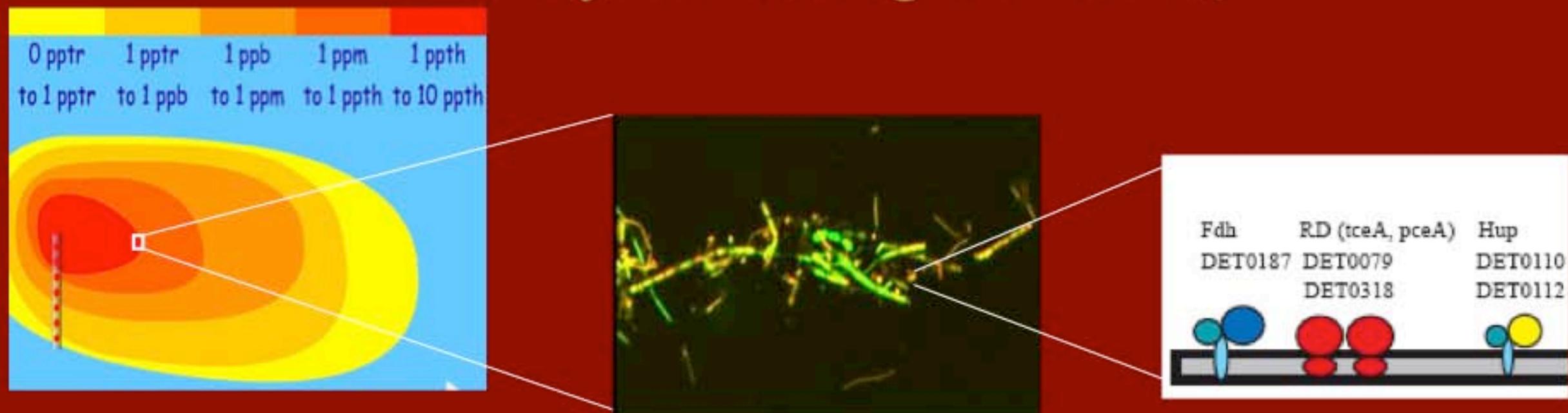


# Metaproteome changes during chloroethene-bioremediation and nutrient-starvation in enrichment cultures assayed using iTRAQ



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1: Cornell University 2. University of Toronto

International Conference on Environmental  
Proteomics, January 20, 2010

# The researchers

**Jeff Werner**

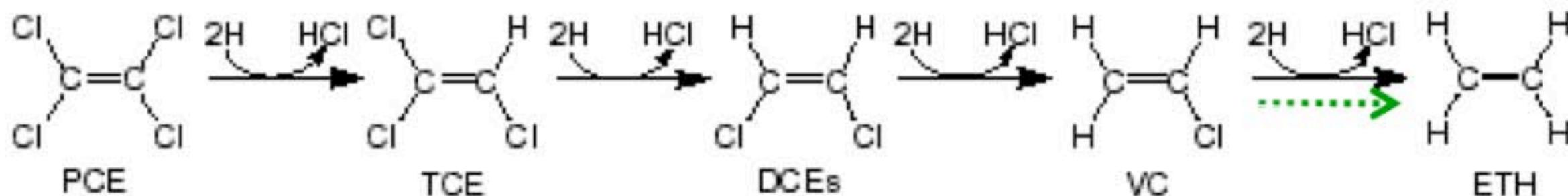


**Annie Rowe**

**Bob Morris**

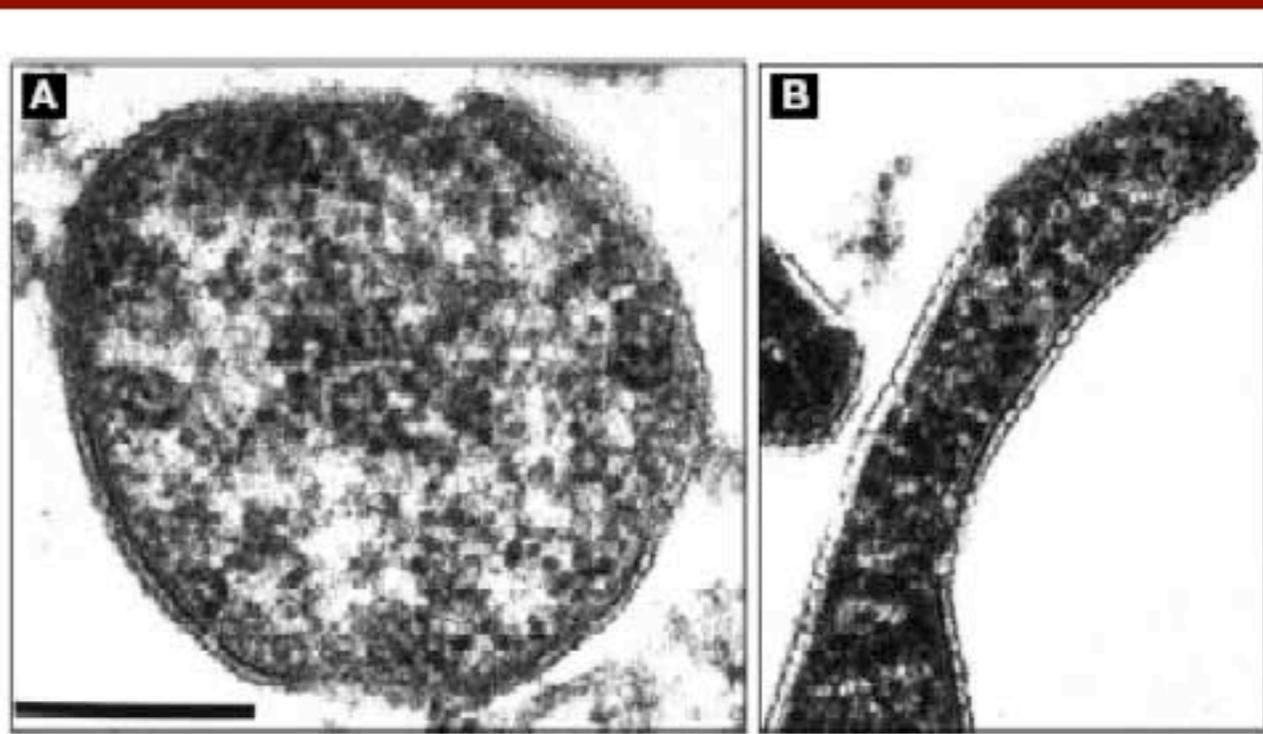
**Steve Zinder**

# *Dehalococcoides ethenogenes* strain 195: First isolate to **dehalorespire** PCE



## Insights gained

- H<sub>2</sub> as only electron-donor
- obligately uses halogenated compounds as e- acceptors
- TceA protein: TCE-dehalogenase enzyme discovered
- complex media requirements (mixed culture extract added)
- Isolated and genome sequenced



(from Maymo-Gatell et al., 1997, *Science*)

# Chloroethene-respiring communities

- Metaproteomics being used to:
  - Better understand microbial ecology
  - Develop diagnostic tools for field site management (biomarkers)
    - specific dechlorination reactions
    - cell stress
    - competing physiologies
- Two cultures being studied: KB1 & DII



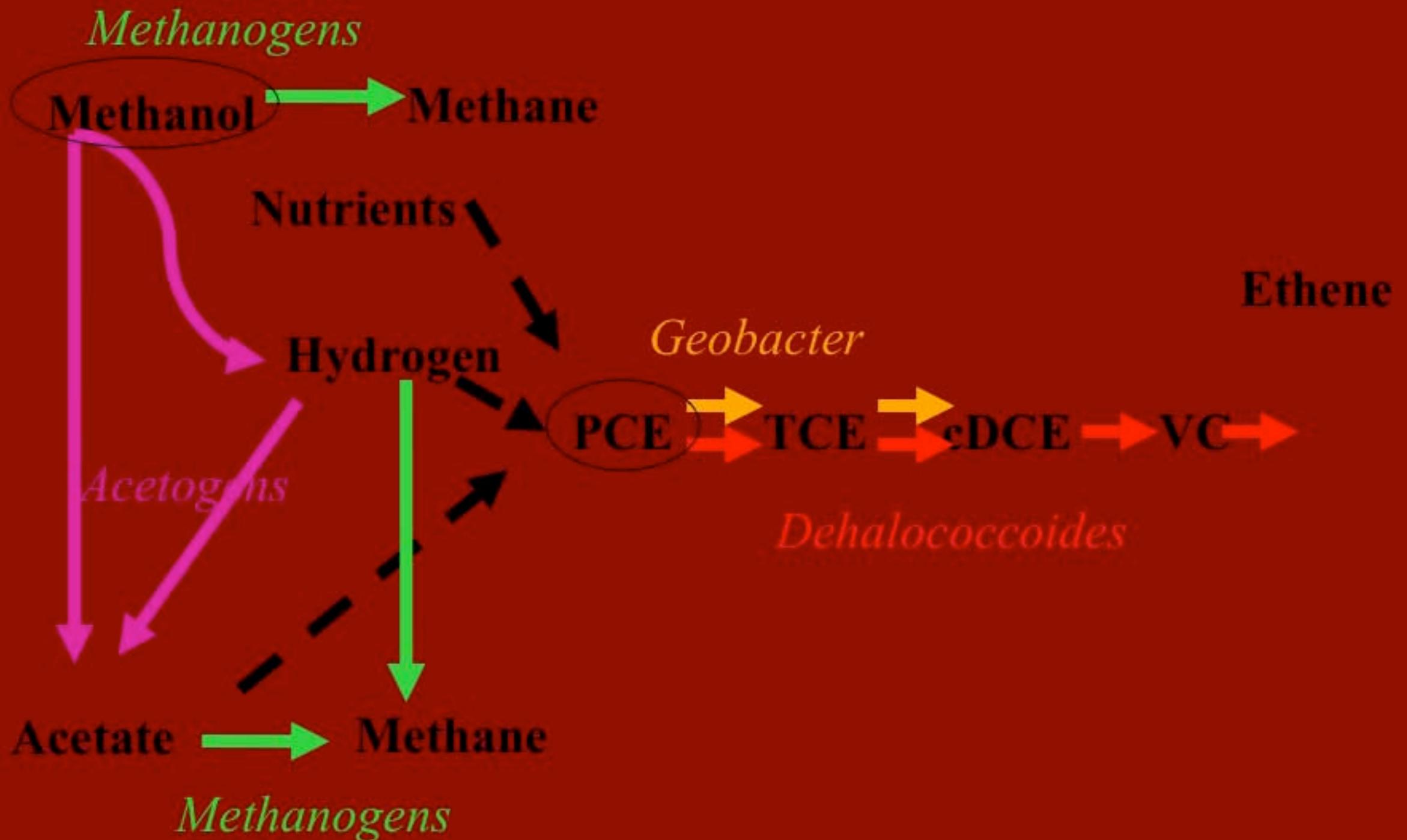
5 microns

Brendan Lazar

## Two dechlorinating mixed cultures

	KB1	DonnaII (DII)
Dehalococcoides pop'ns	Two strains	Strain 195
Other key pop'ns	<i>Geobacter</i> ; <i>Methanosarcina</i> , <i>Methanoregula</i> , <i>Spirochete</i> , <i>Bacteroides</i> , <i>Clostridia</i> , others	<i>Methanospirillum</i> , <i>Methanosaeta</i> , <i>Syntrophomonas</i> , <i>Clostridia</i> , others
Respired substrates	PCE to ethene	PCE to VC
Metagenome (by JGI)	Yes	Yes (in progress)
Experimental variables studied w/iTRAQ	Electron donor type fed to culture (H <sub>2</sub> or MeOH)	Electron Acceptor respiration rate (1 to 50 $\mu\text{mole/L}\cdot\text{hr}$ )

# Mixed culture basic ecology (e.g. KB1)





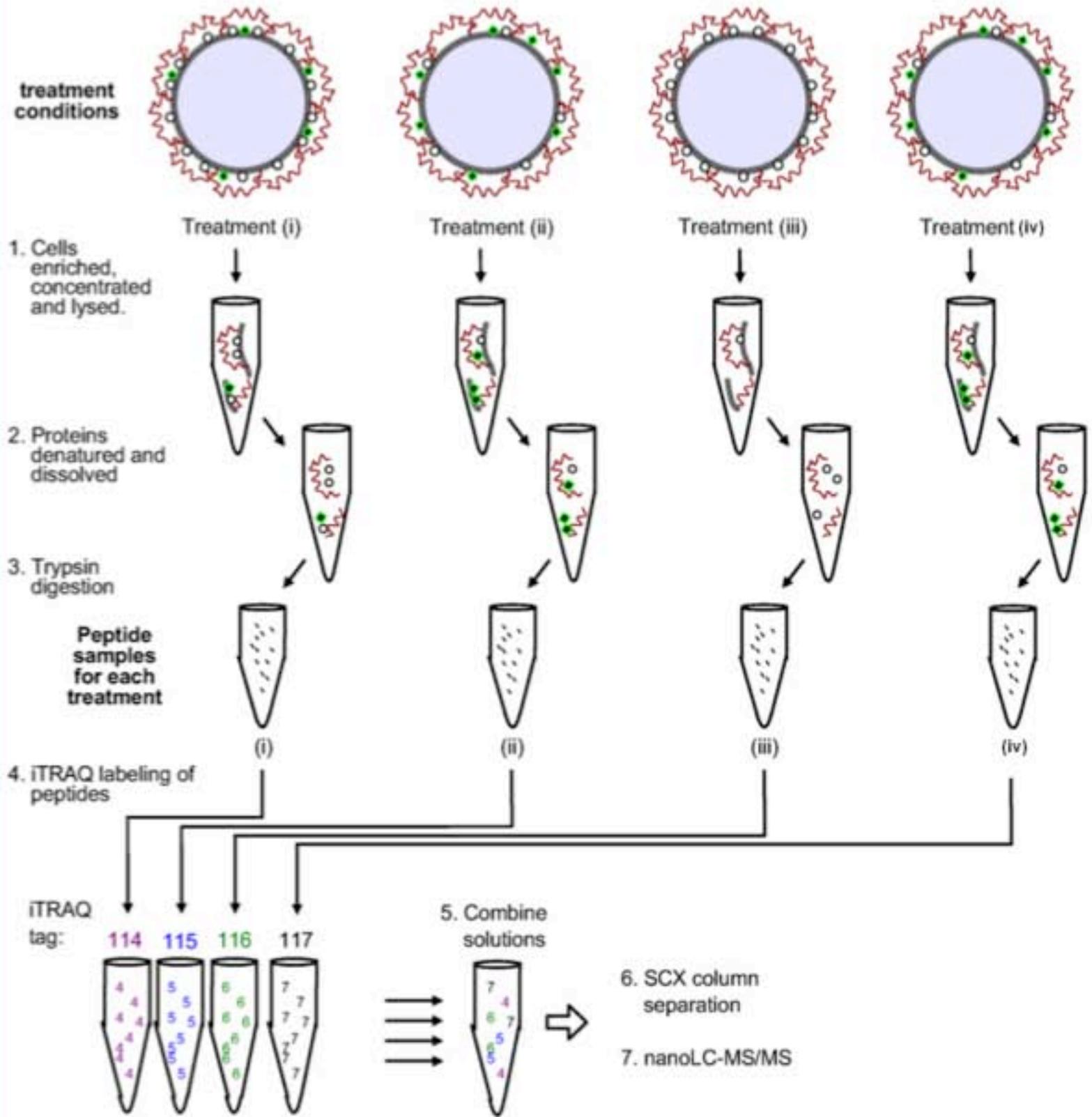
# Comparisons made

- KB1 :
  - MeOH vs H<sub>2</sub> (long term and single feed substrate changes)
- DII:
  - Feeding rate (in continuous feed expts)
  - Starvation
  - Type of electron acceptor (TCE vs PCE)

# Comparisons made

- KB1 :
  - MeOH vs H<sub>2</sub> (long term and single feed substrate changes)
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  - Feeding rate (in continuous feed expts)
  - Starvation
  - Type of electron acceptor (TCE vs PCE)

# iTRAQ= isobaric tags for relative and “absolute” quantification



# Reporter ion peak areas used to infer relative quantification

iTRAQ – label N terminus of same peptide in 4 different samples

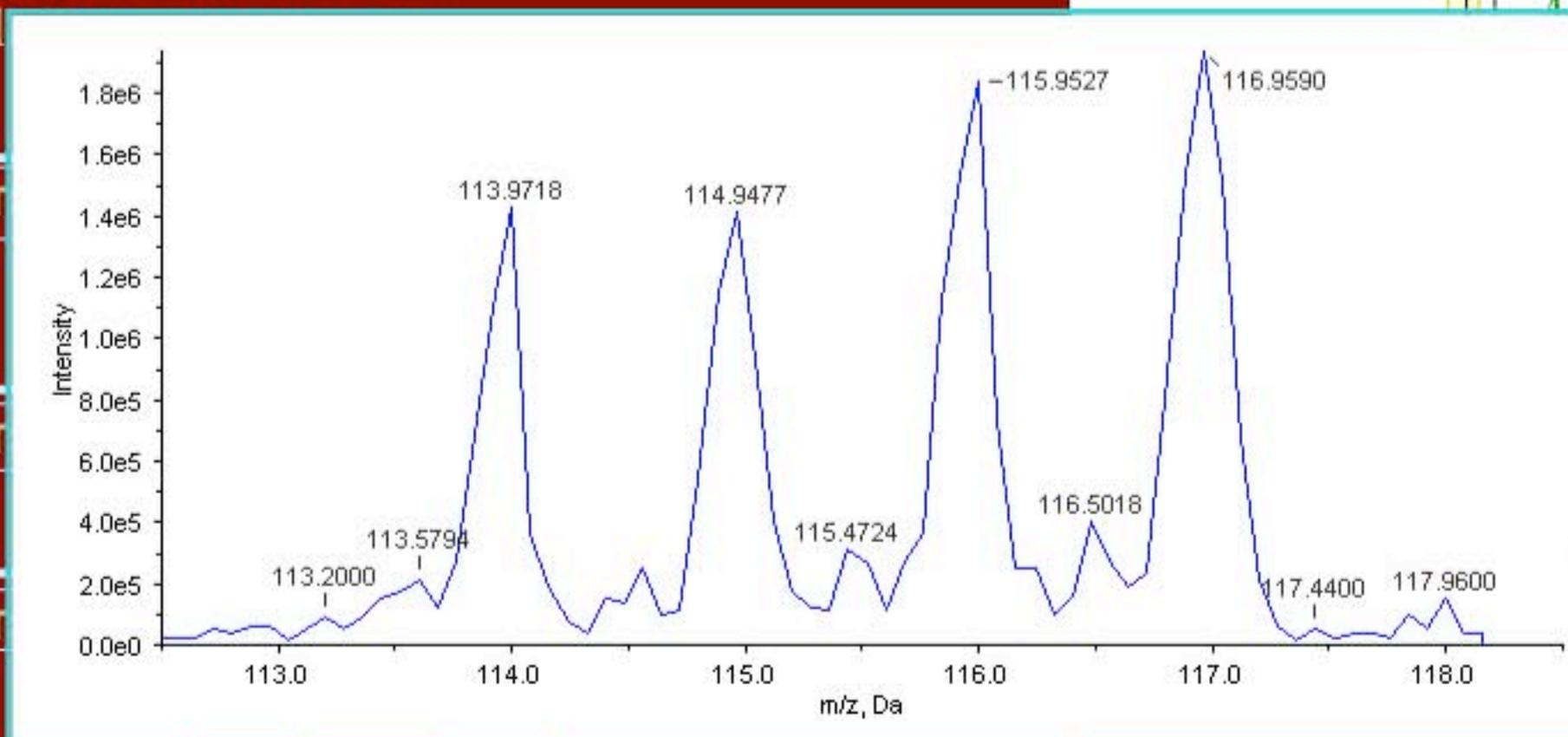
GINT

GINT

GINT

GINTSELTIR

117



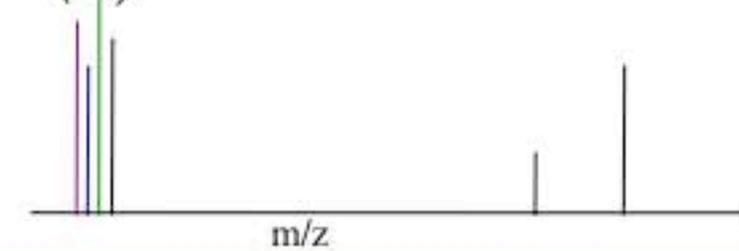
One precursor ion

MS scan

m/z

MS scan

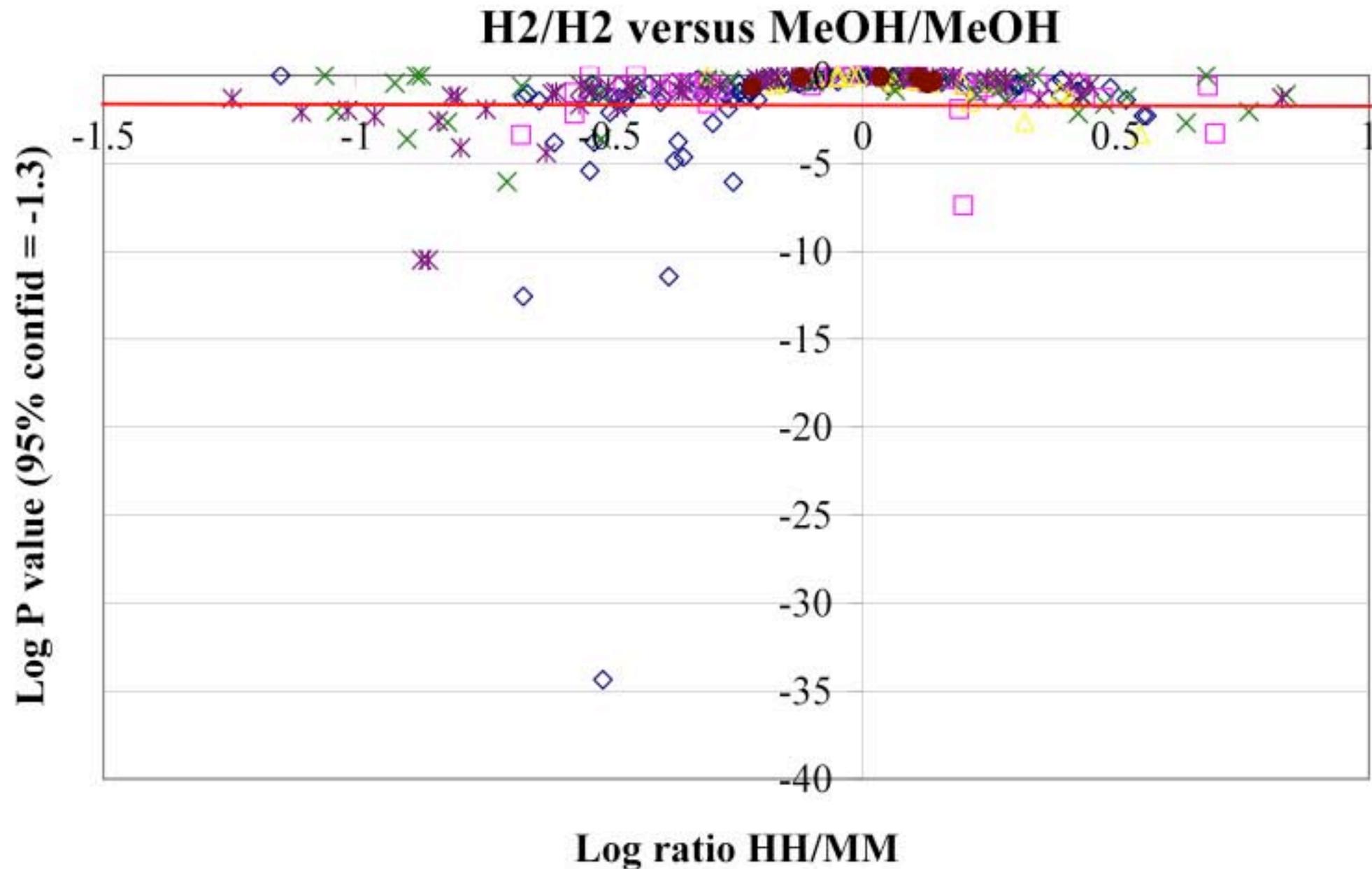
peptides



# Summary of Shotgun & iTRAQ Results

	KB1	DonnaII (DII)
Dehalococcoides pop'ns	Two strains	Strain 195
Other key pop'ns	<i>Geobacter; Methanosarcina, Methanoregula, Spirochete, Bacteroides, Clostridia, others</i>	<i>Methanospirillum, Methanosaeta, Syntrophomonas, Clostridia, others</i>
Respired substrates	PCE to ethene	PCE to VC
Metagenome (by JGI)	Yes	Yes (in progress)
Experimental variables studied w/iTRAQ	Electron donor type fed to culture (H <sub>2</sub> or MeOH)	Electron Acceptor respiration rate (1 to 50 umole/L*hr)
proteins IDed w Metagenome (95% confid)	322	267
# of proteins IDed w Swiss Prot or IMG Genomes (95% confid)	264	347
proteins showing diff'l expression (metagenome db)	55	53

# KB1 – effect of electron donor



◇ *Dehalococcoides*

□ *Methanogens*

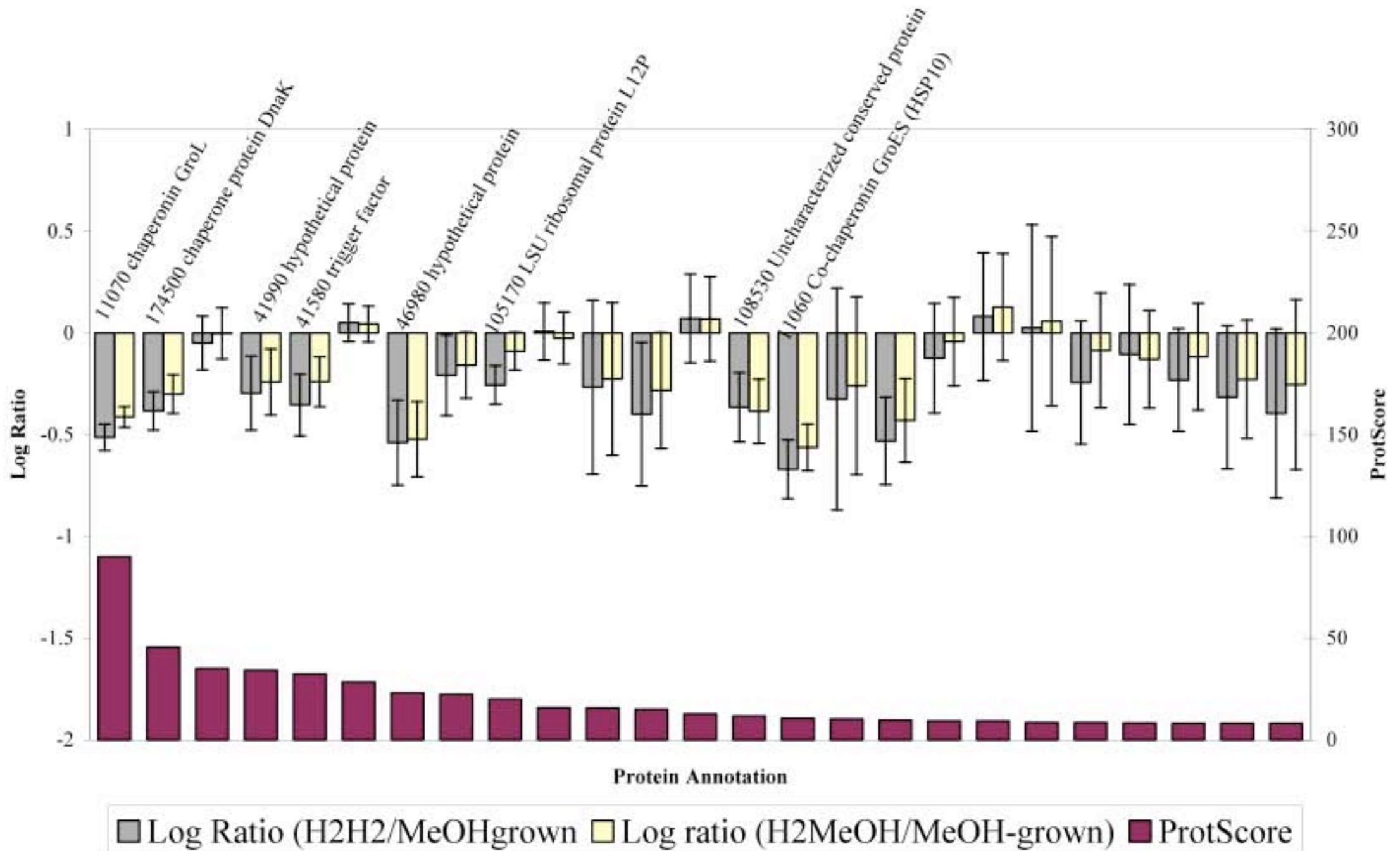
△ *Geobacter*

× Other organisms/unknown

\* Clostridia

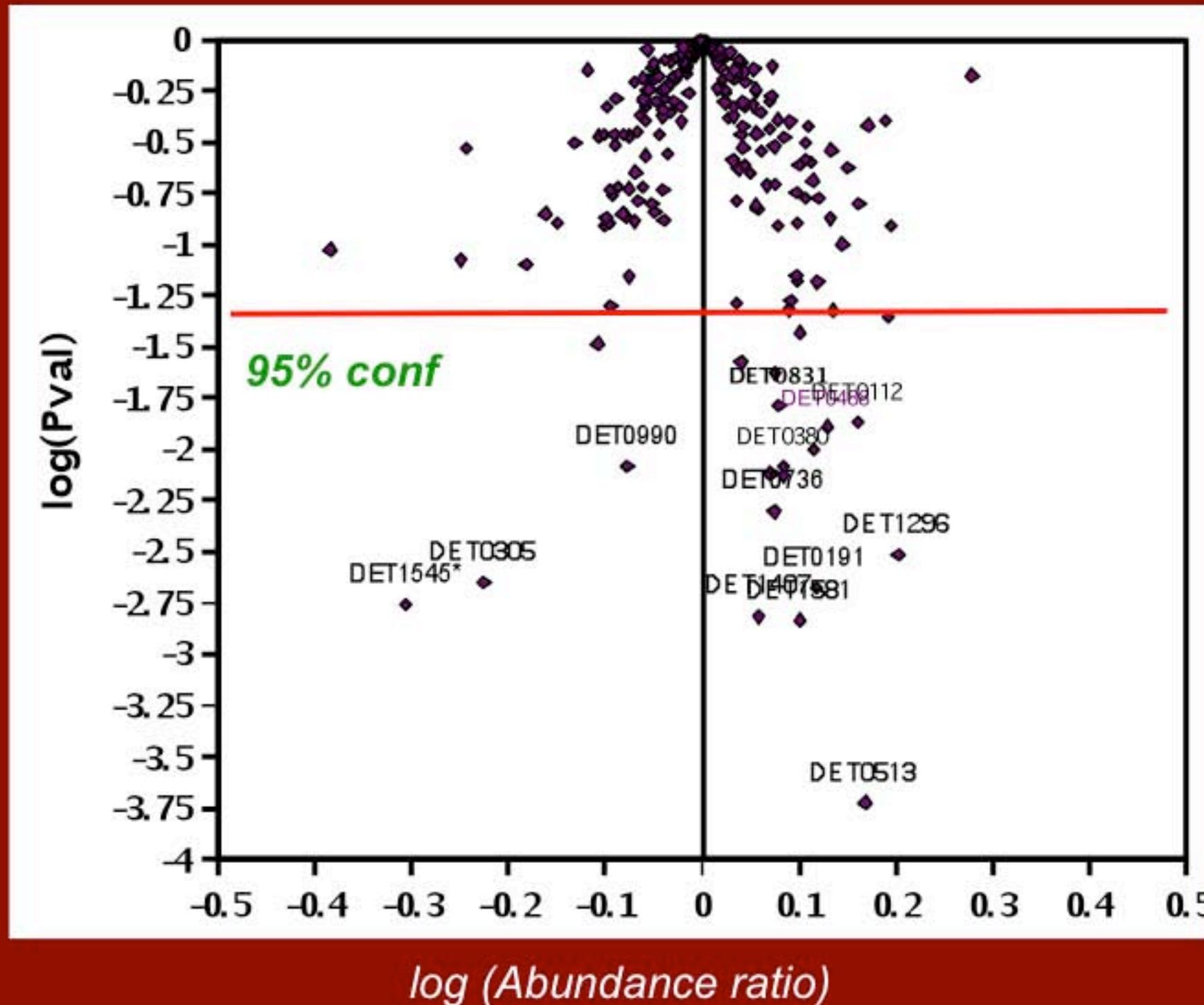
● *Bacteroidetes*

## *Dehalococcoides* Proteins' Log Ratio w/95% CI

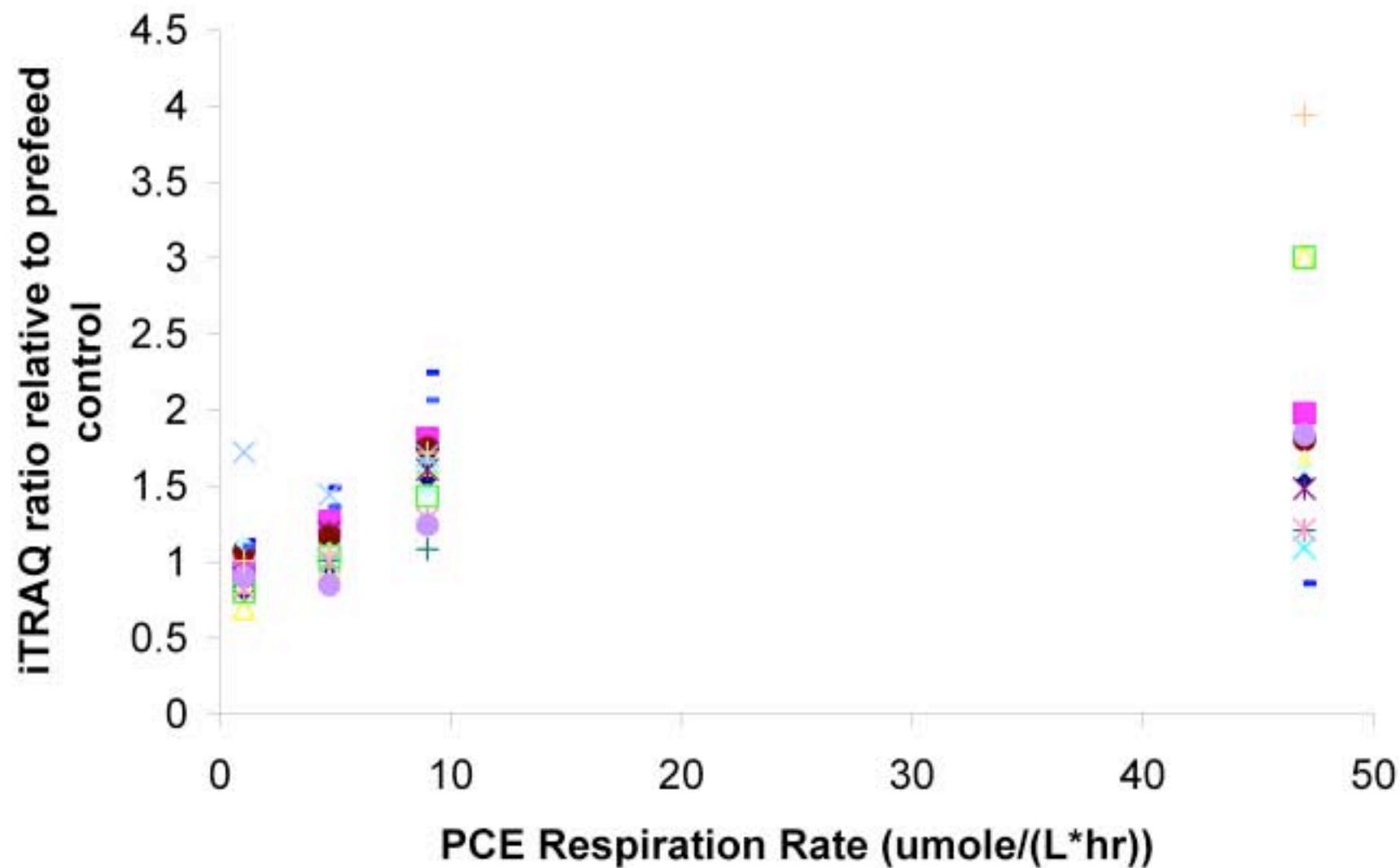


Chaperonins and Ribosomal proteins lower when fed H2 directly

# DII results: *Dehalococcoides* protein changes as a function of feeding rates



# DII: Changes in proteins involved in respiration, housekeeping or cell wall as function of respiration rate

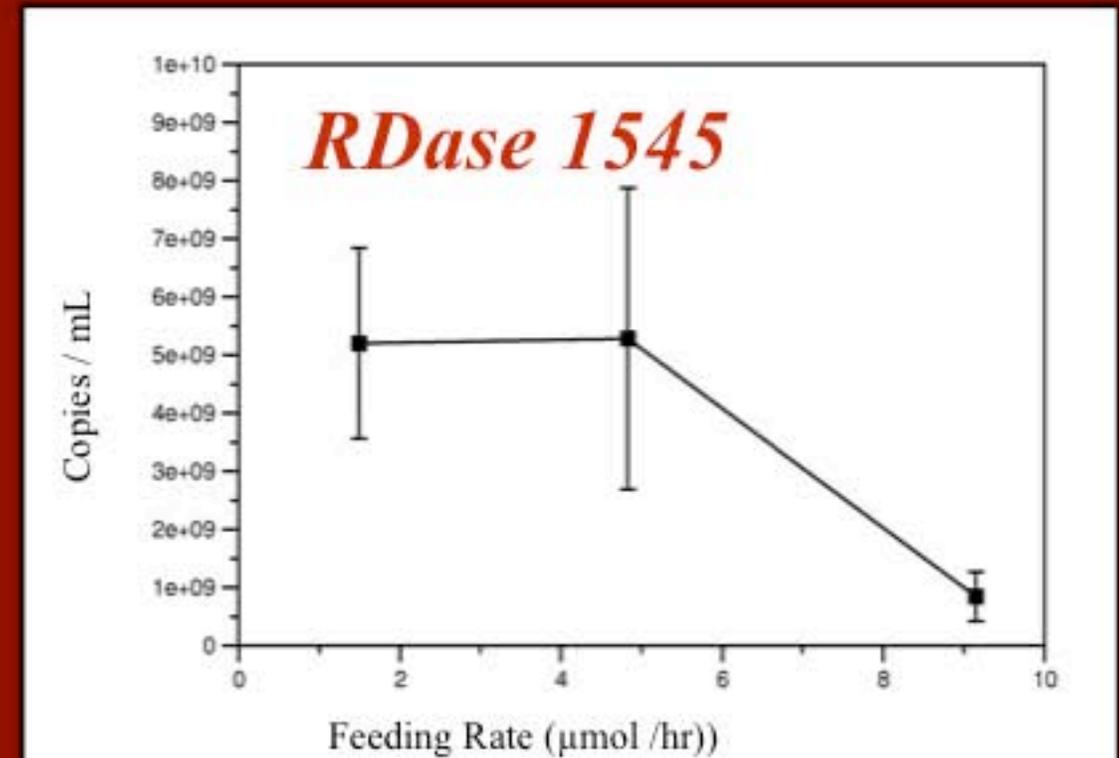
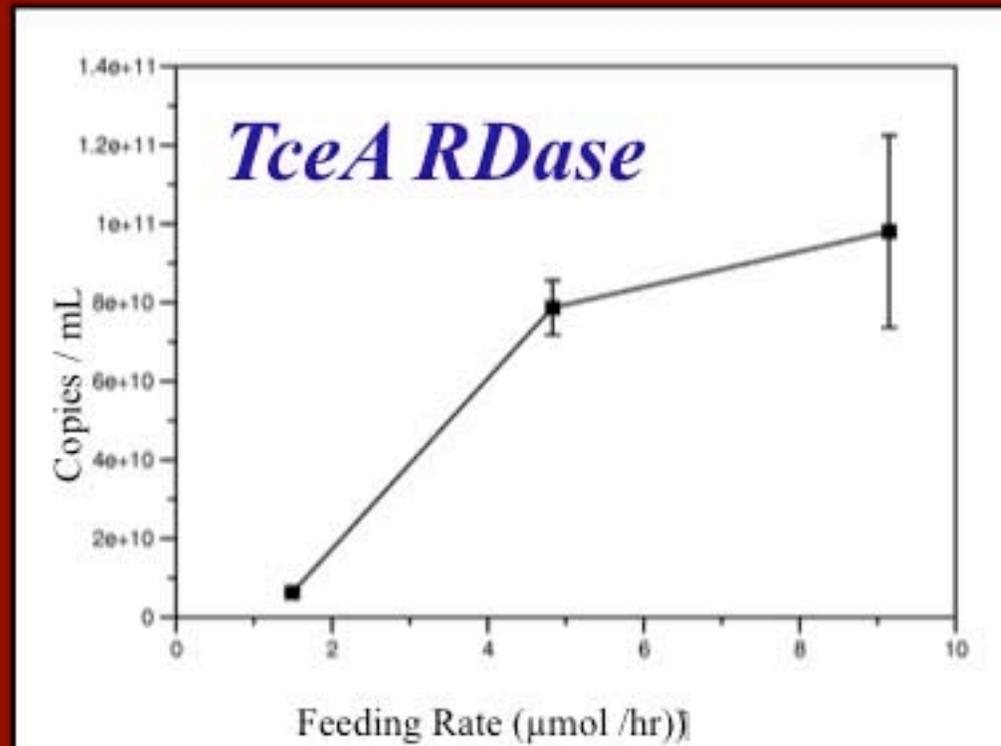


- |   |                              |                            |
|---|------------------------------|----------------------------|
| ◆ Chaperonin GroEL                      | ■ Rdase TceA                 | △ Hydrogenase subunit HupL |
| × putative "Formate Dehydrogenase" FdhA | × S-Layer cell wall protein  | ● ATPase                   |
| + Ribosomal protein L7/L12              | - Rdase PceA                 | - RpoB                     |
| ● RpoB'                                 | □ Hydrogenase subunit HupS   | ● EF-TU                    |
| × DET 1545                              | × EF-G                       | ● 30S S4                   |
| + Hydrogenase subunit HymB              | - Cell Division protein FtsZ |                            |

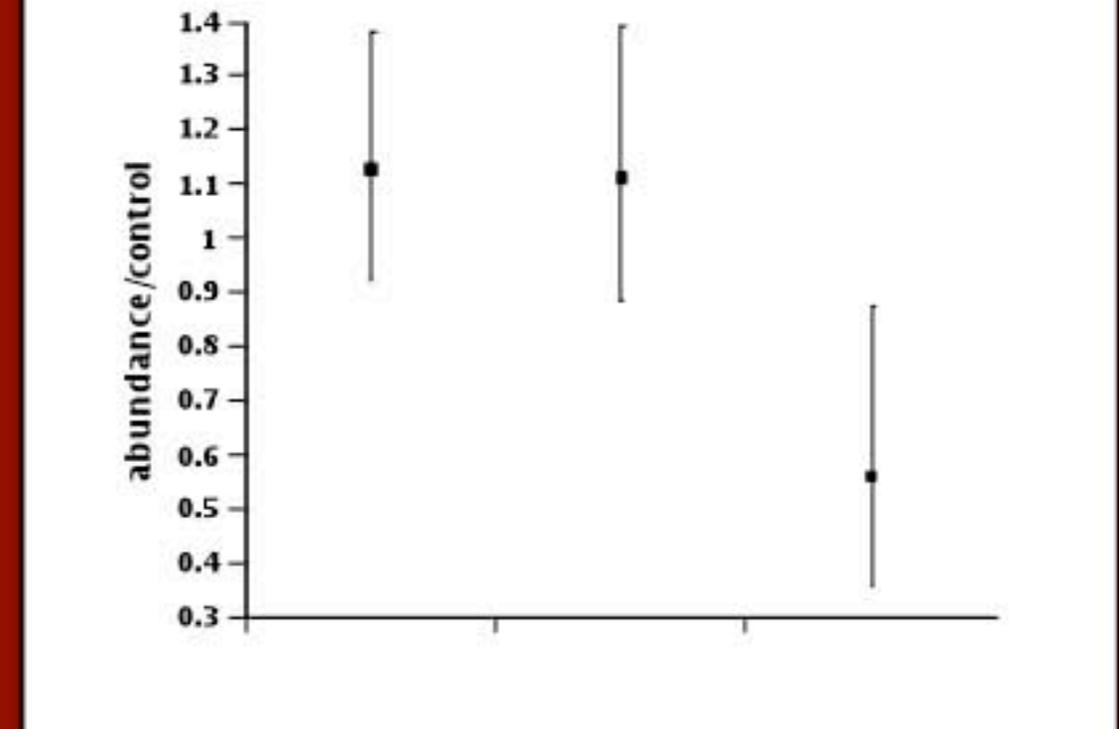
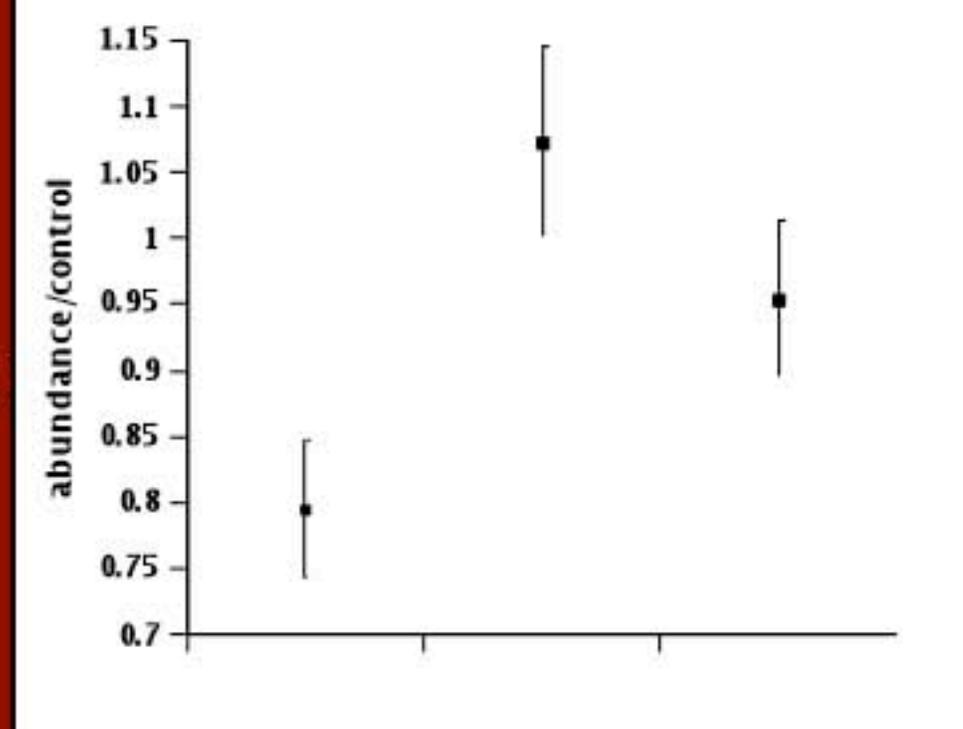
# Key Biomarkers: RDases

Protein and RNA both suggest rate-dependent expression

*RNA*

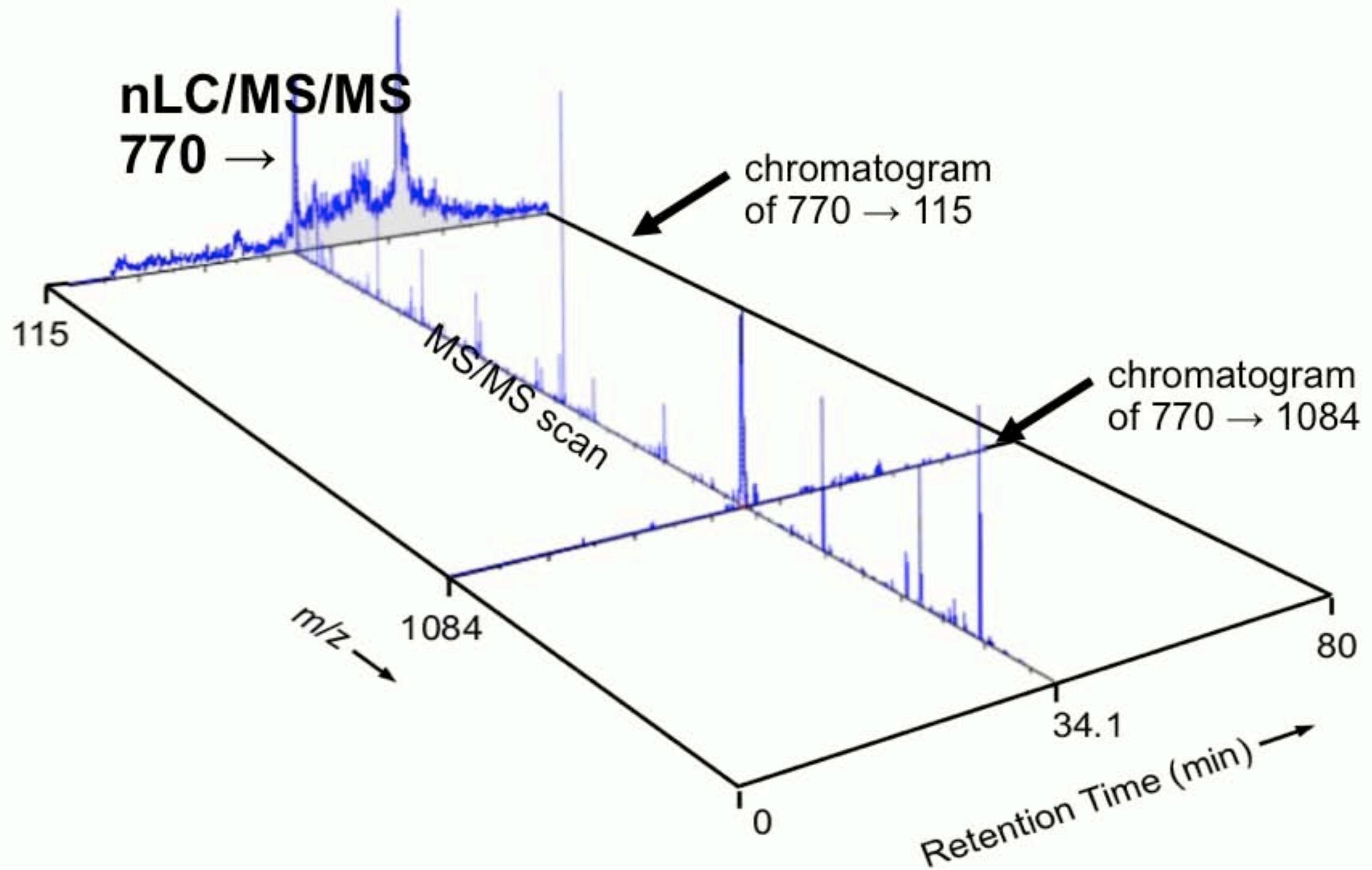


*protein*

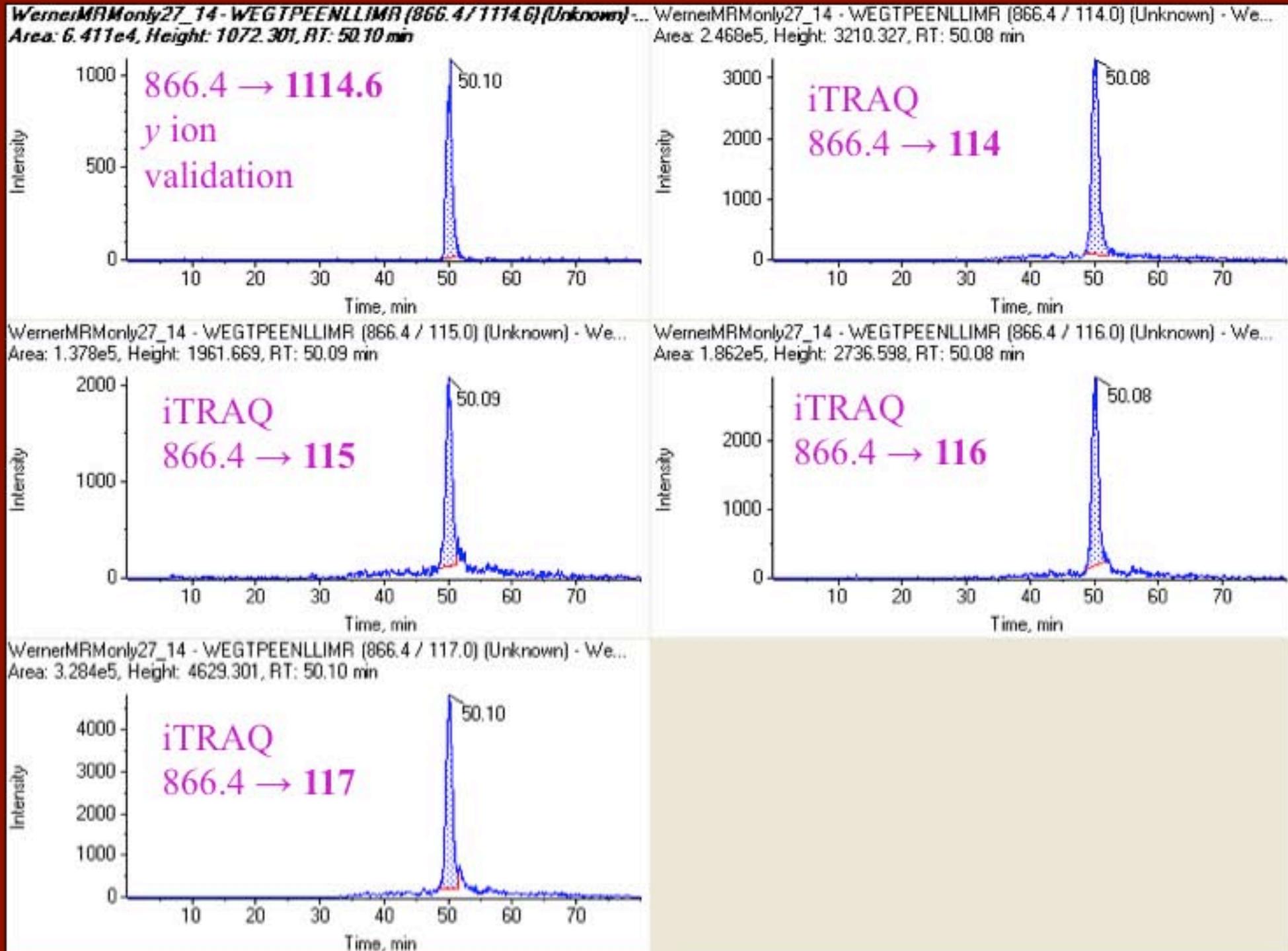




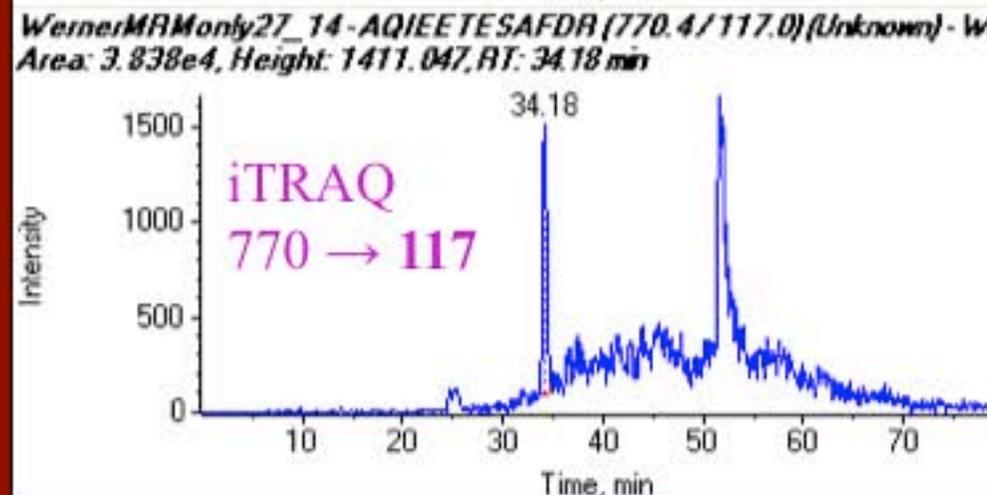
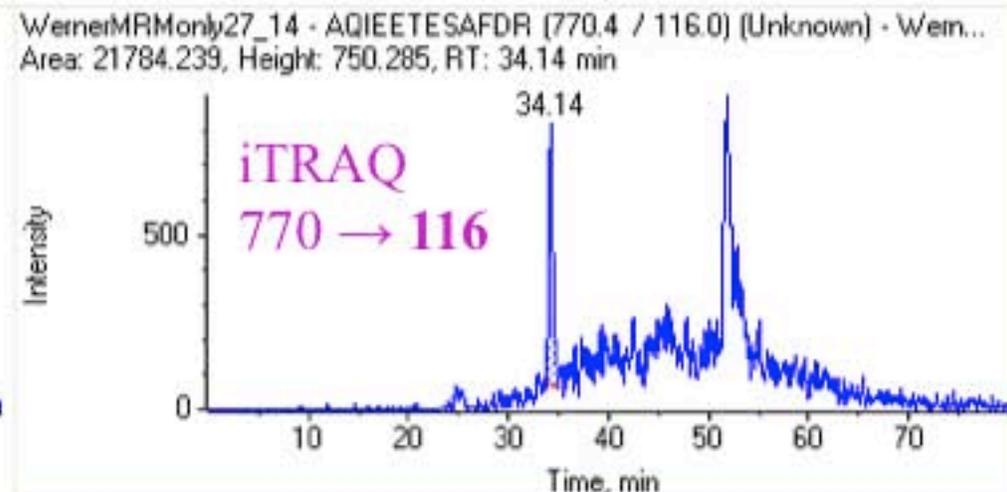
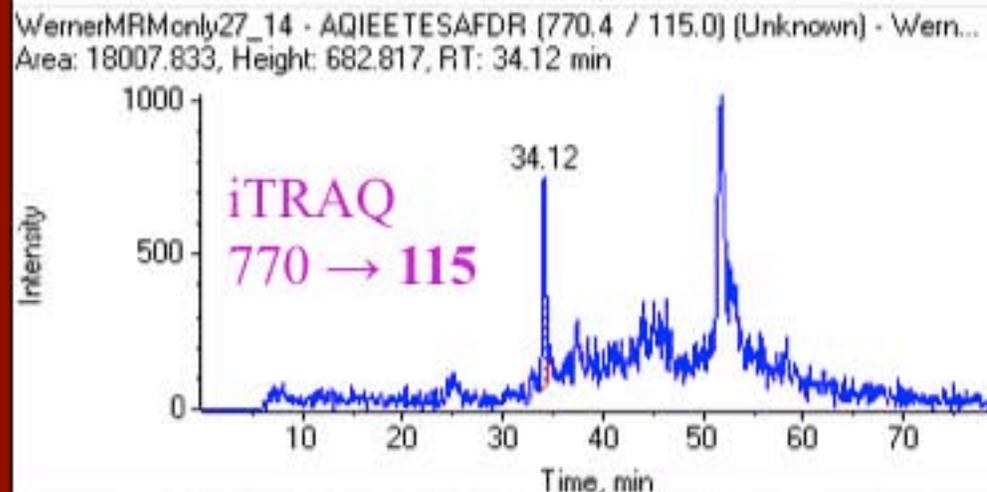
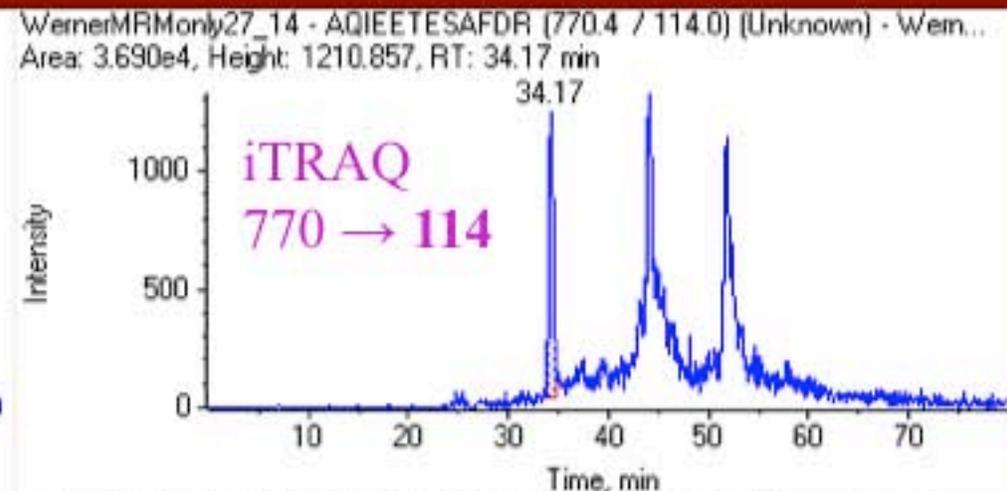
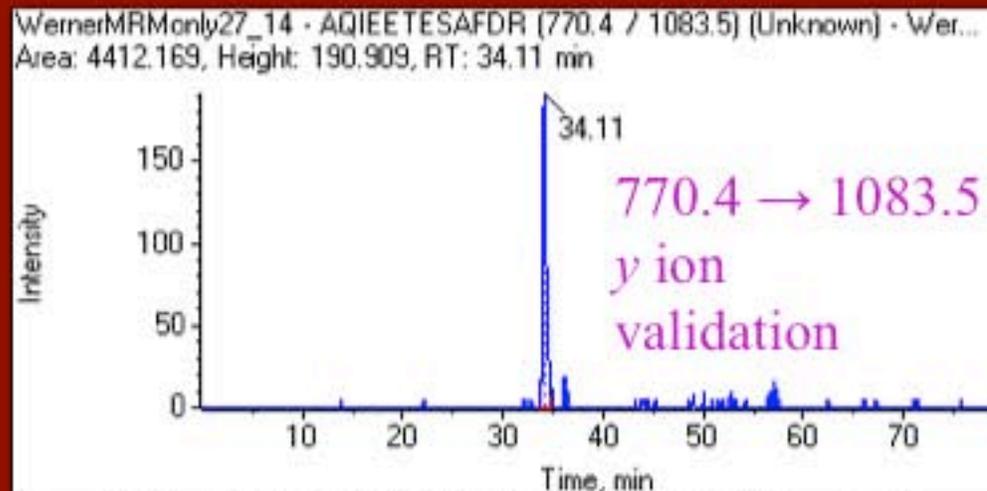
# Combining iTRAQ w/ MRM for multiplex targeted quantification



# tceA biomarker *WEGTPEENLLIMR* Validated by MRM nLC/MS/MS assay

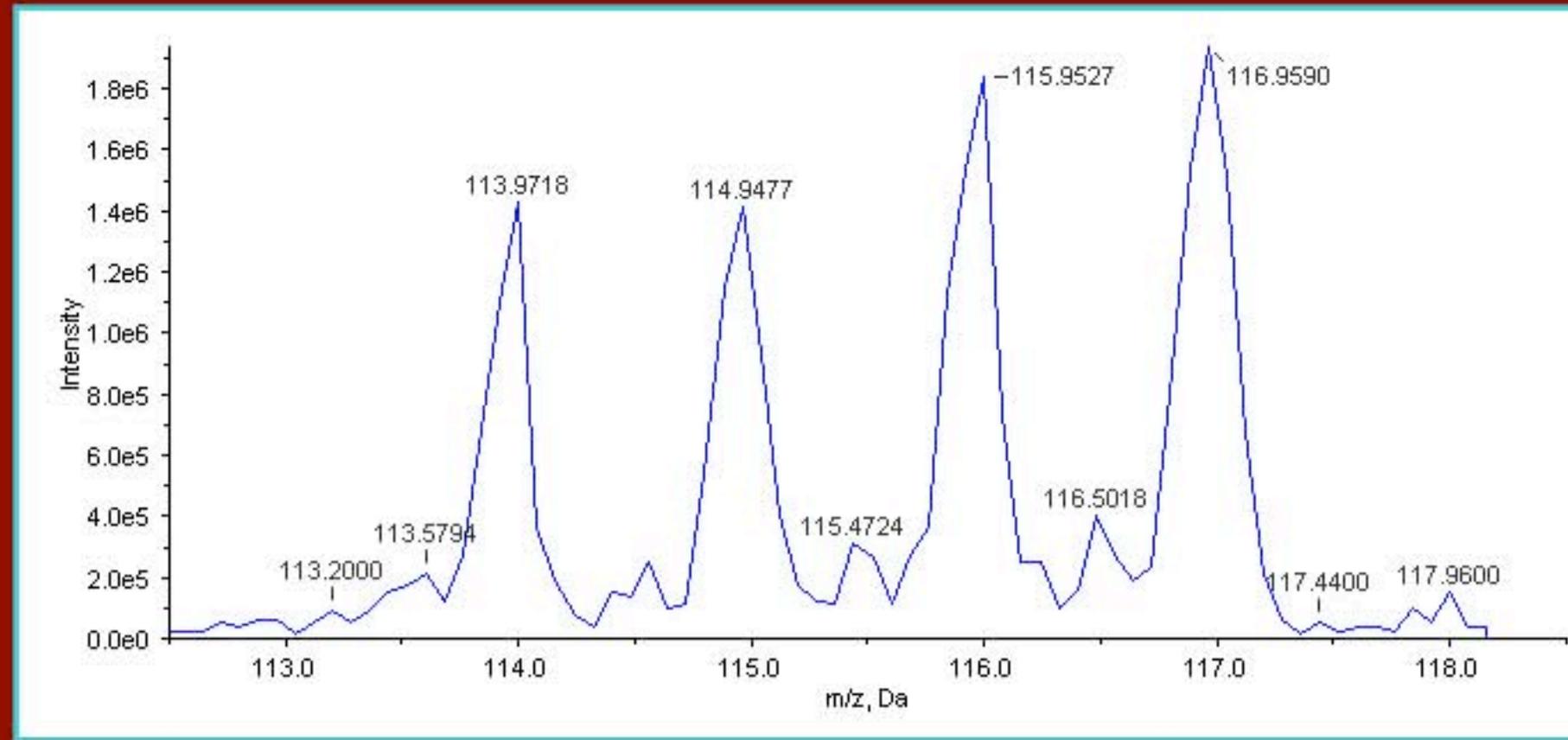


# GroEL biomarker *AQIEETESAFDR* Validated by MRM nLC/MS/MS assay

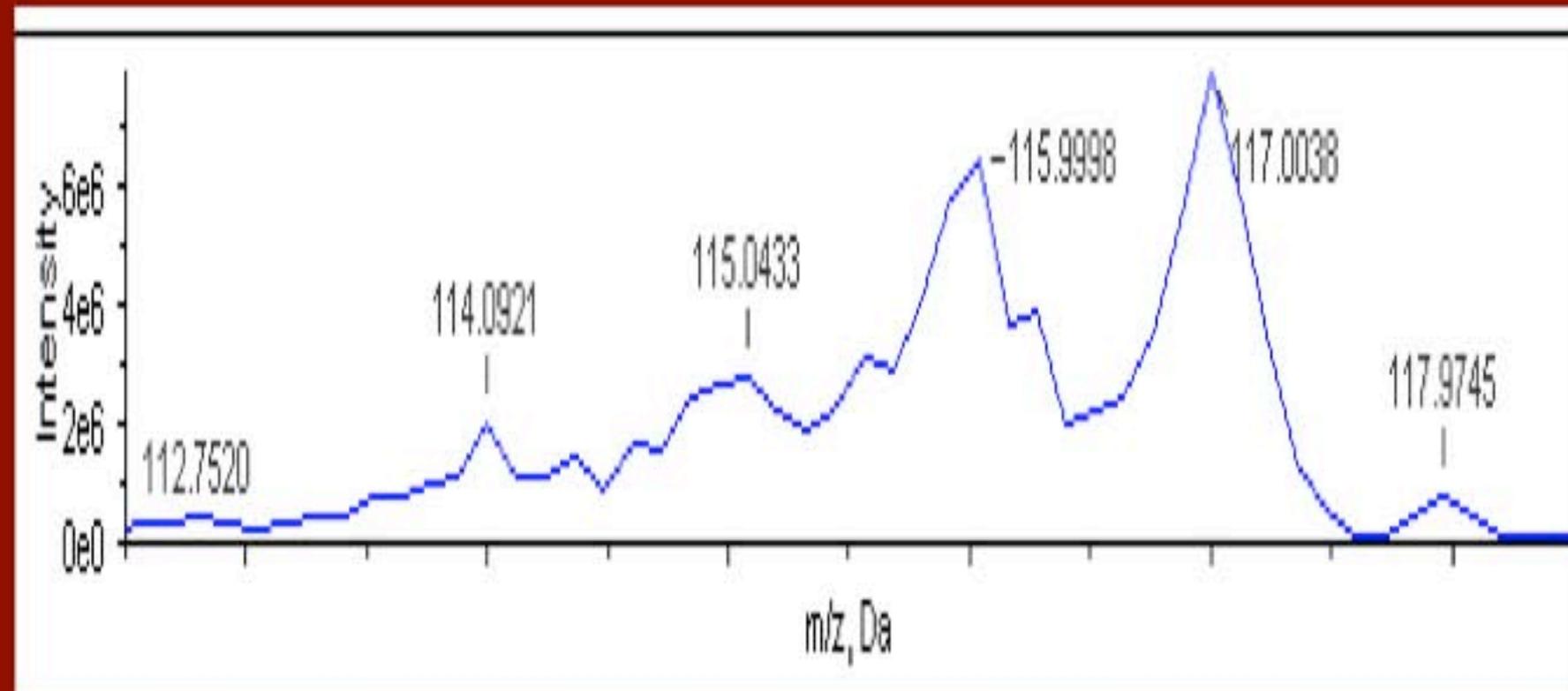


Note that in this case the signal-to-noise ratio for the validation ion is much better than for the iTRAQ reporter ions.

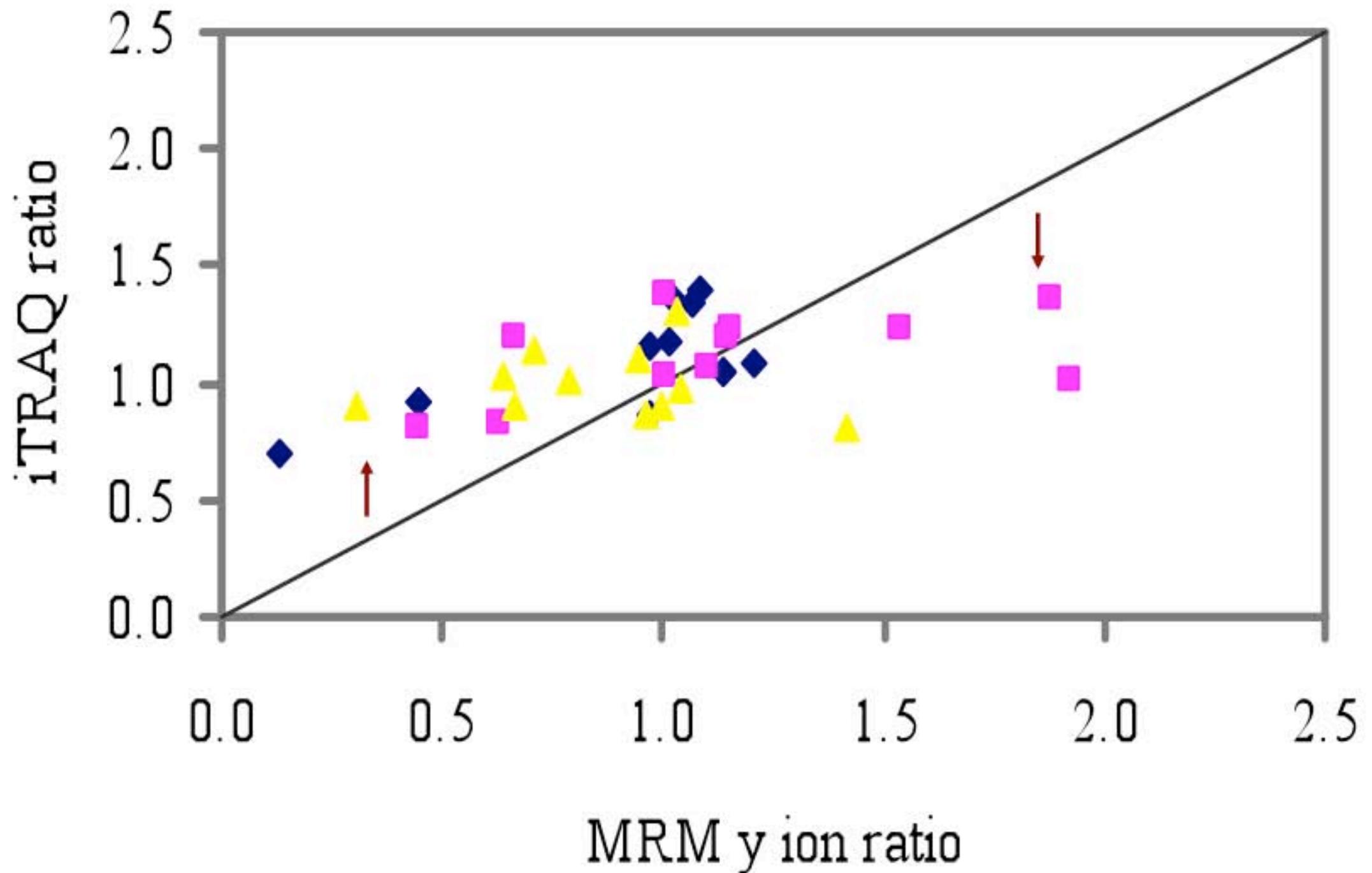
Nice  
Resolved  
iTRAQ  
peaks



Dubious  
iTRAQ  
peaks –  
Baseline  
issues?



# Comparing ratios from MRM-iTRAQ to iTRAQ (all targets)



# Remaining Challenges

- iTRAQ specific issues:
  - Low dynamic range (could be improved w/instrument improvements and by better peptide chromatography)
  - Comparing across multiple iTRAQ experiments
    - Some newer algorithms/pipelines (e.g. iQuantitator, Quant) poised to help here.
- Database issues leading to false negatives
  - Metagenomes most appropriate but not incomplete assembly issue
  - Microbial Genomes (IMG, NCBI, SwissProt) complete but are not usually specific to environment of interest
- Mapping proteins to function (metabolic, regulatory, structural)

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- Cornell Proteomics & Mass Spec Facility (Sheng Zhang; Celeste Ptak)
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- Brian Rahm (PhD, 2007)

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