ?)

U(VI) inhibited SO_4-reduction (Elias et al., 2004)

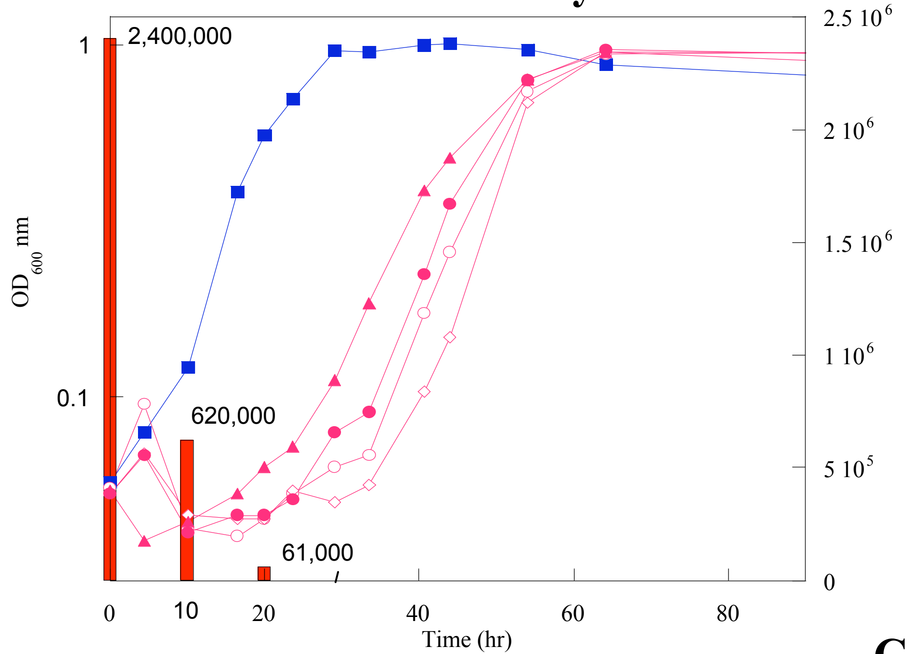
Growth Effects of Cr(VI) Exposure



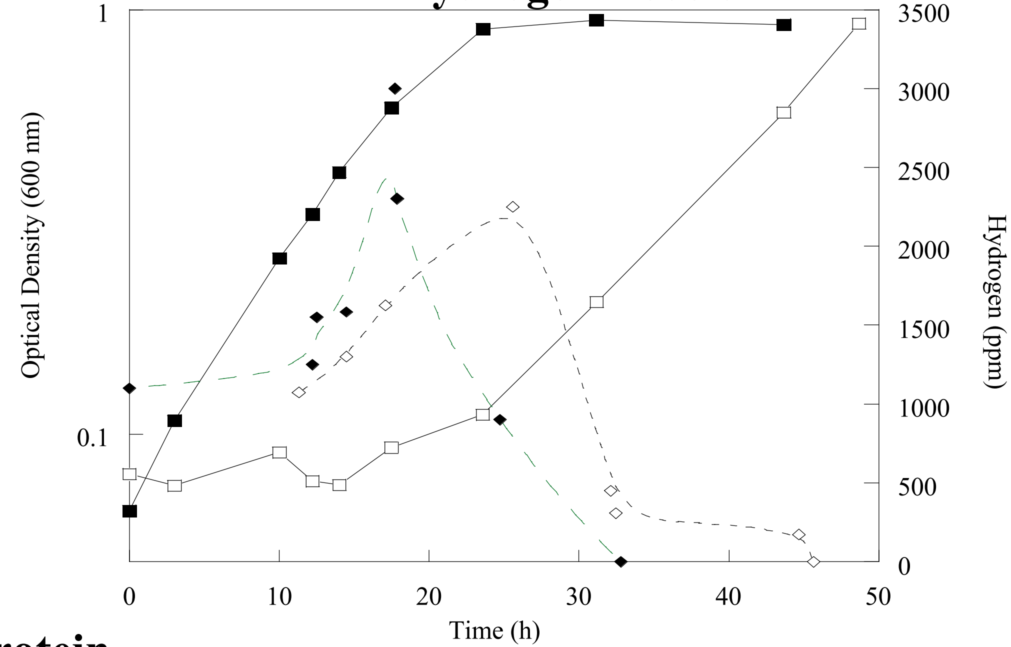
(Klonowska et al., in prep)

Growth Effects of Cr(VI) Exposure

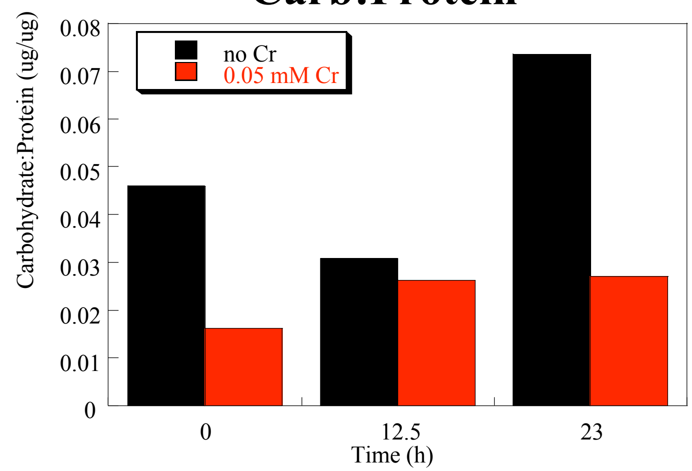
Cell Viability



Hydrogen Levels



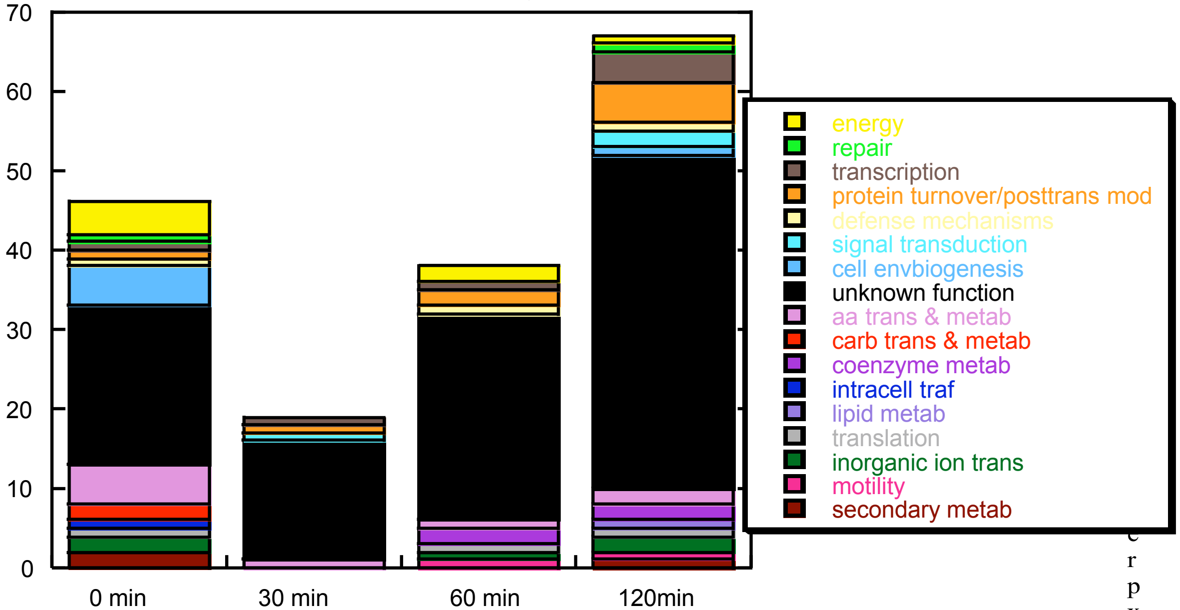
Carb:Protein



(Klonowska et al., in prep)

Cr(VI) Responses

Up-Expression by COGs

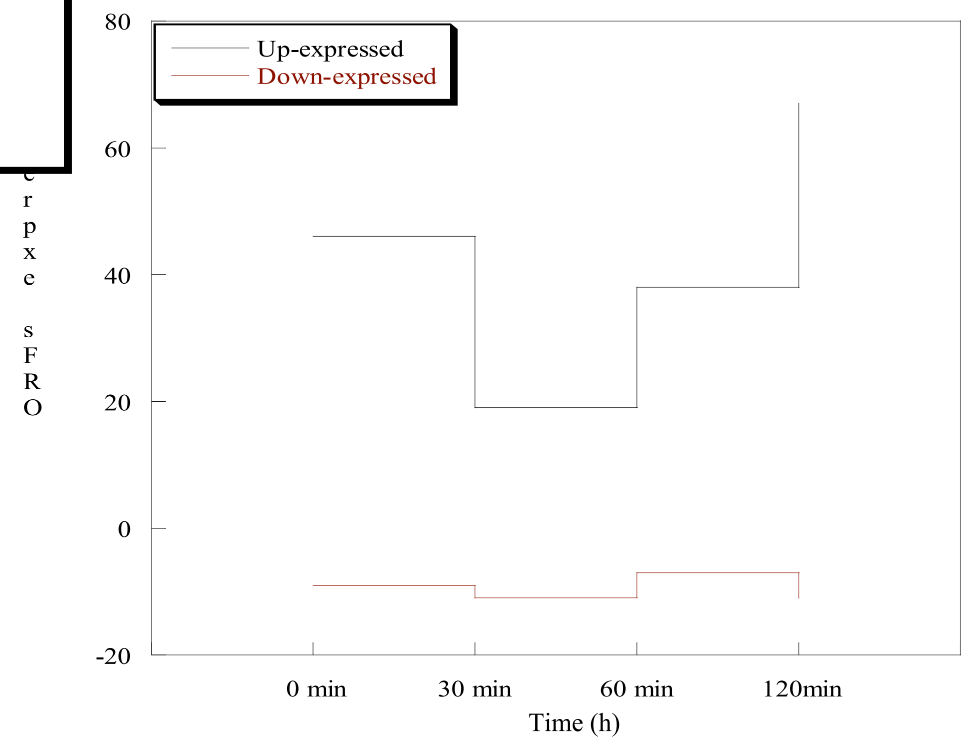


Expression levels decline (30 min):
Carbohydrate and amino acid metabolism; inorganic ions; envelope synthesis ; energy

Expression levels increase (60 min):
Coenzymes; translation; defense; protein turnover; energy

Expression levels increase (120 min):
protein turnover; defense; repair; signaling; envelope synthesis

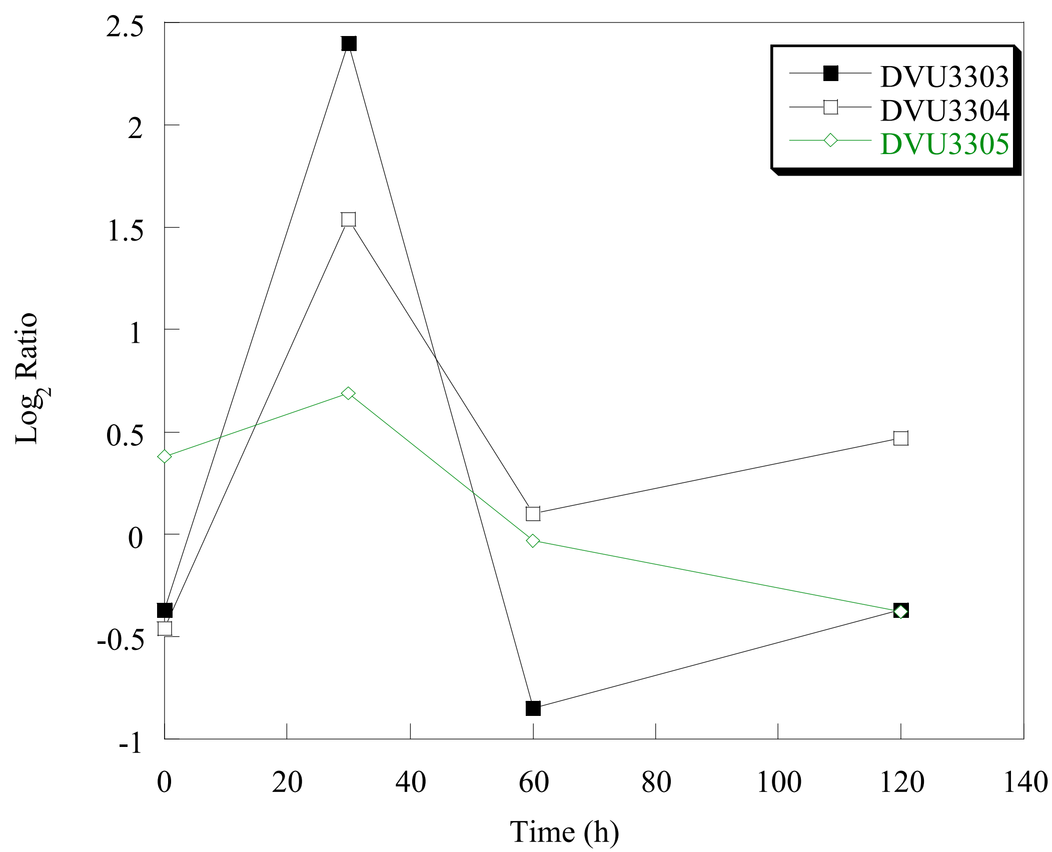
Temporal Up- and Down-Expression



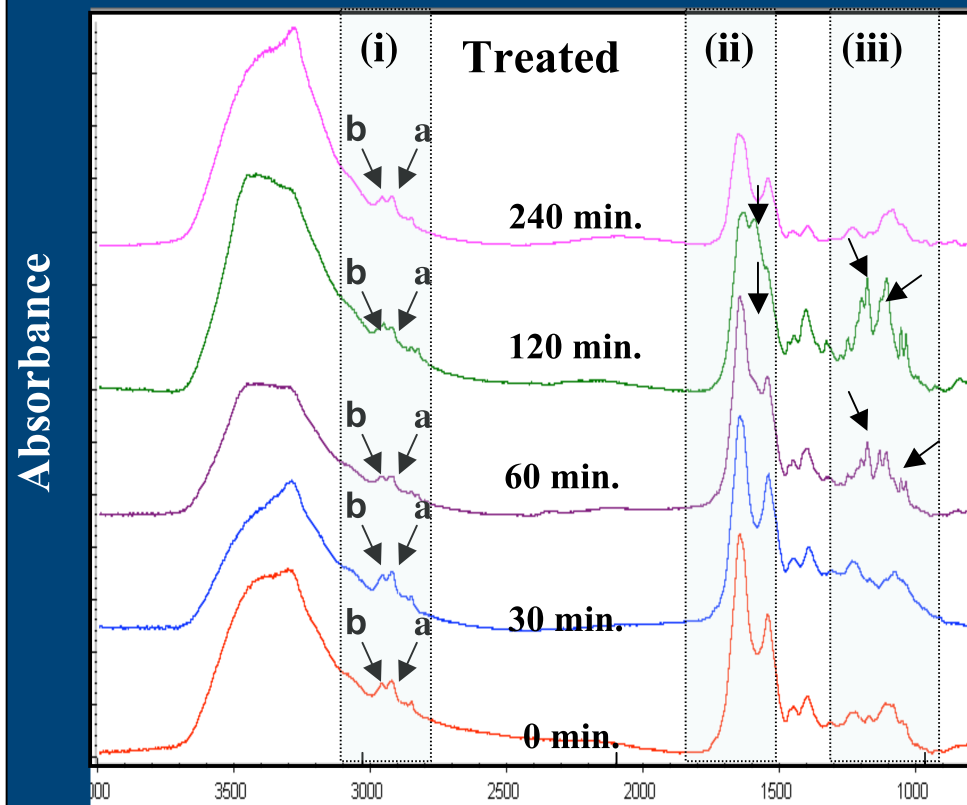
(Fields et al., in prep)

Cr(VI) Responses

Response-Regulator and Protease



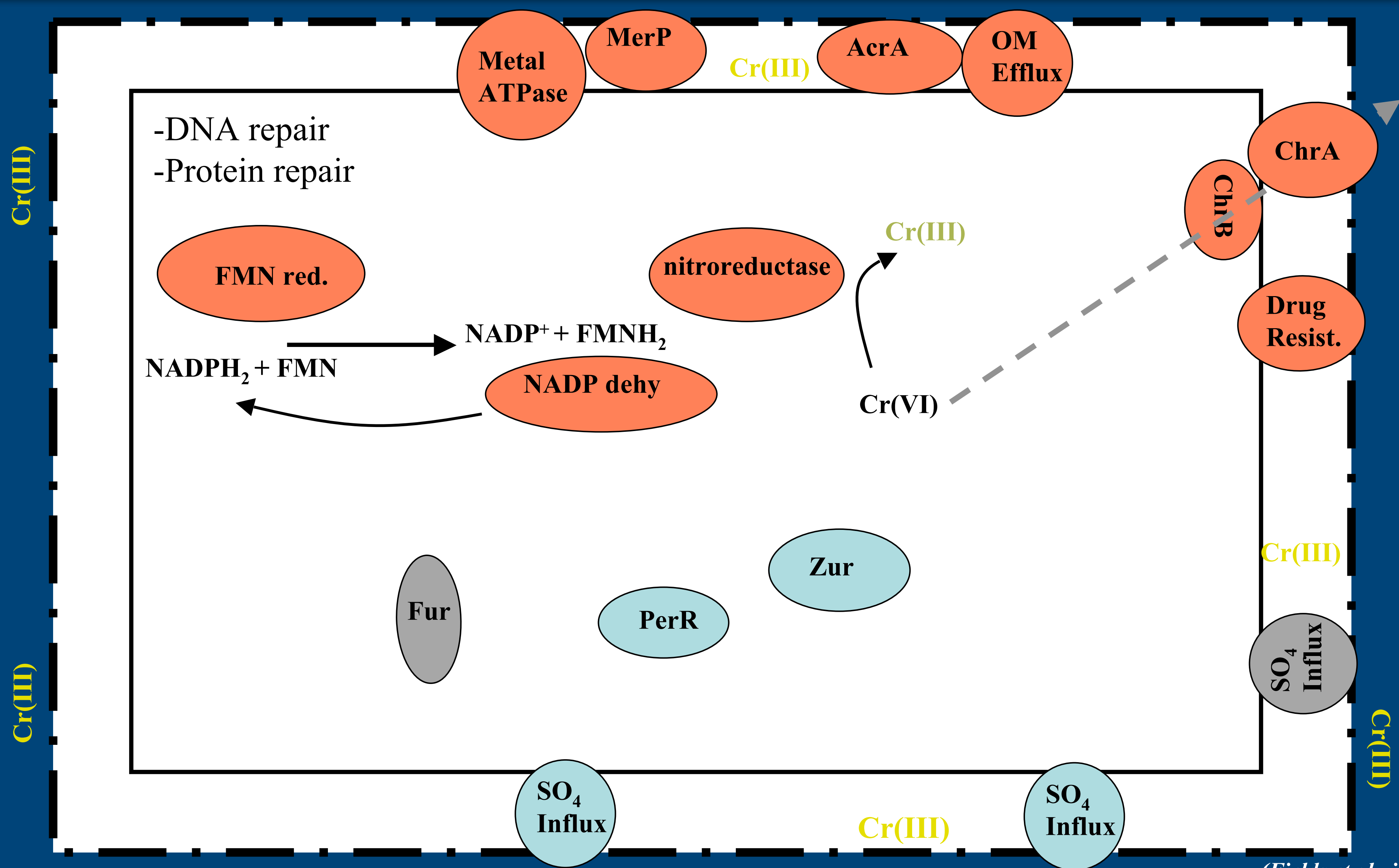
FTIR After Cr-Treatment



Holman et al.

- Changes in the membrane lipids
- Changes in secondary protein structures
- Changes indicative of the PO_2^- groups in nucleic acids
- Changes in the C-O-C and C-O-P groups in various oligo- and polysaccharides

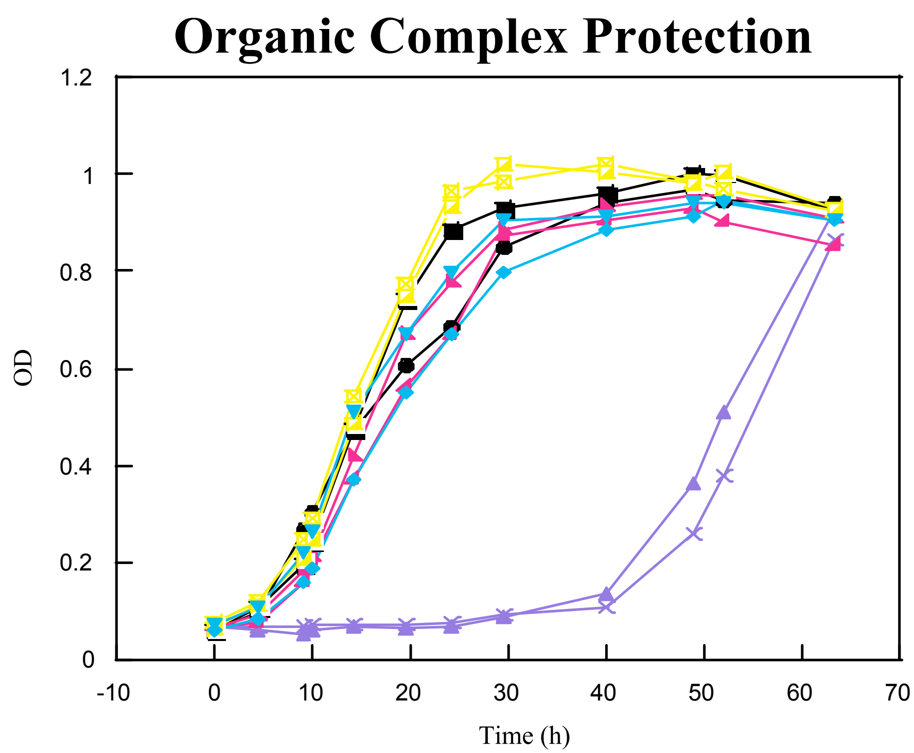
Model for Cr(VI) Responses Based Upon Transcriptomics



(Fields et al., in prep)

Cr(VI) Exposure and Organo-Ligand Protection

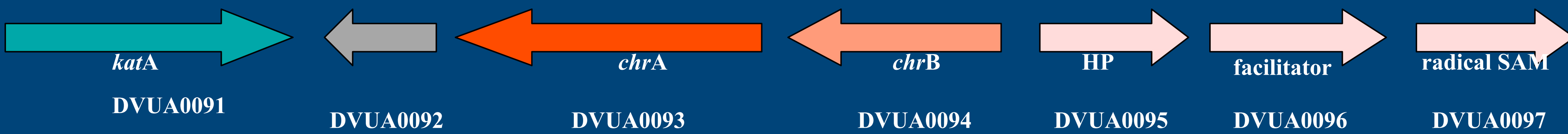
- Role(s) for 1e$^-$ and/or 2e$^-$ transfers to Cr(VI)
- Cr(III) is considered less toxic (?)
- Carbon routing to produce specific ligand (?)
- Non-specific Cr(III) adducts (?)

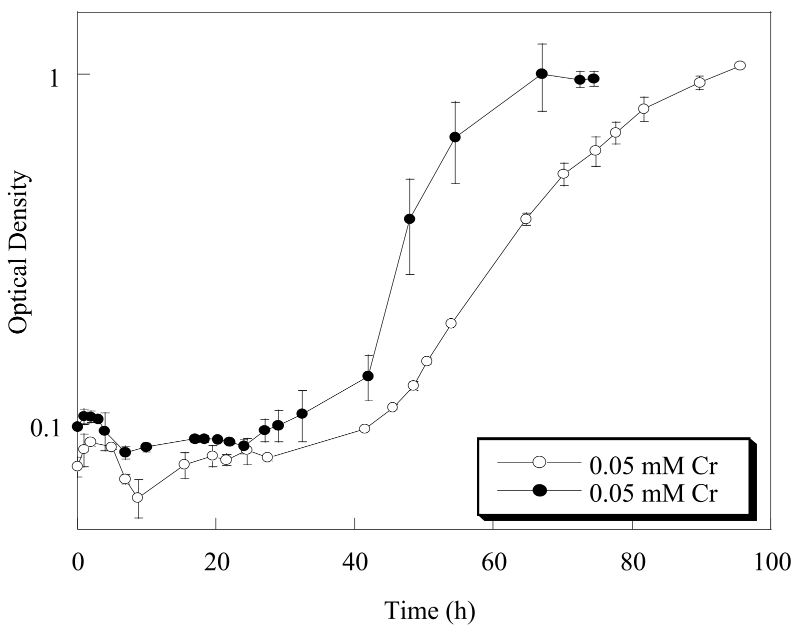


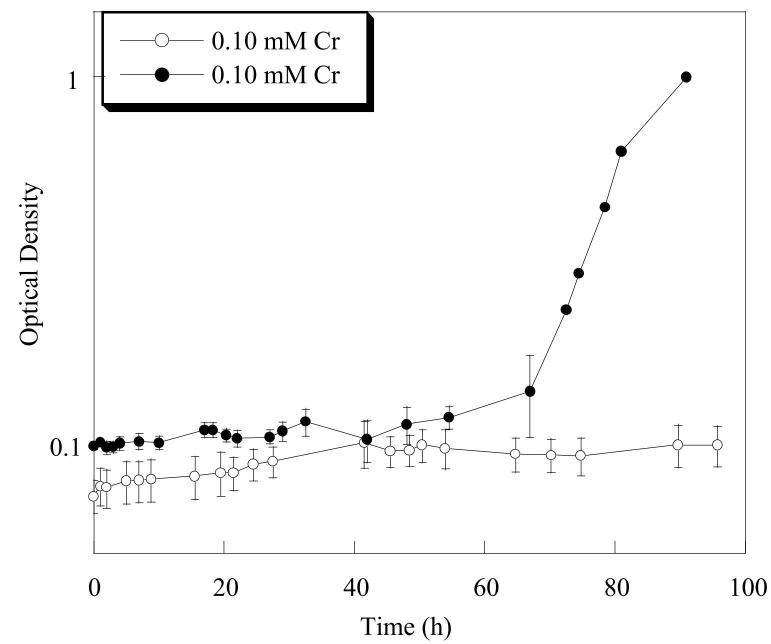
Ascorbate acts as a highly potent inducer of chromate mutagenesis via DSBs in epithelial cells (NAR 35:465-76. 2007).

(Klonowska et al., in prep)

*chr*AB, Megaplasmid, and Cr(VI) Tolerance



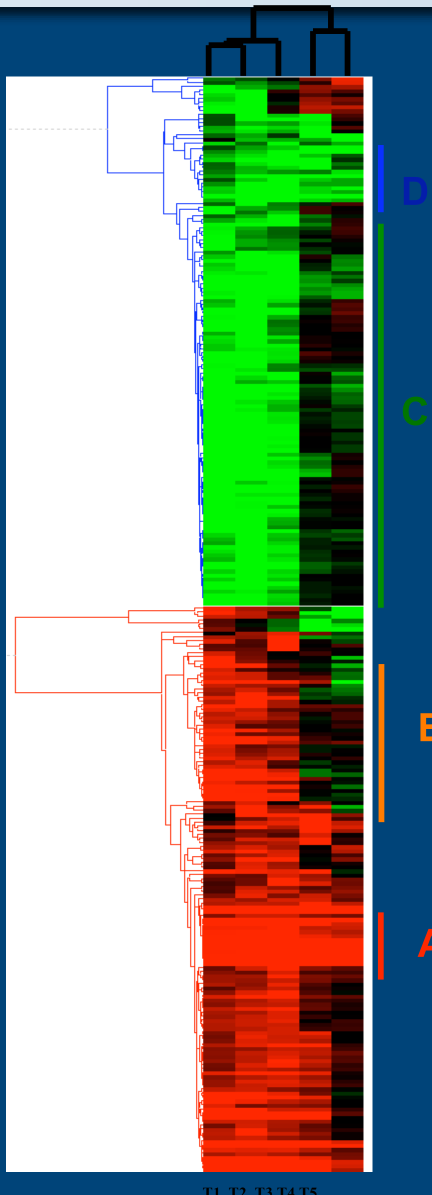




• Strain without the megaplasmid is more susceptible to Cr(VI) exposure

(Fields et al., in prep)

NO_2 Exposure



DVU0775 ATP synthase F1 beta subunit
DVU0776 ATP synthase F1 gamma subunit
DVU0777 ATP synthase F1 alpha subunit
DVU0778 ATP synthase F1 delta subunit
DVU0917 ATP synthase F0, C subunit
DVU0918 ATP synthase F0, A subunit
DVU1286 reductase, transmembrane subunit, putative
DVU1287 reductase, iron-sulfur binding subunit, putative
DVU1290 nitrate reductase, gamma subunit, putative

↓ ATPase synthase
↓ NO_3 reductase

DVU0927 ribosomal protein L21
DVU1211 ribosomal protein L28
DVU1303 ribosomal protein L3
DVU1310 ribosomal protein L16
DVU1319 ribosomal protein L18
DVU1574 ribosomal protein L25
DVU2518 ribosomal protein L13
DVU2924 ribosomal protein L11
DVU2925 ribosomal protein L1
DVU2926 ribosomal protein L10

↓ ribosomal proteins

DVU0303 hypothetical protein
DVU0304 hypothetical protein
DVU2383 tonB dependent receptor domain protein
DVU2571 ferrous iron transport protein B
DVU2572 ferrous iron transport protein A, putative
DVU2573 hypothetical protein
DVU2574 ferrous iron transport protein
DVU2680 flavodoxin

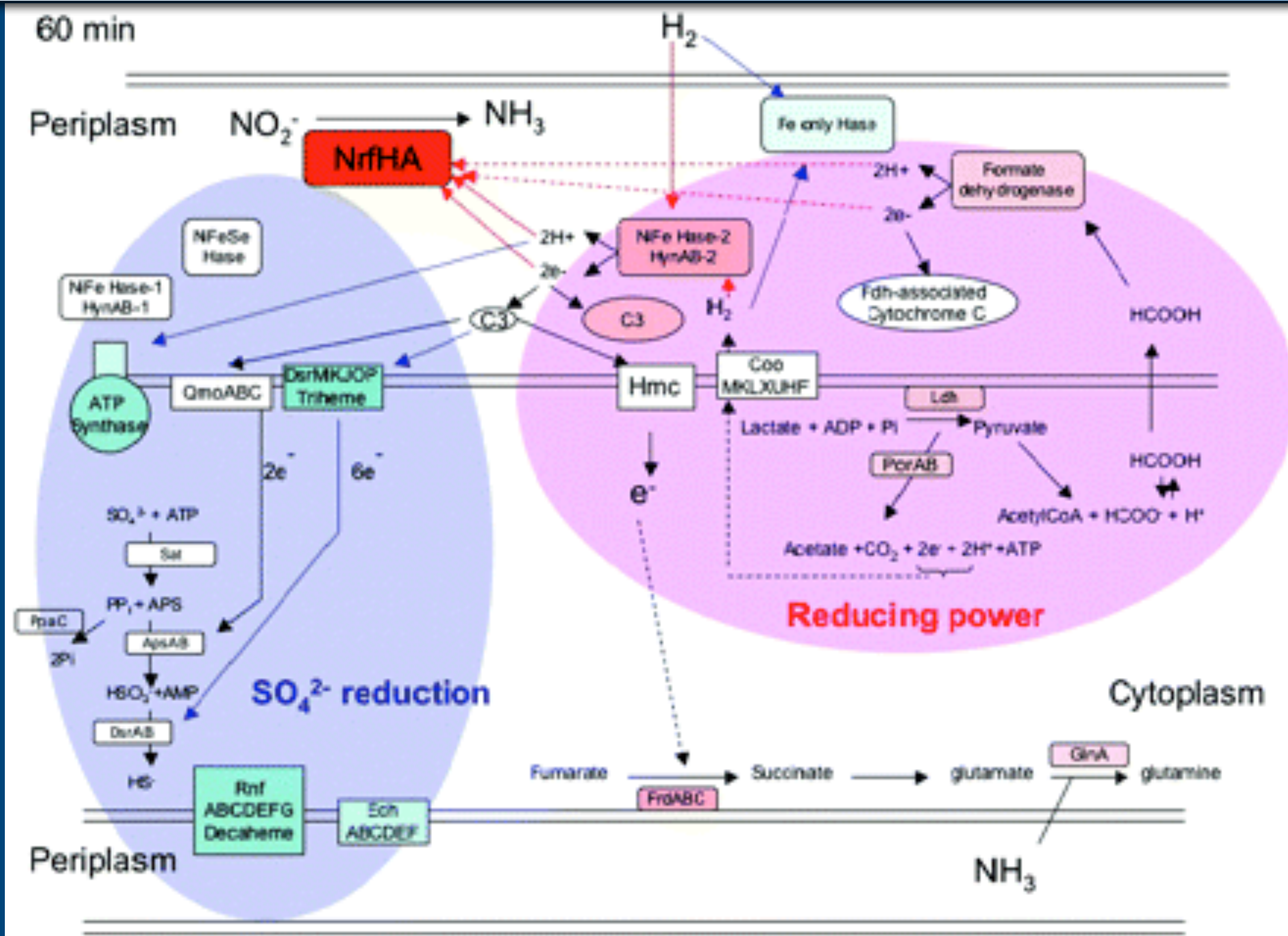
↑ HP
↑ *feo*AB
↑ Fe(II) transport

DVU0624 NapC/NirT cytochrome *c* family protein
DVU0625 cyt *c* nitrite reductase, catalytic subunit NfrA
DVU1080 iron-sulfur cluster-binding protein
DVU1081 iron-sulfur cluster-binding protein
DVU2543 hybrid cluster protein
DVU2544 iron-sulfur cluster-binding protein

↑ NO_2 reductase
↑ cytochrome

(He et al., 2006)

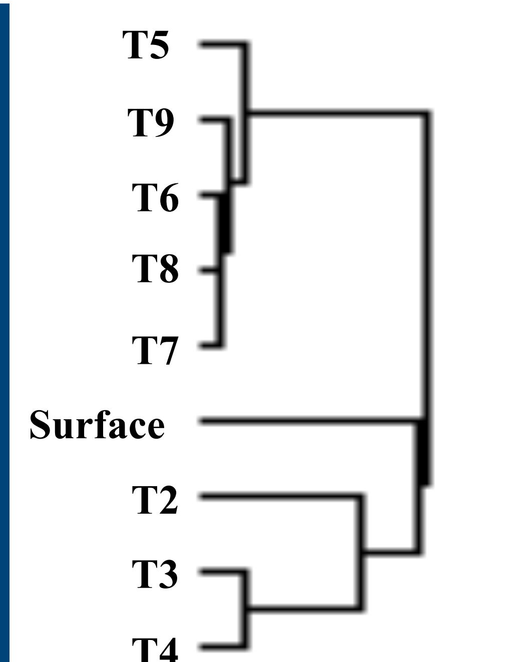
NO_2 Exposure



(He et al., 2006)

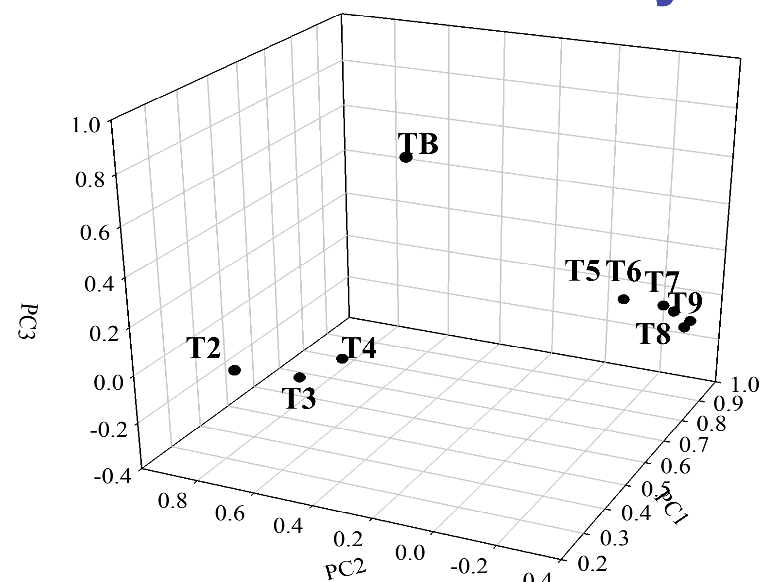
Gene	Name	Expression
DVU3025	*por*	-3.09
DVU3027	*glc*D	-2.06
DVU3030	*ack*A	-2.62
DVU2286	*coo*M	-4.30
DVU2288	*coo*L	-3.68
DVU2289	*coo*X	-3.00
DVU2290	*coo*U	-3.12
DVU2291	*coo*H	-3.13
DVU2291	*hyp*A	-1.97
DVU2293	*coo*F	-1.98
DVU1944	*oor*D	2.16
DVU1945	*oor*A	1.96
DVU1946	*oor*B	1.58
DVU2792	*rnf*C	1.97
DVU2793	*rnf*D	2.61
DVU2794	*rnf*G	2.70
DVU2795	*rnf*E	1.71
DVU2796	*rnf*A	2.51
DVU2797	*rnf*B	1.42
DVU2798	*apb*E	2.94

Metabolic Differentiation Between Surface-Adhered and Planktonic Populations



Cluster Analysis

Factor and PC Analysis

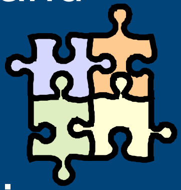


(Fields et al., in prep)

More to Heavy Metal Stress than Just Heavy Metal

What can organismal biology and ecology do for mineral and contaminant biotransformation?

If one wants to understand and predict carbon and energy utilization (mass balance)----then we need to understand how cells respond to stressful conditions by altering carbon and energy flow.



At the cellular level and upward through the community.

What we want and what the bugs want may be two different things. Bug wants: grow efficiently - increase biomass – reproduce

Our wants: efficient activity of interest with minimal input

More biomass? More reducing power? More carbon? What type of precipitates? Aqueous-phase or the solid-phase? How do microbes affect flow paths?

The more we know about how the cell (community) works as a system----the more we will be able to predict and control.

Biochemical capacity f(t) and f(p)

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