Title
Application of Proteomics and Lipid Studies in Environmental Biotechnology

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Application of Proteomics and Lipid studies in Environmental Biotechnology

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ACKNOWLEDGEMENT

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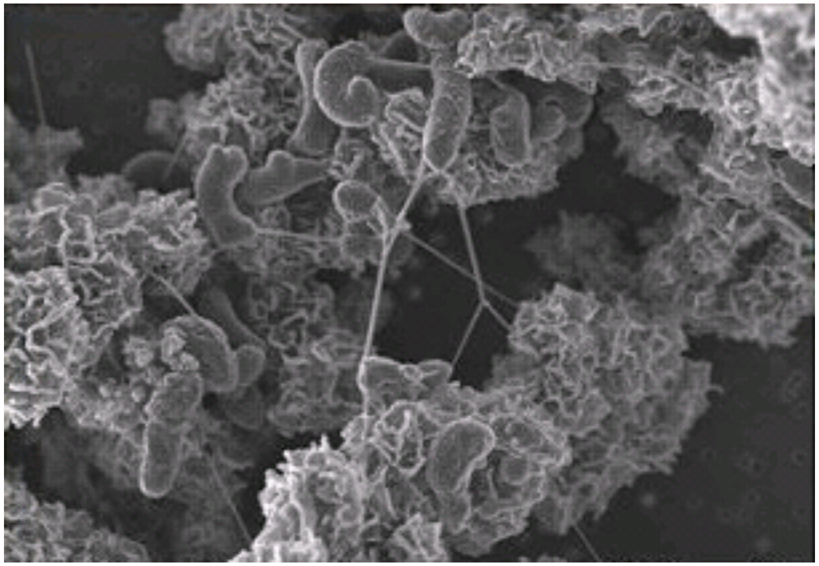
Microbial metal reduction



Desulfovibrio vulgaris

Shewanella oneidensis

$$U^{(VI+)} \xrightarrow{\text{Microbial metal reduction}} U^{(IV+)}$$

(Soluble in water)

(insoluble mineral uraninite)

Figure 1: *Desulfovibrio vulgaris* Biofilm on metal surface (Matthew Fields, Montana state university)

Figure 2: *Shewanella oneidensis* CN32 cells on the surface hematite (Alice Dohnalkova, Pacific Northwest National Laboratory)

Sulfate and Metal reduction: Biofouling

Pollution 99

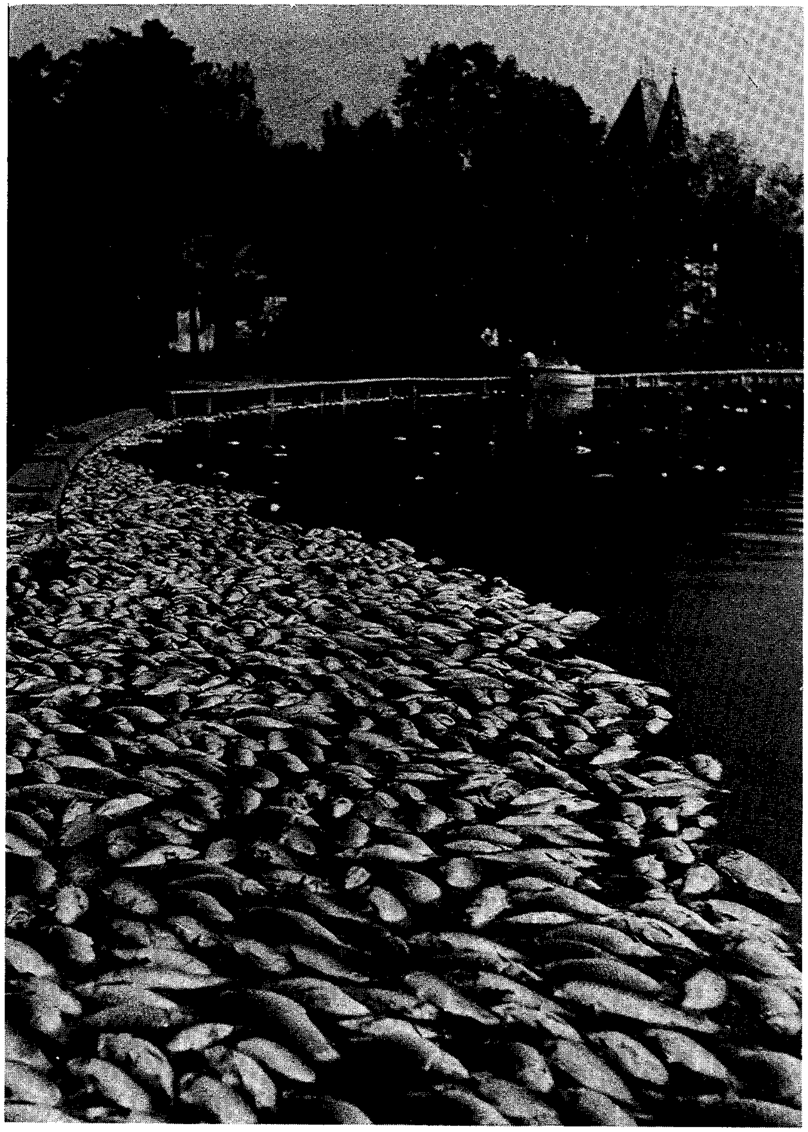


Fig. 16. Fish killed by sulphide pollution. Dead carp killed by biogenic H_2S as a result of pollution in Lake Palic, Yugoslavia, in 1971 (courtesy of Dr R. Vamos).

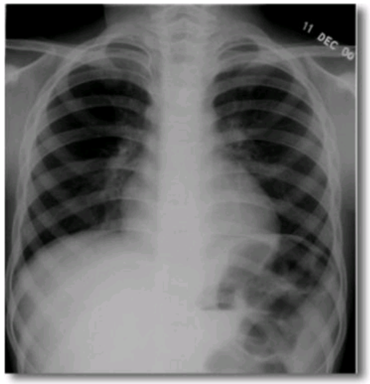
- Biomagnification of mercury – Methyl mercury formation.
- Biocorrosion
- Biofouling of crude oil

Radioactive and heavy metal waste management

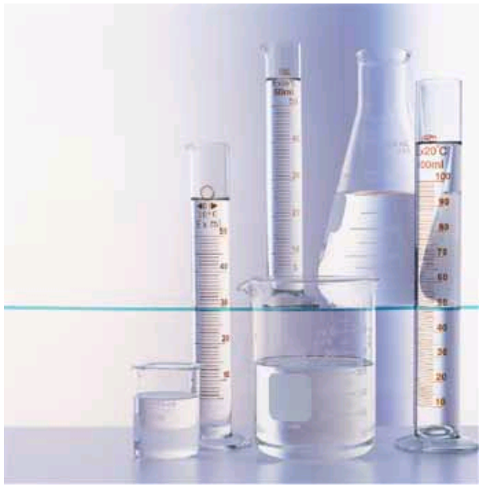
Mining

Stored legac

Power Plants



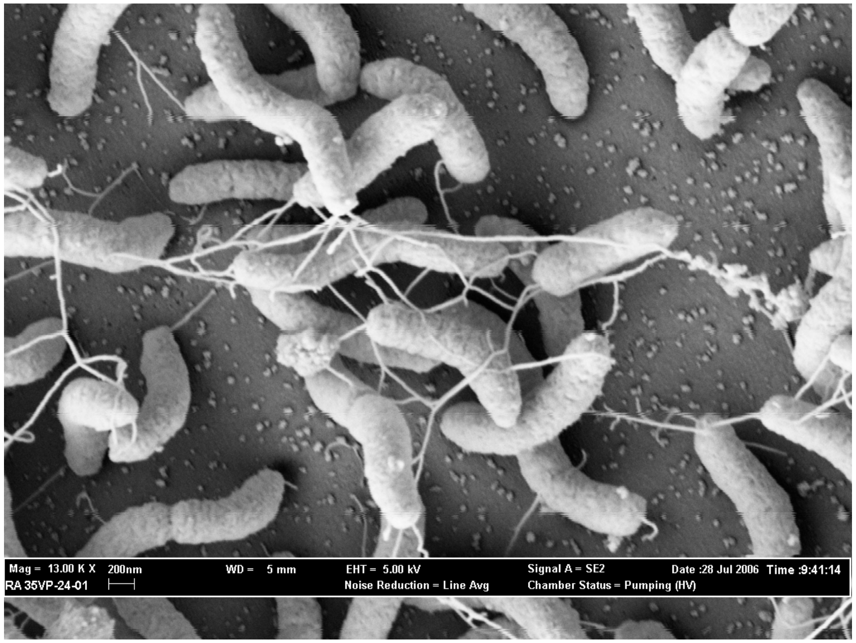
Medicine



Research

Figure credits
www.greatbasinminewatch.org
www.fas.org/irp/imint/doe_hanford_fftf_01c.jpg
http://nc.sierraclub.org/images/NuclearPowerPlant.jpg

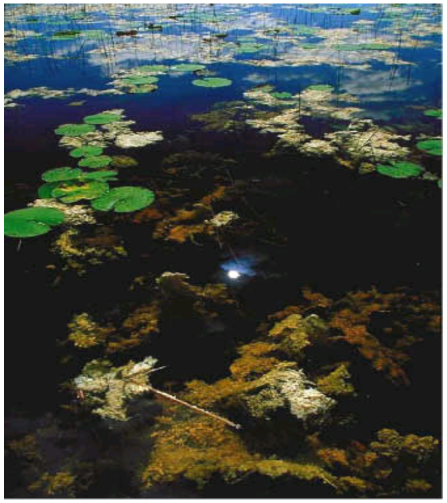
Desulfovibrio vulgaris Hildenborough



- Sulfate reducing bacterium
- Anaerobic organism
- Found in heavy metal and nuclear waste sites
- Genome was sequenced in 2003

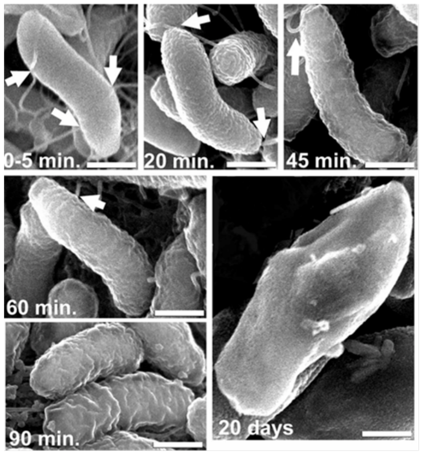
Heidelberg Nat. Biotechnol **22:**554-9

Using Environmental Microbes



Understand the affect of environmental factors on bioremediation potential of *D. vulgaris*

Apply this knowledge to accelerate the bio-containment of heavy metal waste or limit adverse impact of such organisms in the environment

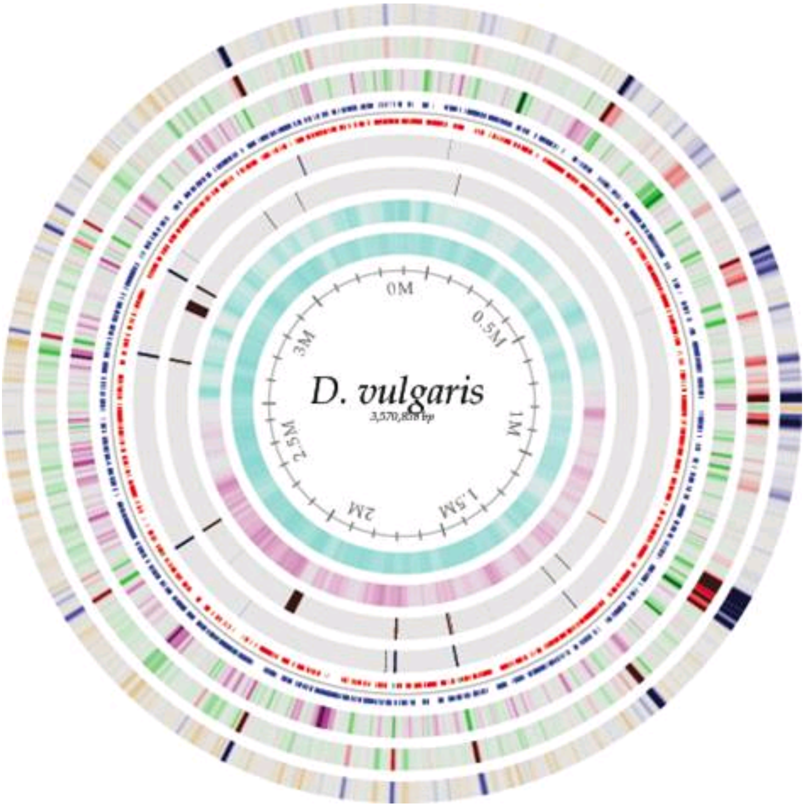


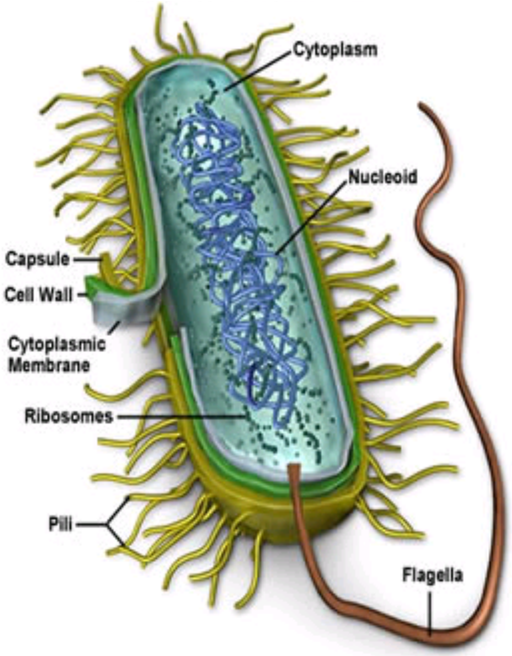
Exposure to Air

Physiologically Relevant environmental stresses

- Salt
- pH
- Oxygen
- Nitrate
- Heavy metals

Cell wide data ??





Genome sequence indicates
the presence of 3480 genes

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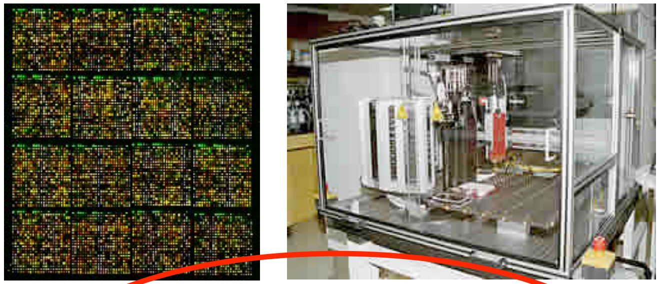
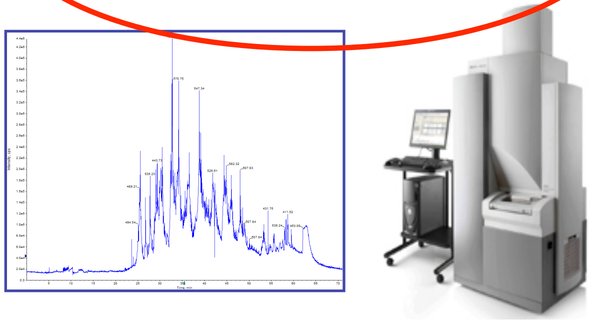
Functional genomics pipeline

Biomass production

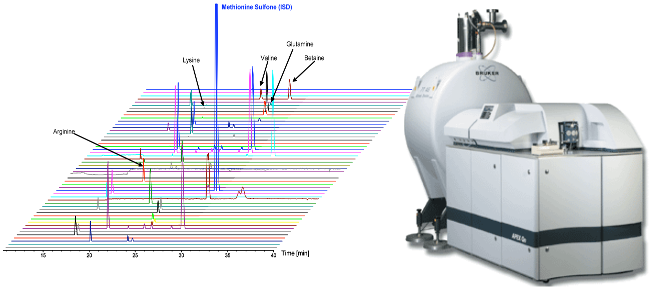
Transcripts



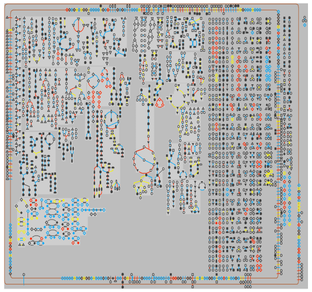
Proteins/ lipids



Metabolites



Analysis

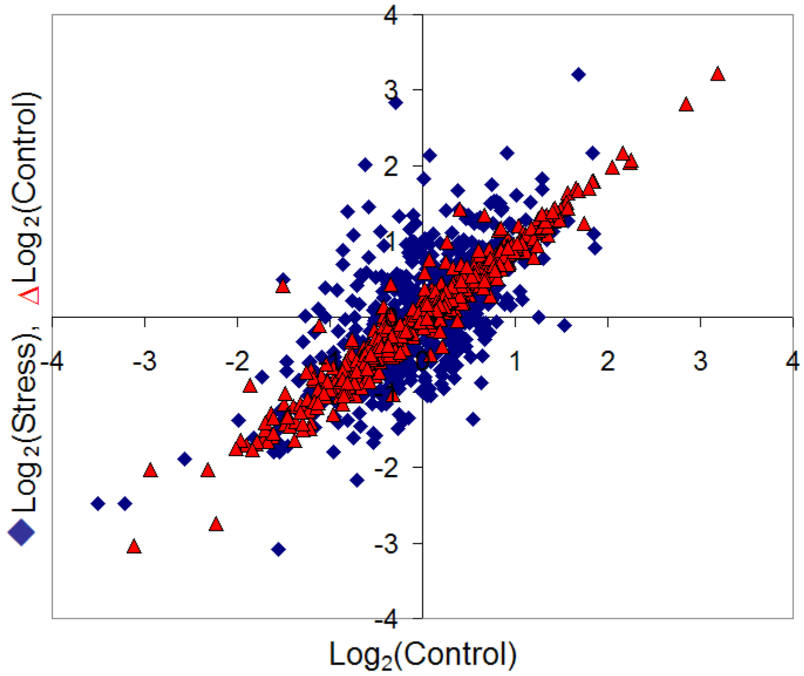


Why proteomics?

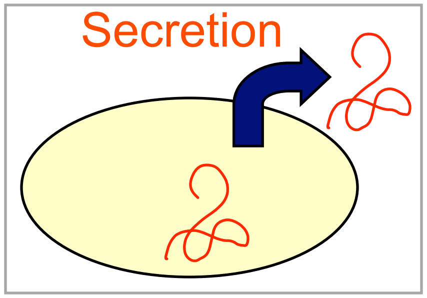
Proof of expression/ Change

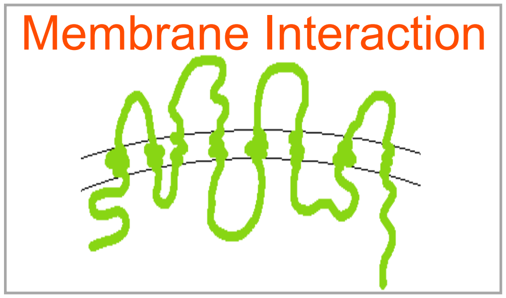
Activation State

Differential Expression:

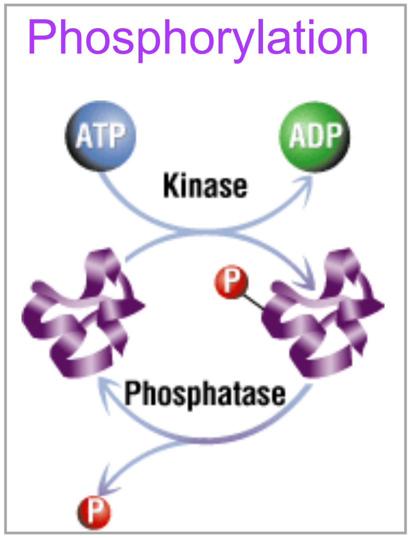


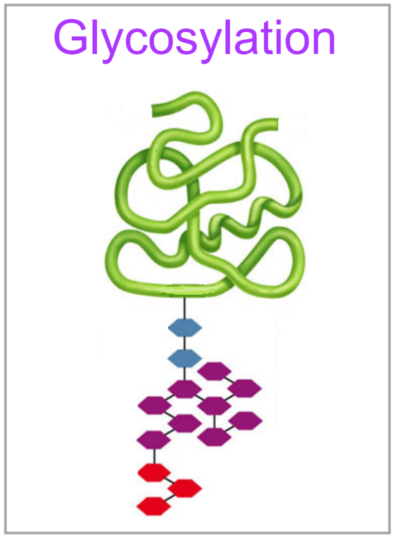
Localization:





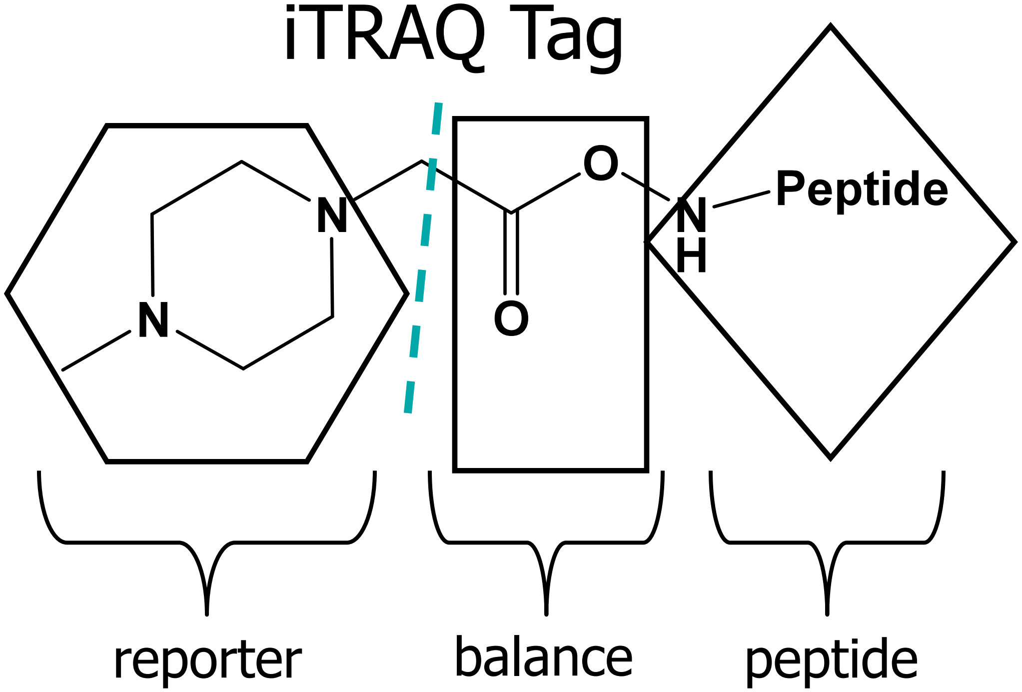
Post translational modifications:

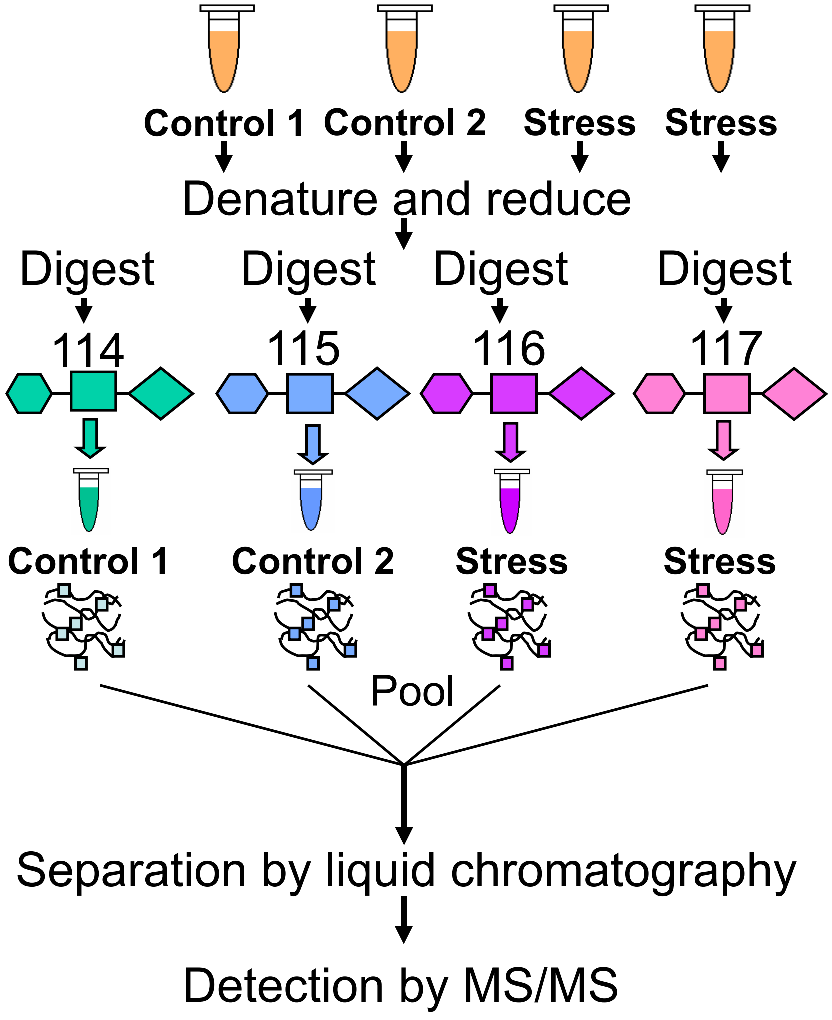




iTRAQ Peptide Labeling Strategy

iTRAQ Tag





Control 1 **Control 2** **Stress** **Stress**

Denature and reduce

Digest Digest Digest Digest

Control 1 **Control 2** **Stress** **Stress**

Pool

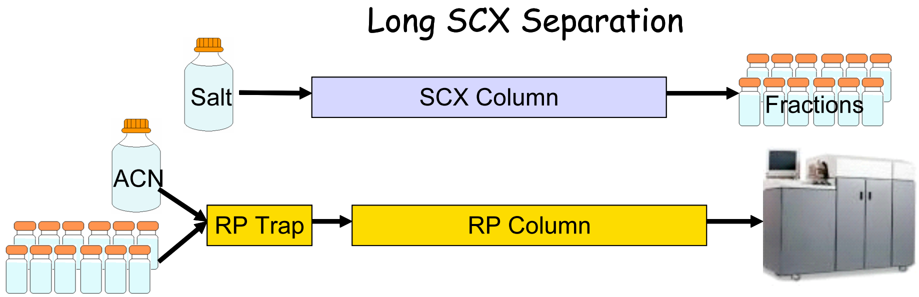
Separation by liquid chromatography

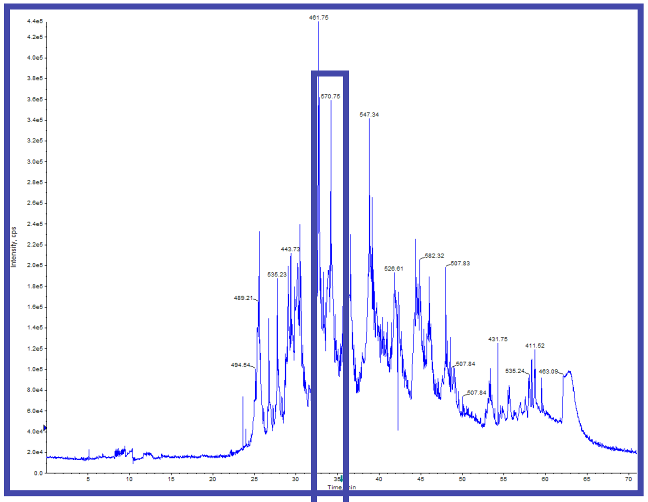
Detection by MS/MS

Mass	Reporter	Balance
114	^{13}C	^{13}C, ^{18}O
115	$^{13}C_2$	^{18}O
116	$^{13}C_2$, ^{15}N	^{13}C
117	$^{13}C_3$, ^{15}N	--

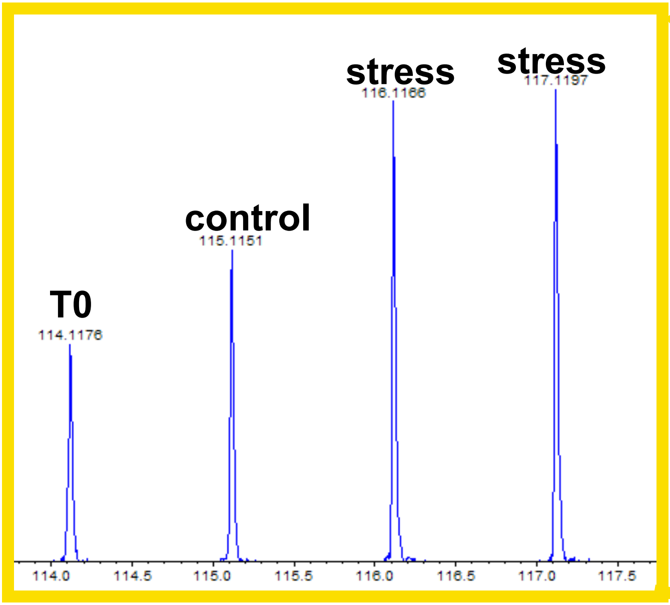
Ross PL, et al., Mol Cell Proteomics, 2004; 3(12):1154-69

Tandem LC-MS Proteomics

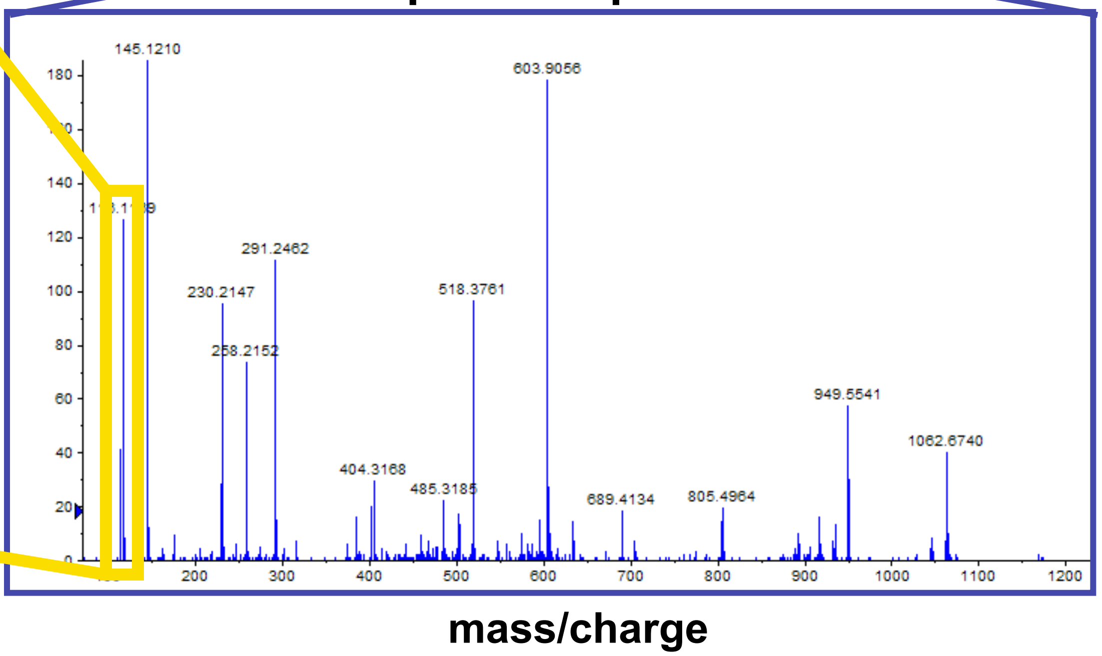




Quantitation

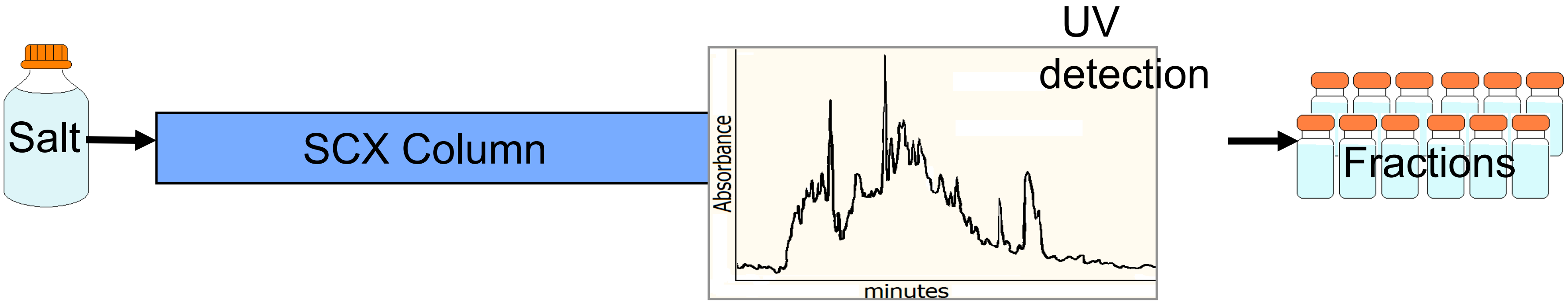


Peptide Sequence



2-D Liquid Chromatography

Strong Cation Exchange (SCX) Separation

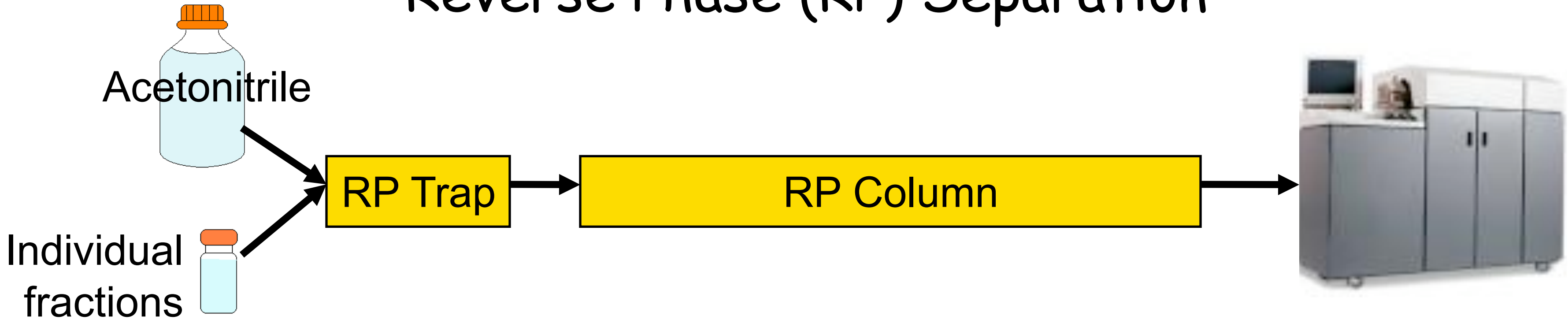

Salt

SCX Column

UV detection

Fractions

Reverse Phase (RP) Separation


Acetonitrile

Individual fractions

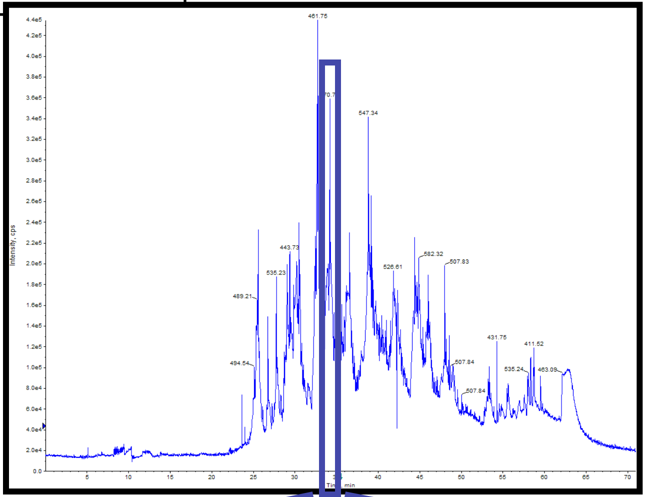
RP Trap

RP Column

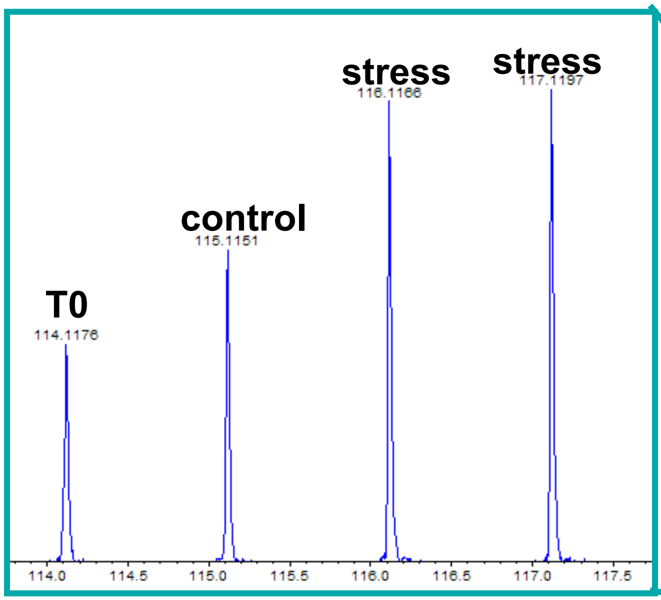
Wolters DA, Washburn MP, Yates JR, 3rd. Anal Chem, 2001; 73(24)):5683-90

Comparative Proteomics

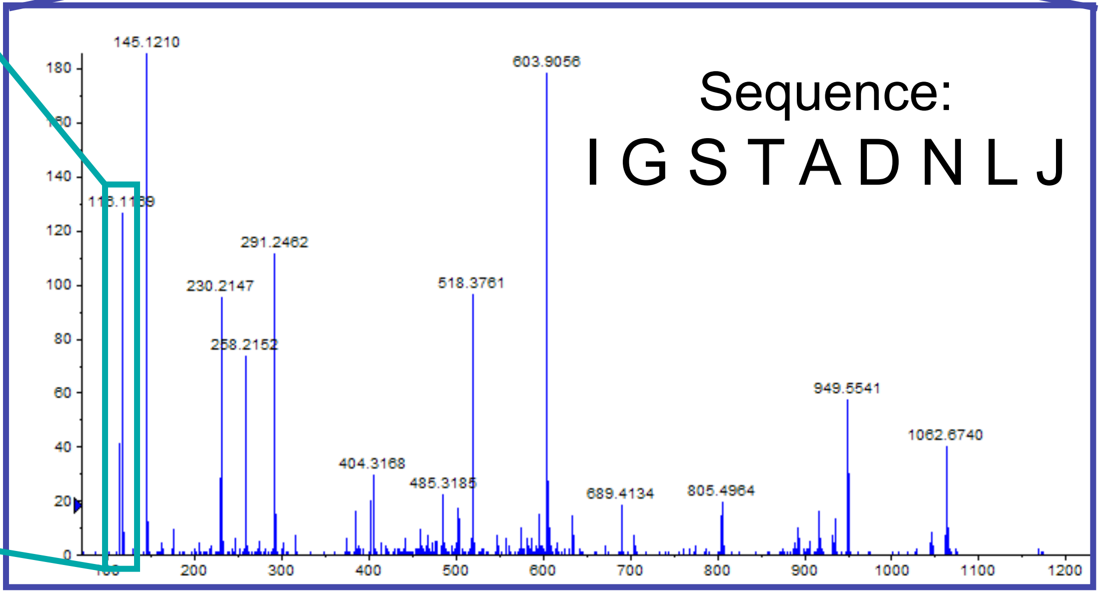
Total Ion Chromatogram



Quantitation



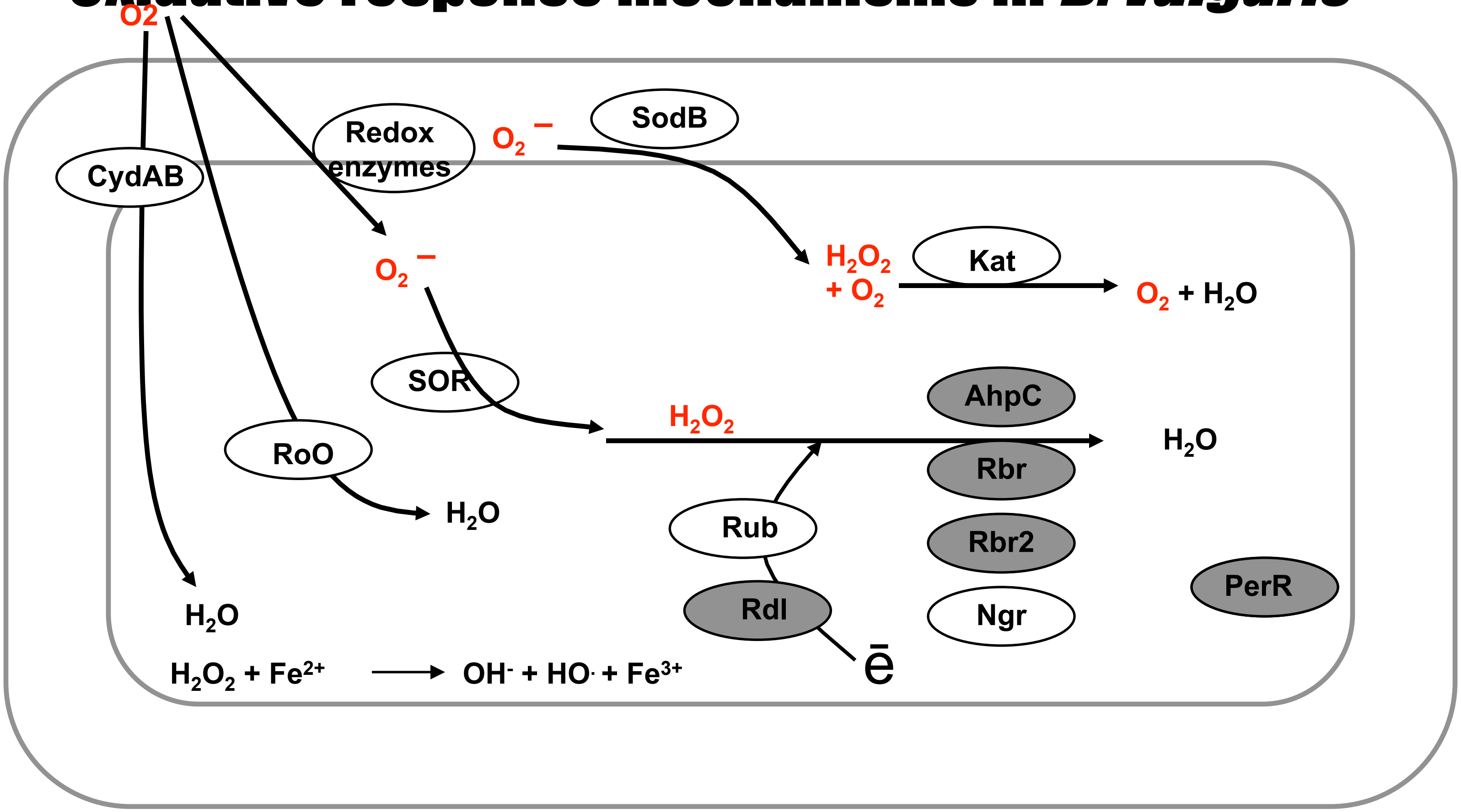
Peptide Sequence



Sequence:
I G S T A D N L J

mass/charge

Oxidative response mechanisms in *D. vulgaris*



$O2$

CydAB

Redox enzymes

O_2^-

SodB

O_2^-

$H_2O_2 + O_2$

Kat

$O_2 + H_2O$

SOR

H_2O_2

AhpC

Rbr

Rbr2

Ngr

H_2O

RoO

H_2O

Rub

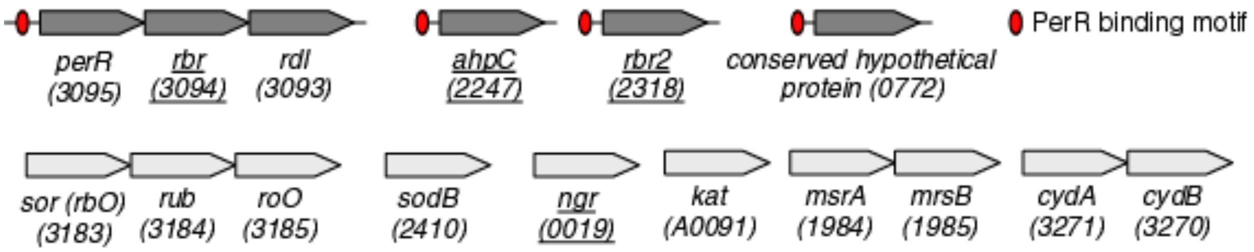
Rdl

ē

PerR

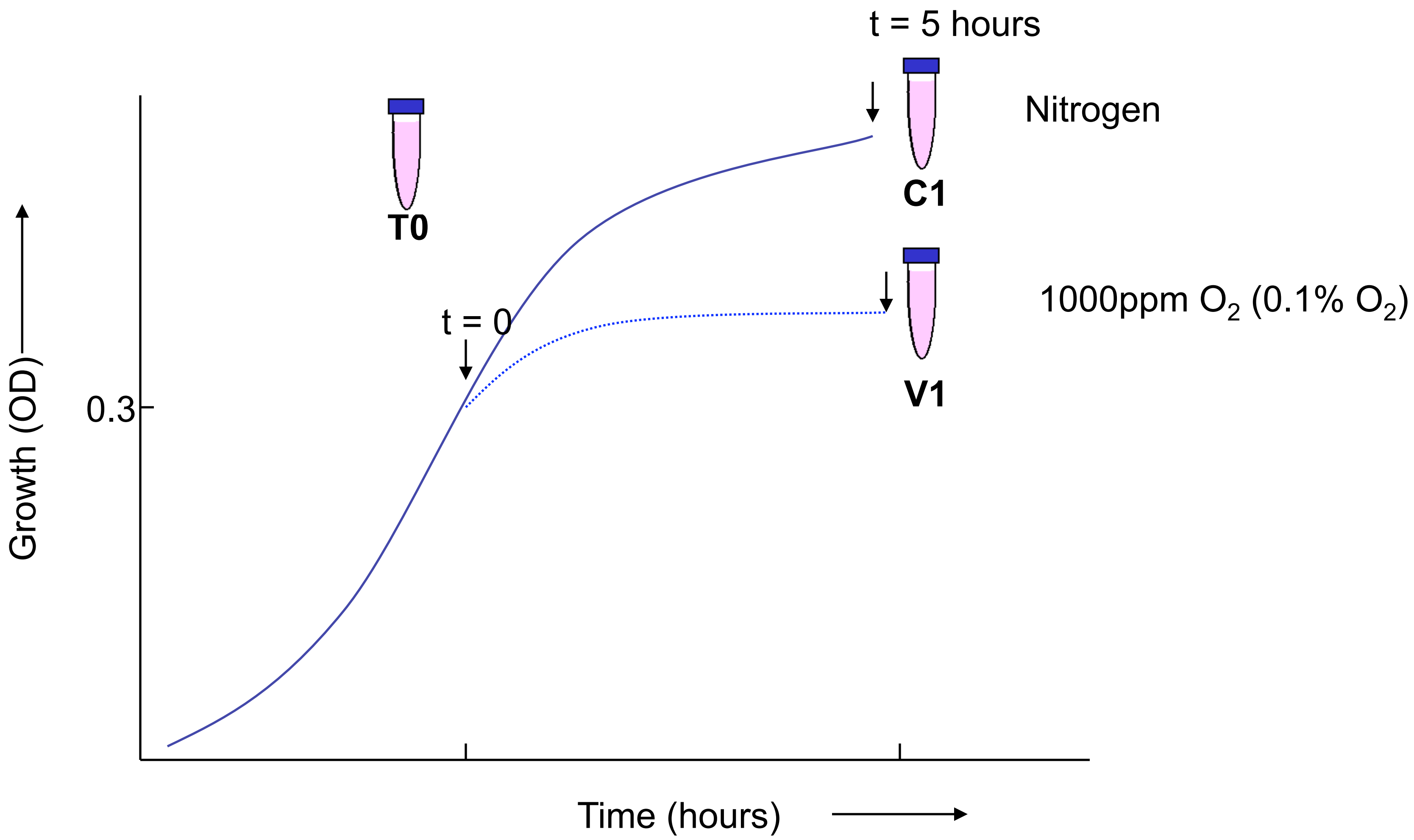
H_2O

$H_2O_2 + Fe^{2+} \longrightarrow OH^- + HO^\cdot + Fe^{3+}$

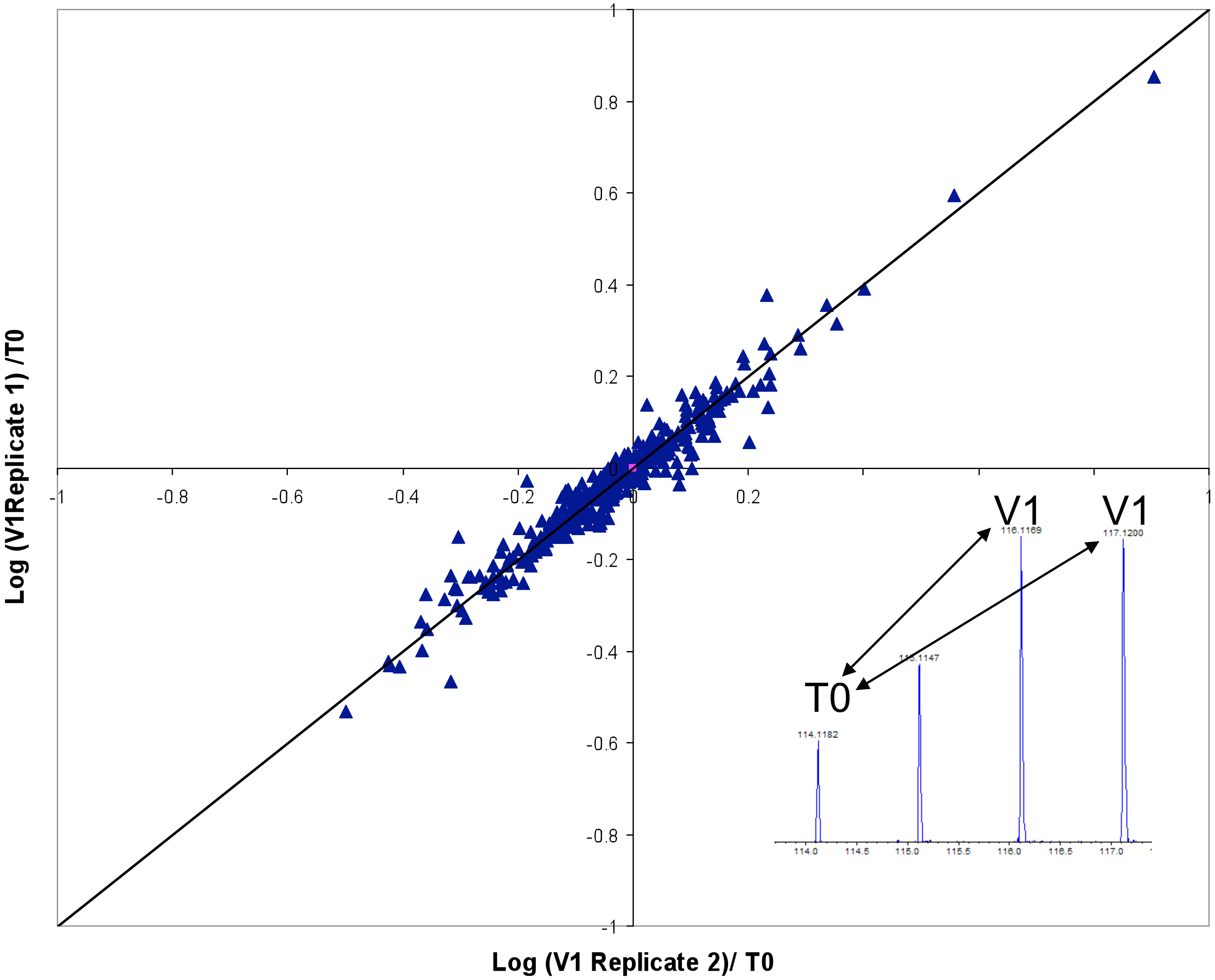


Lumppio et al J Bacteriol (2001) 101-8; 183
Dolla et al J Biotechnol **126:**87-100
Rodionov Genome Biol **5:**R90.

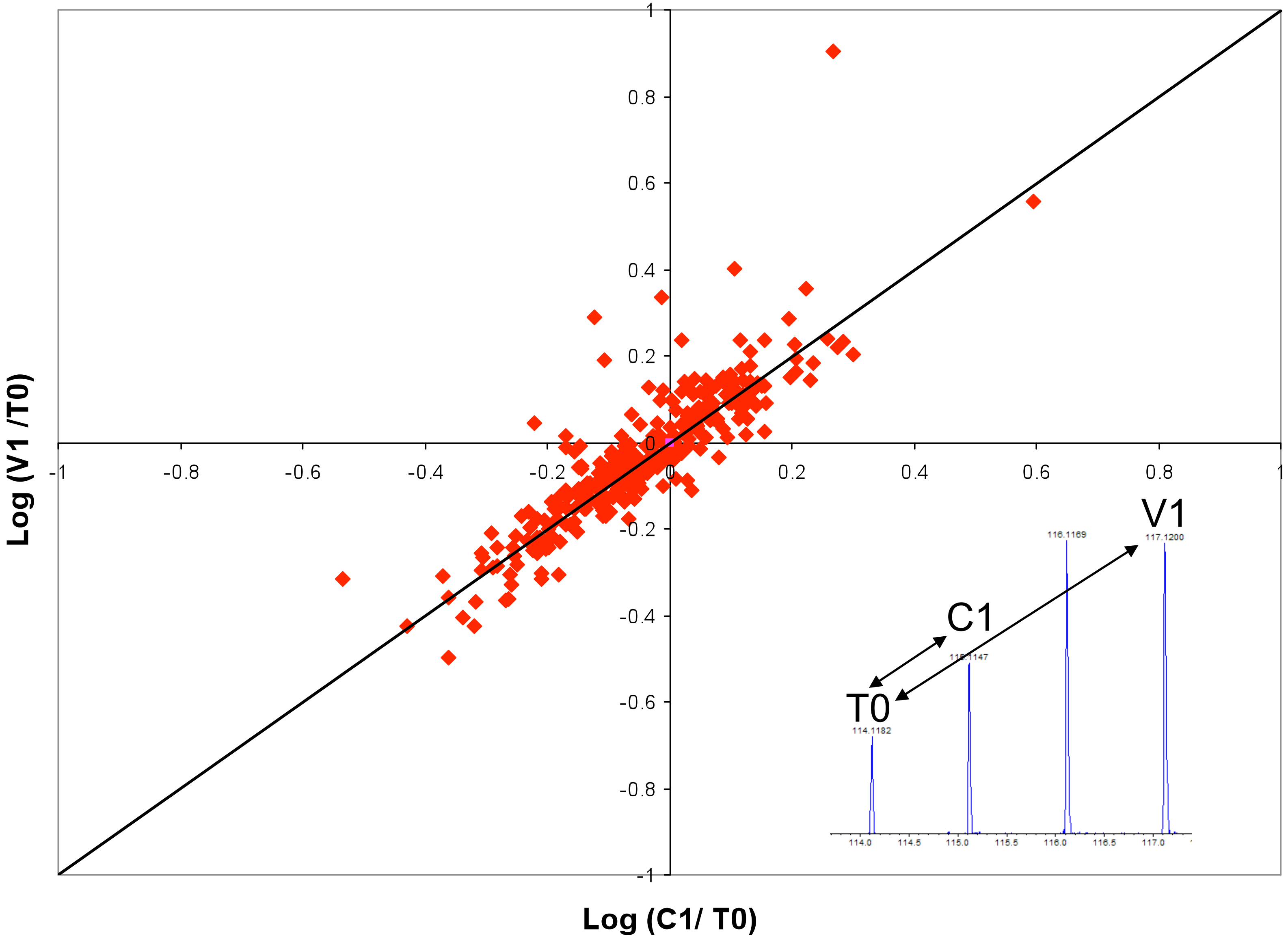
iTRAQ analysis of 0.1% O_2 exposure in *D. vulgaris*



Use of internal replicates



Comparison of Stressed and Control



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Use of error to determine significant changers

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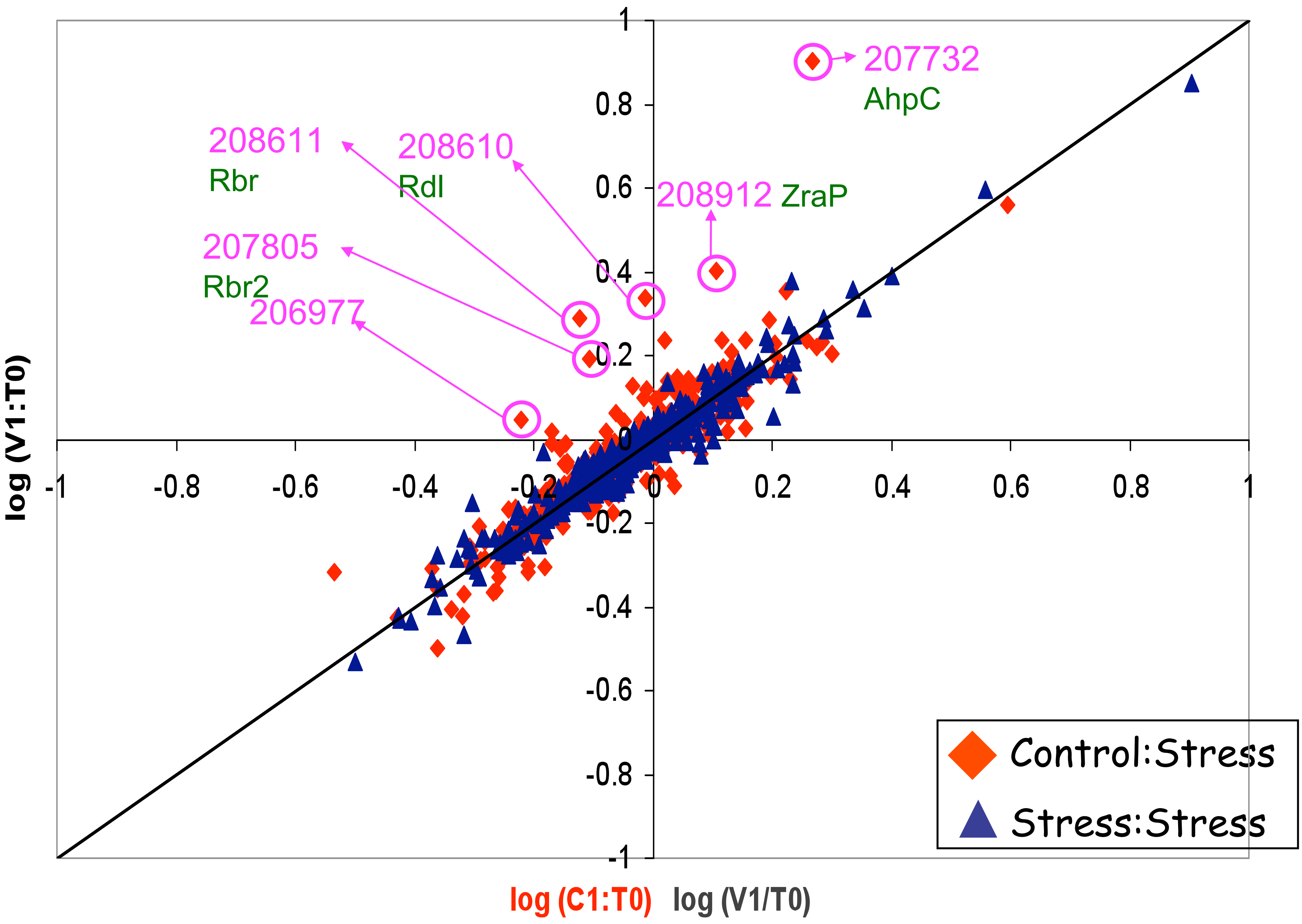
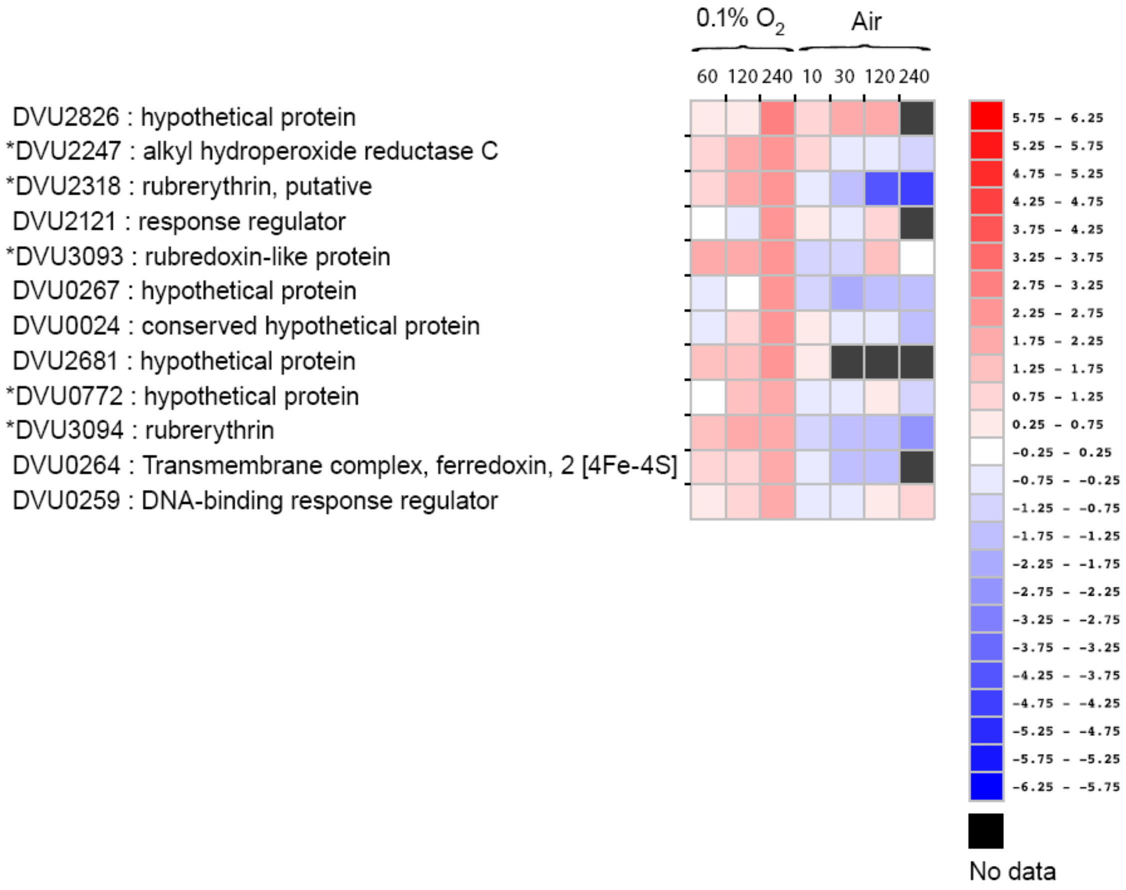
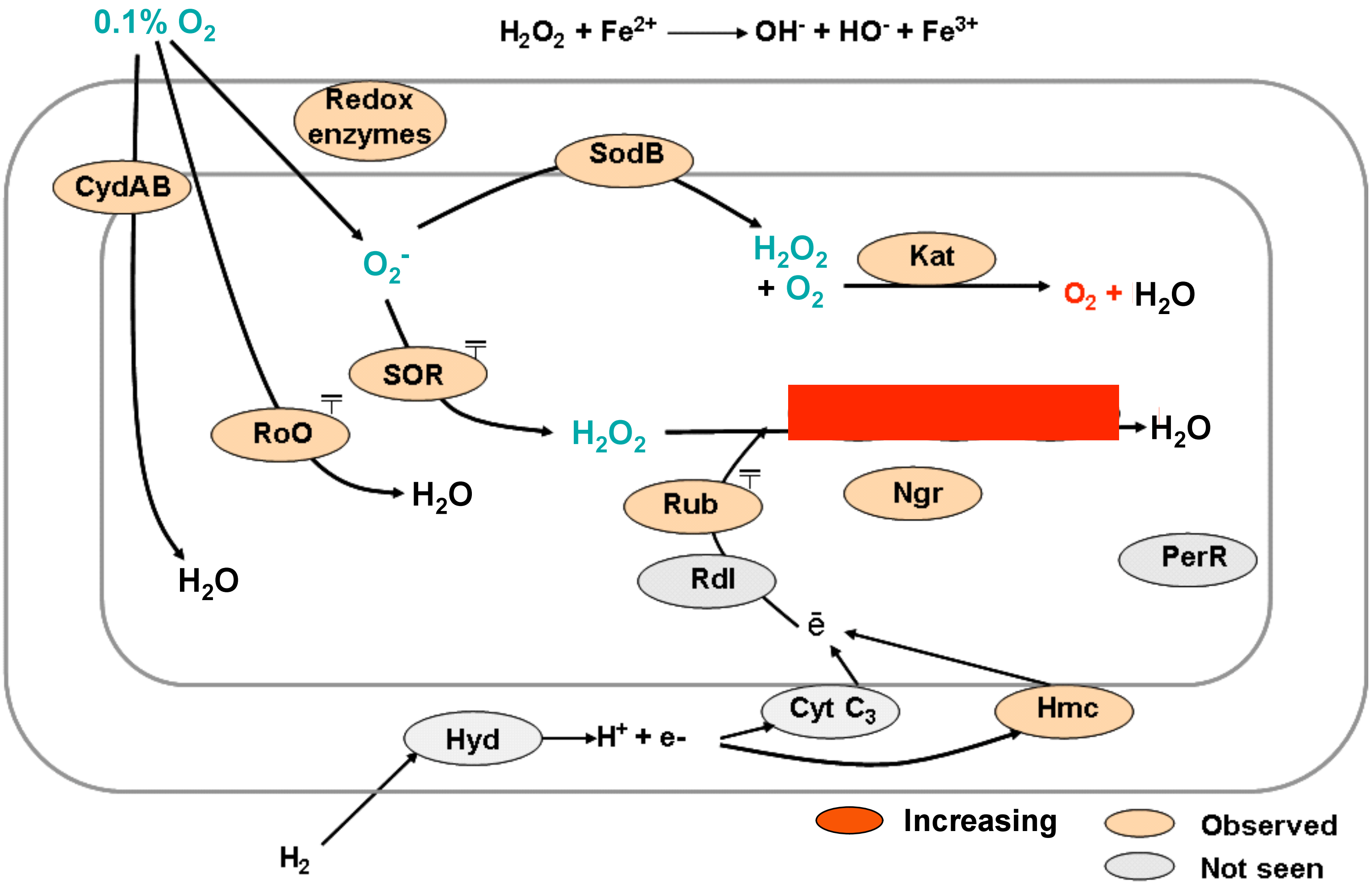


Both transcript and protein data pointed to PerR



Response to low O_2



Mukhopadhyay et al J Bacteriol (2007) 5996-6010; 189

Some interesting hypothesis to follow up on

- *D. vulgaris* adapted to occupy sub-oxic environment ?

- Per regulon in *D. vulgaris* provides additional mitigation over and above SOR and SOD based activity

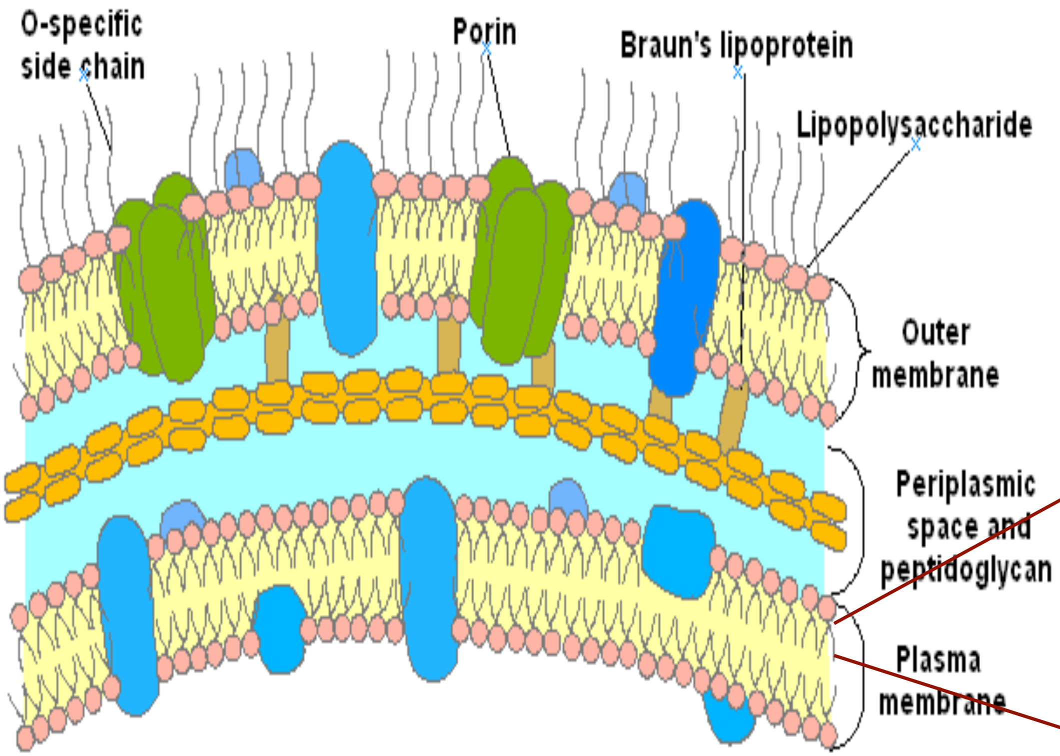
- The *D. vulgaris* Per regulon may contain additional members than initially predicted
- And can be experimentally tested

Importance of Cell wall in environmental stresses

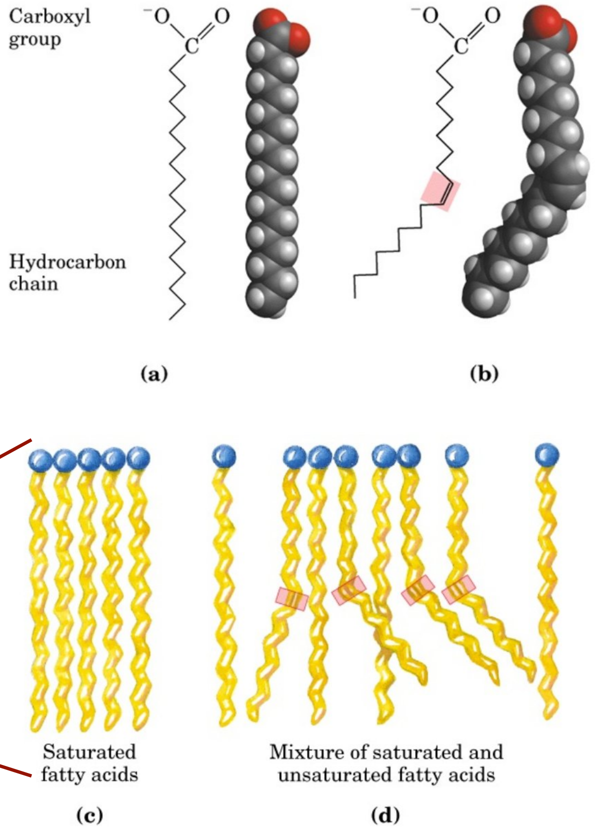
- The cell wall presents the first line of defense for microorganisms
  - Salt stress/ cold stress/ heat/ solvent
  - Optimal homeoviscocity is determined by the distribution of fatty acids in the cell wall and readjusted as the environment changes

Fatty Acid distribution

Schematic of Cell wall (Gram negative)

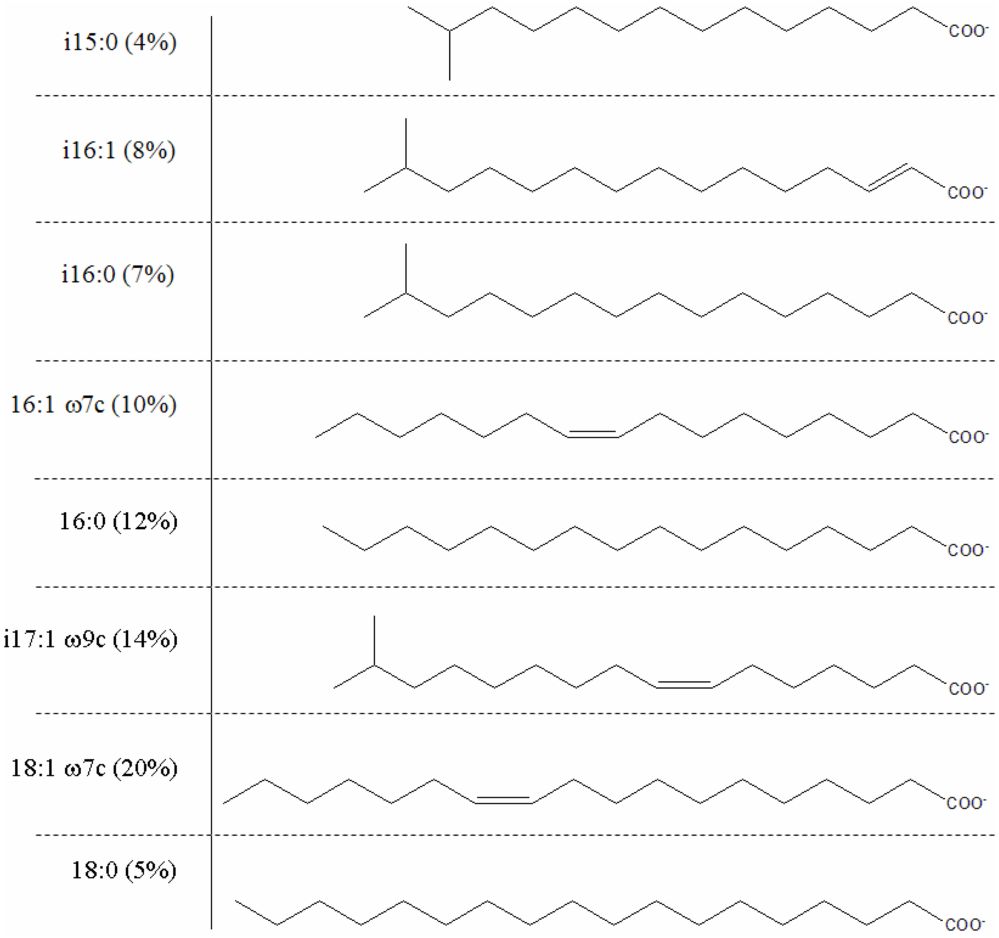


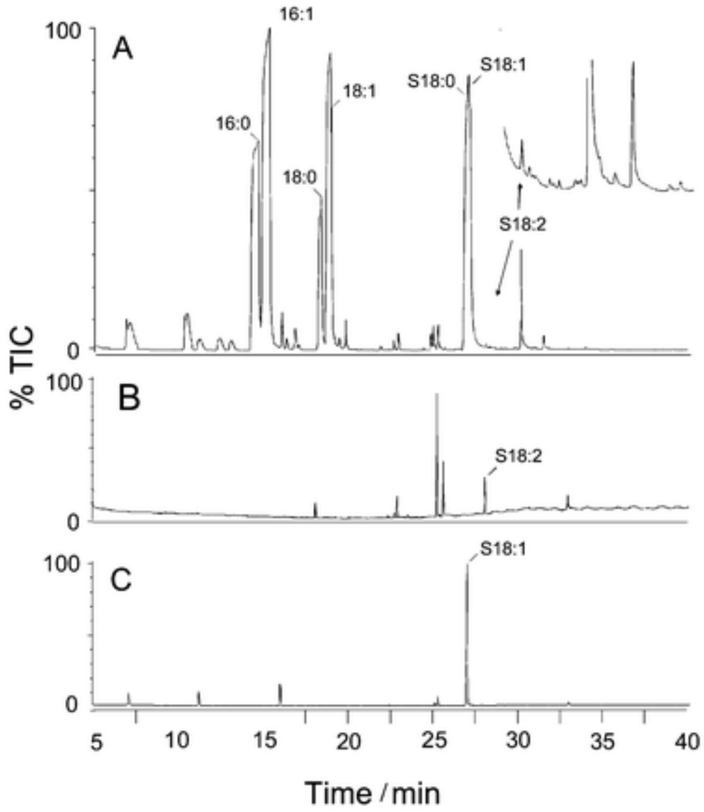
Fatty Acid Structure



FAME analysis for PLFA quantification

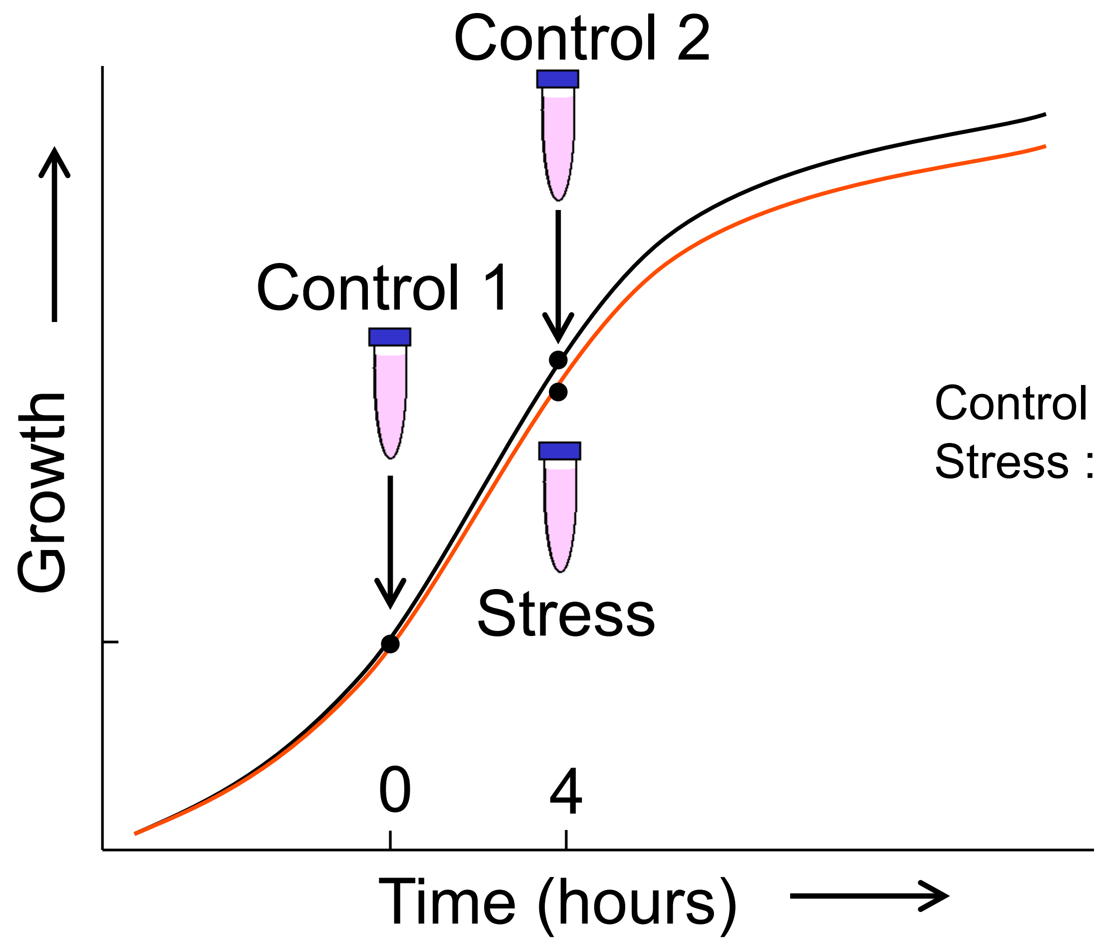
PLFA extraction → Methyl Esterification → GCMS detection and quantification





Note: The Phospholipid Fatty acid (PLFA) distribution of a microbe is unique

Salt stress in *D. vulgaris*



Control : No stress
Stress : 250 mM NaCl

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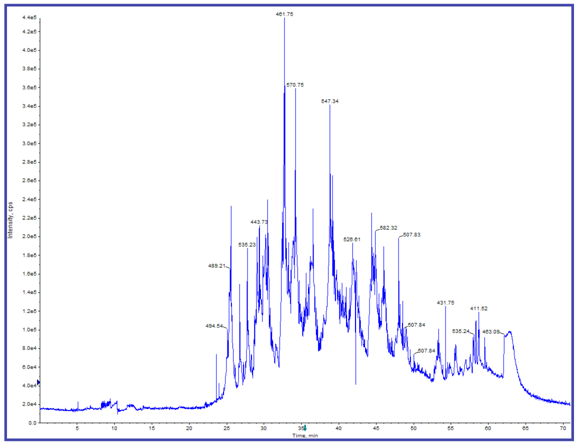
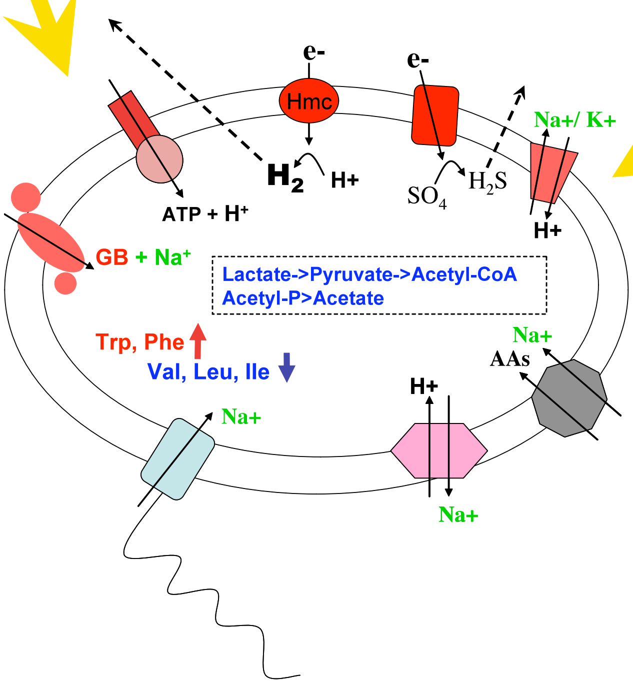
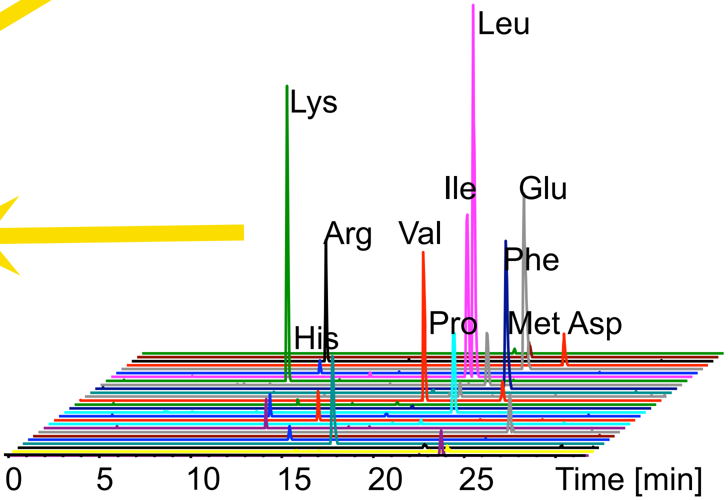
Integrating Functional Genomics

Proteomics



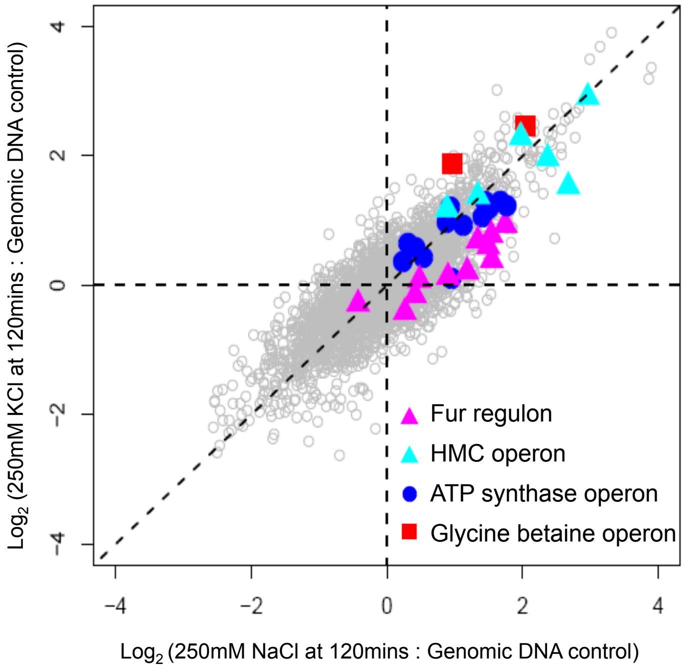
Transcriptomics

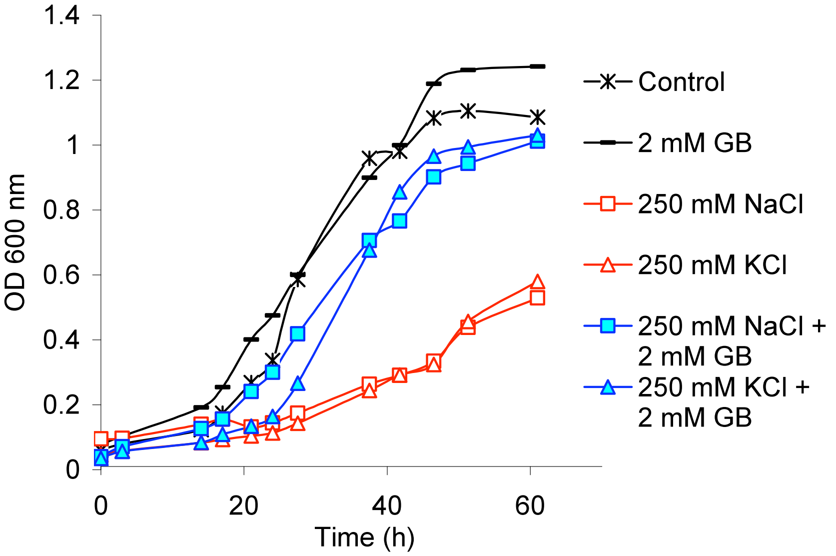




Metabolomics

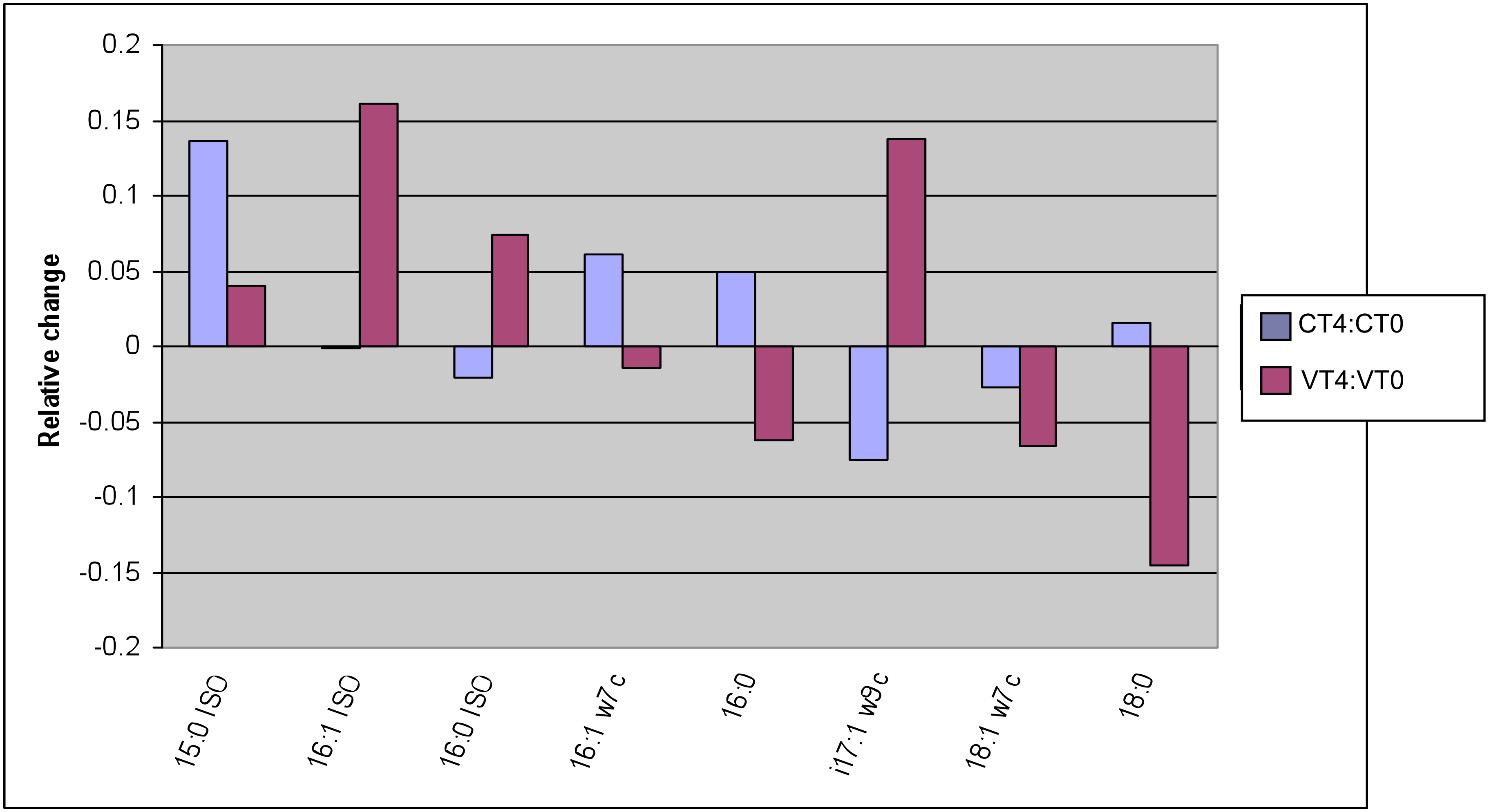
Uptake of Osmolyte is a primary mechanism of stress response





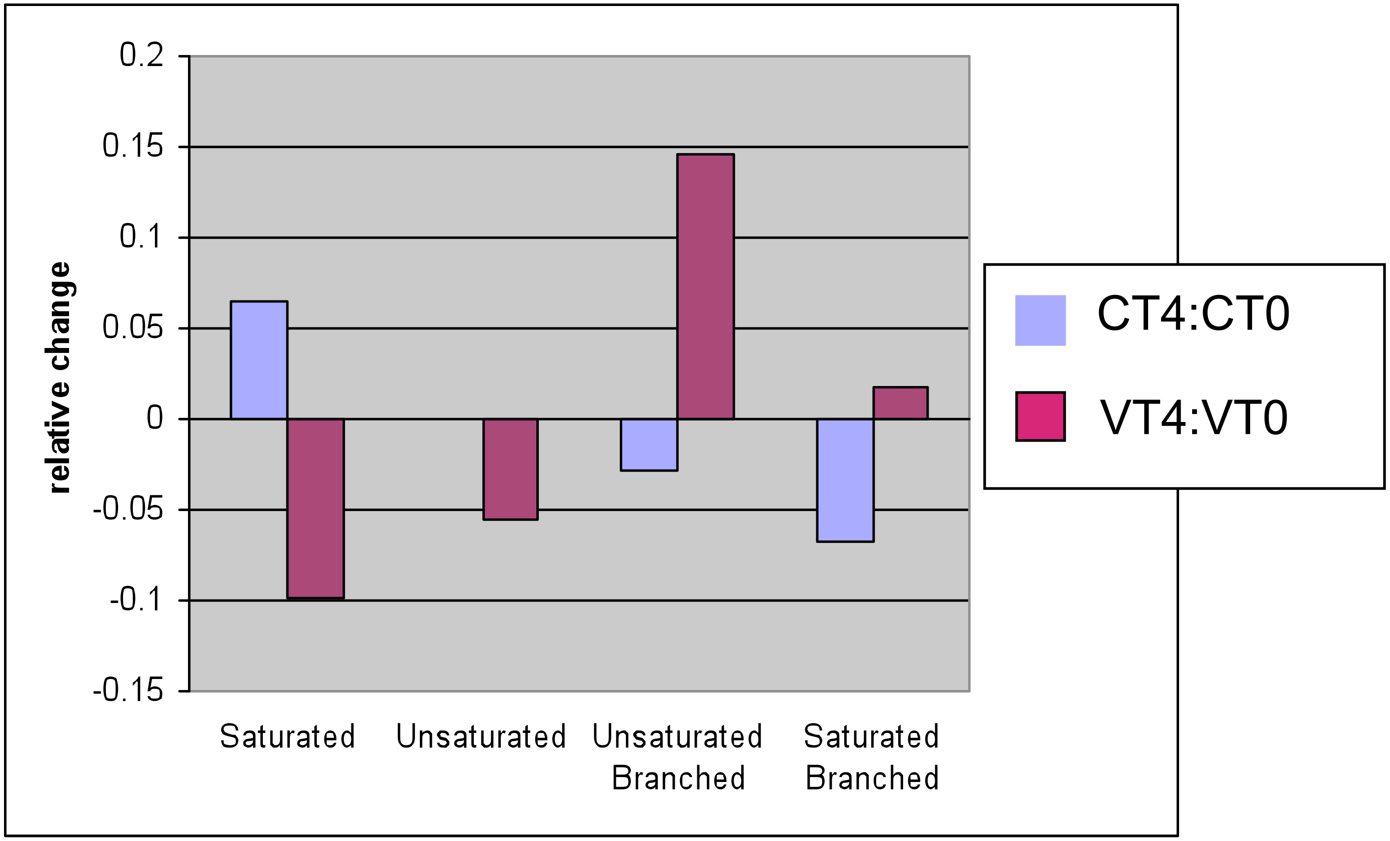
But we were missing some important aspects of cell wide data

Lipid analysis : PLFA studies



Relative change of the 8 major types PLFA after stress

Critical change in PLFA profile



Changes in PLFA to increase fluidity are documented for many bacteria in both Salt and Cold stress.

Increase in branched PLFA also reduces packing and increases fluidity of the membrane

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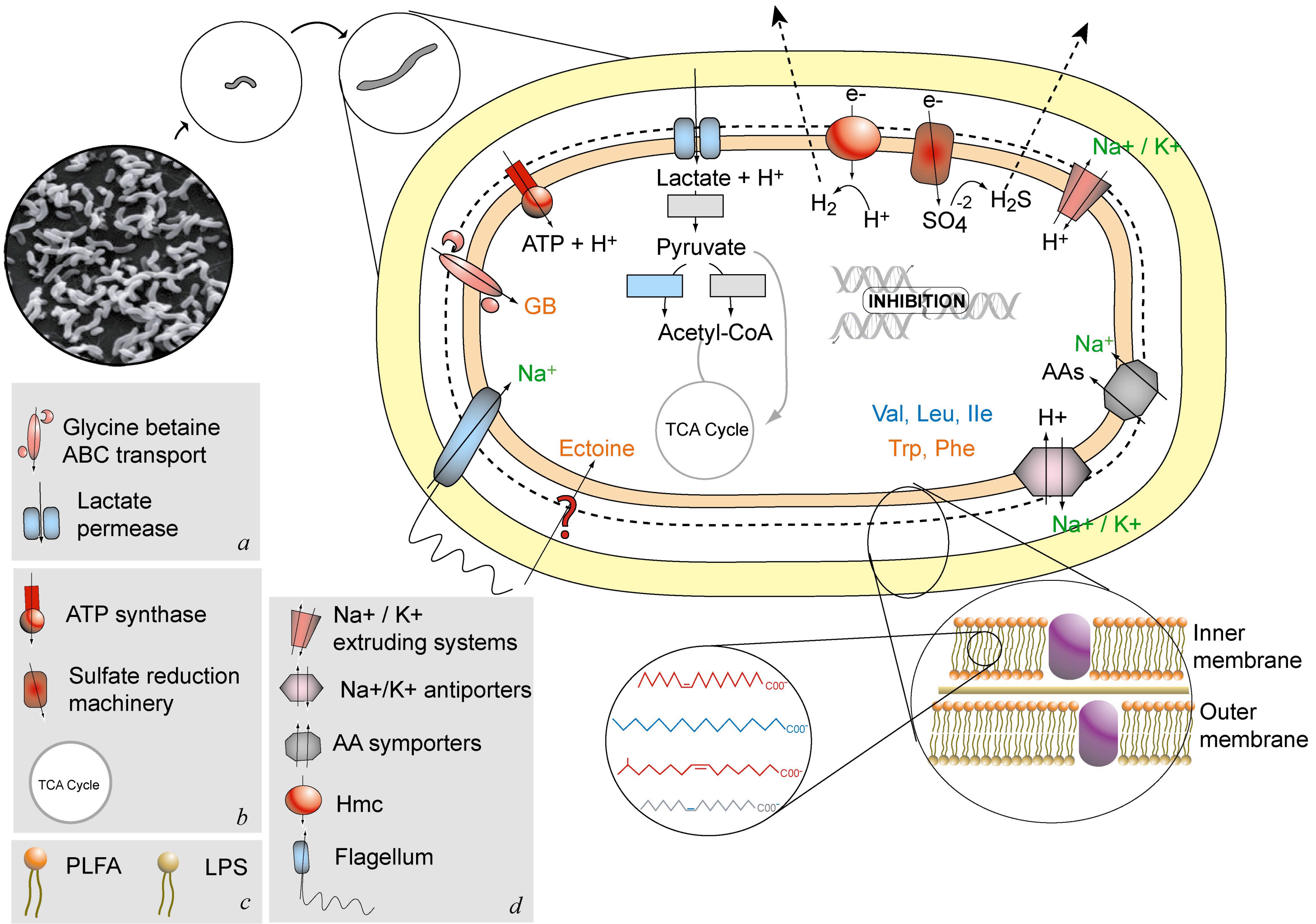
Salt stress in *D. vulgaris*



Mukhopadhyay et al J Bacteriology (2006) 4068-4078;188

PLFA Studies

- Not only is a change in PLFA profile a significant marker of stress response
- PLFA studies can help ID microbes in an ecosystem and provide a excellent HT method to assess the biotechnological potential of a microbial community for a particular function

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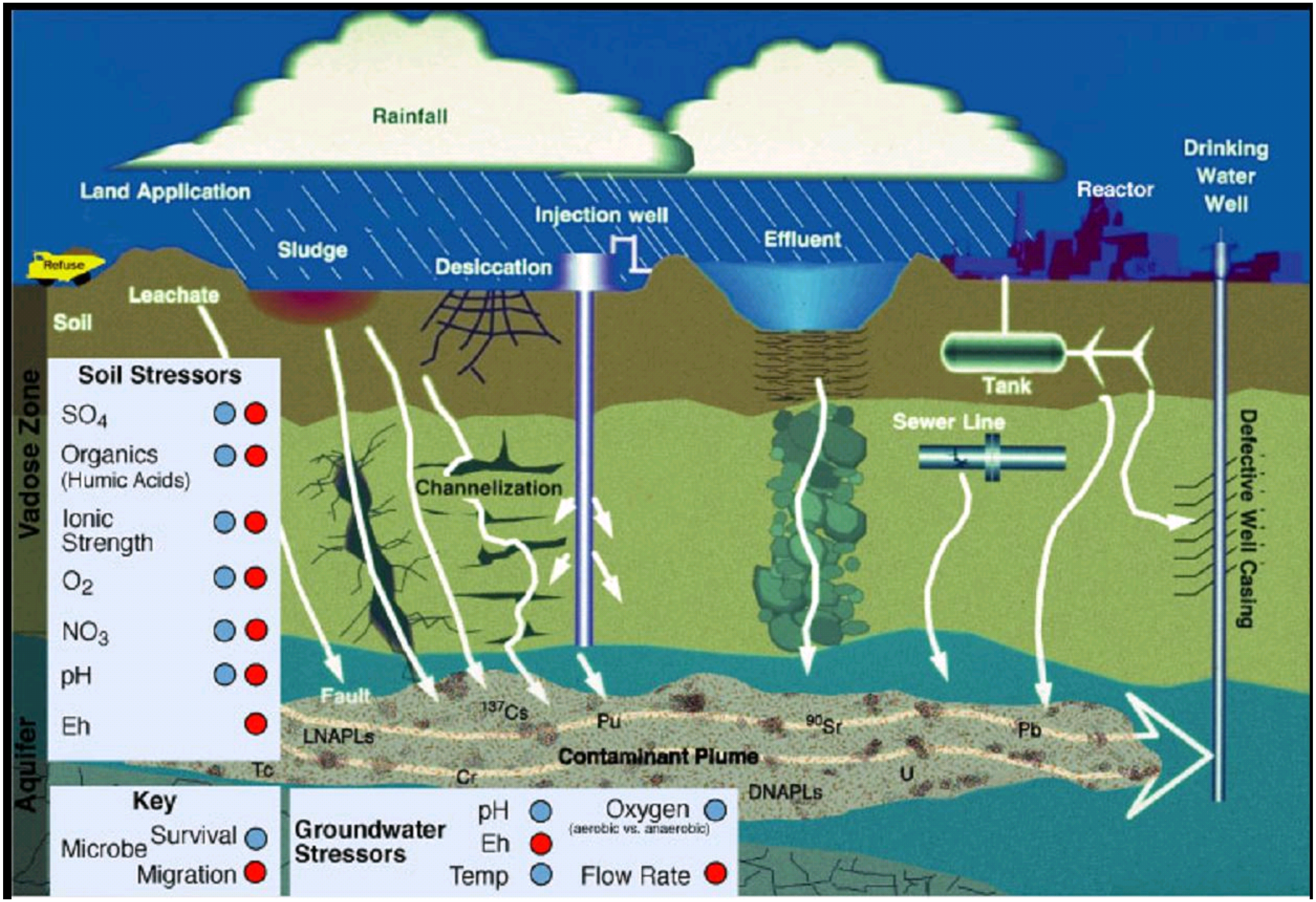
Environmental Stress factors



Summary

- Systems biology approaches allow us to obtain cell wide data that can be used to build models for how an organism interacts with its environment.

- These models provide the foundation to build bio-remedial approaches in the environment.

Acknowledgements

Keasling Group
U C Berkeley and
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Dominique Joyner
Kat Huang
Marcin Joacimiak

Wall Lab
University of Missouri, Columbia
Prof. Judy Wall
Bill Yen
Grant Zane

Zhou Lab
Oklahoma University
Jizhong Zhou
Zhili He
Qiang He

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