

BUSINESS SENSITIVE

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Battelle Memorial Institute

Internal Research and  
Development Project

# Demonstration of Metaproteomic and Metagenomic Technologies for Advanced Monitoring of Bioremediation Performance

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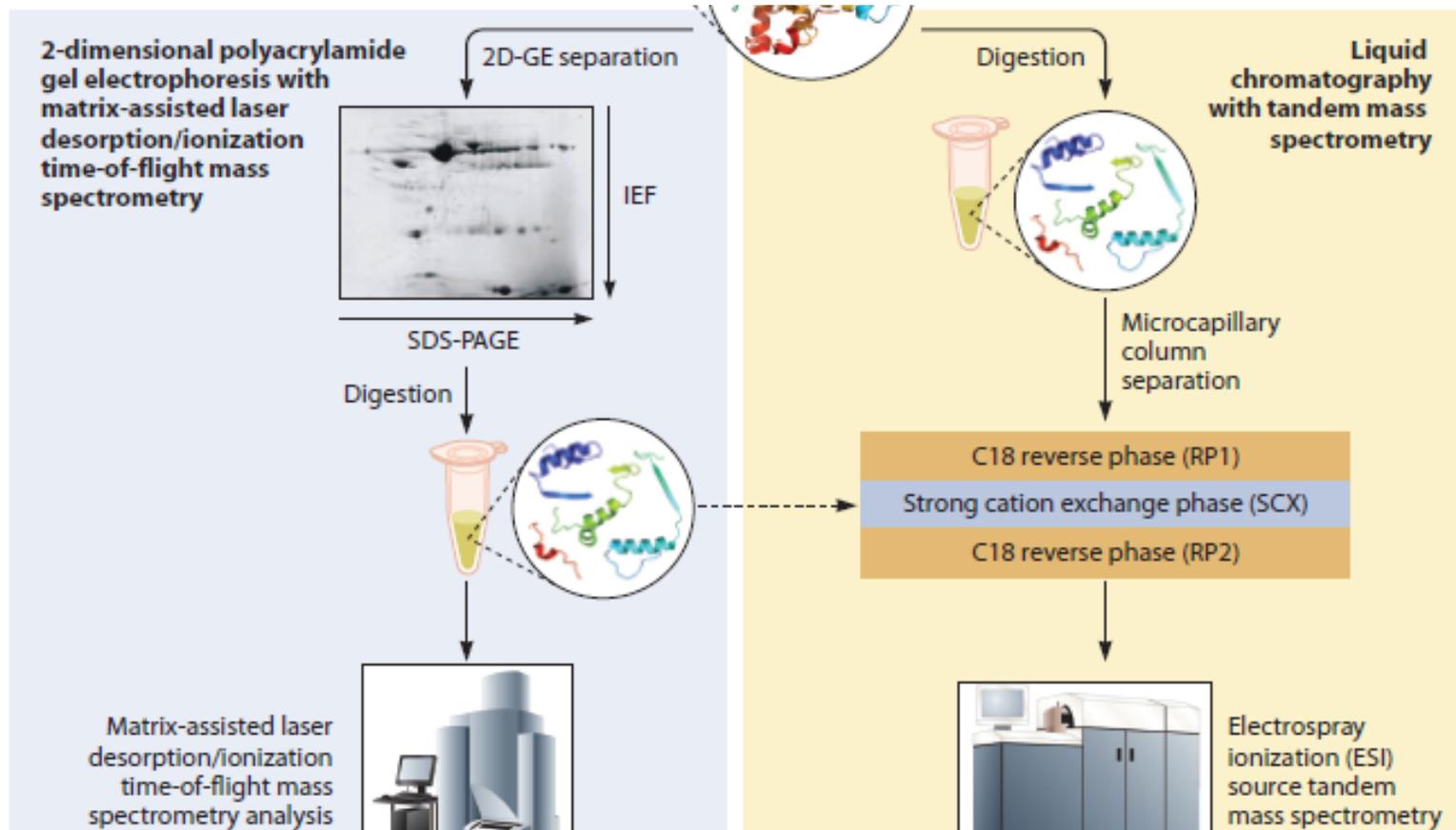
# Environmental Proteomics

- Post-environmental genomics tool
- Designed to provide quantitative (qProteomics) and qualitative (sProteomics) measurements of final gene products (proteins) as biomarkers of metabolic activity
- 16S rRNA work and deep metagenome sequencing are key for success of proteomics

Experimental system	Research goal	Complexity	Feasibility
<i>Isolate in the laboratory</i>	Metabolic pathways Stress responses and adaptation	↓	↑
<i>Community in the laboratory</i>	Community function under controlled conditions Model communities to study interactions		
<i>Community in the environment</i>	Community function under complex, ecologically relevant conditions		

Relationship between feasibility of proteomic studies and sample complexity. Different complexity levels can be used to accomplish certain study goals.

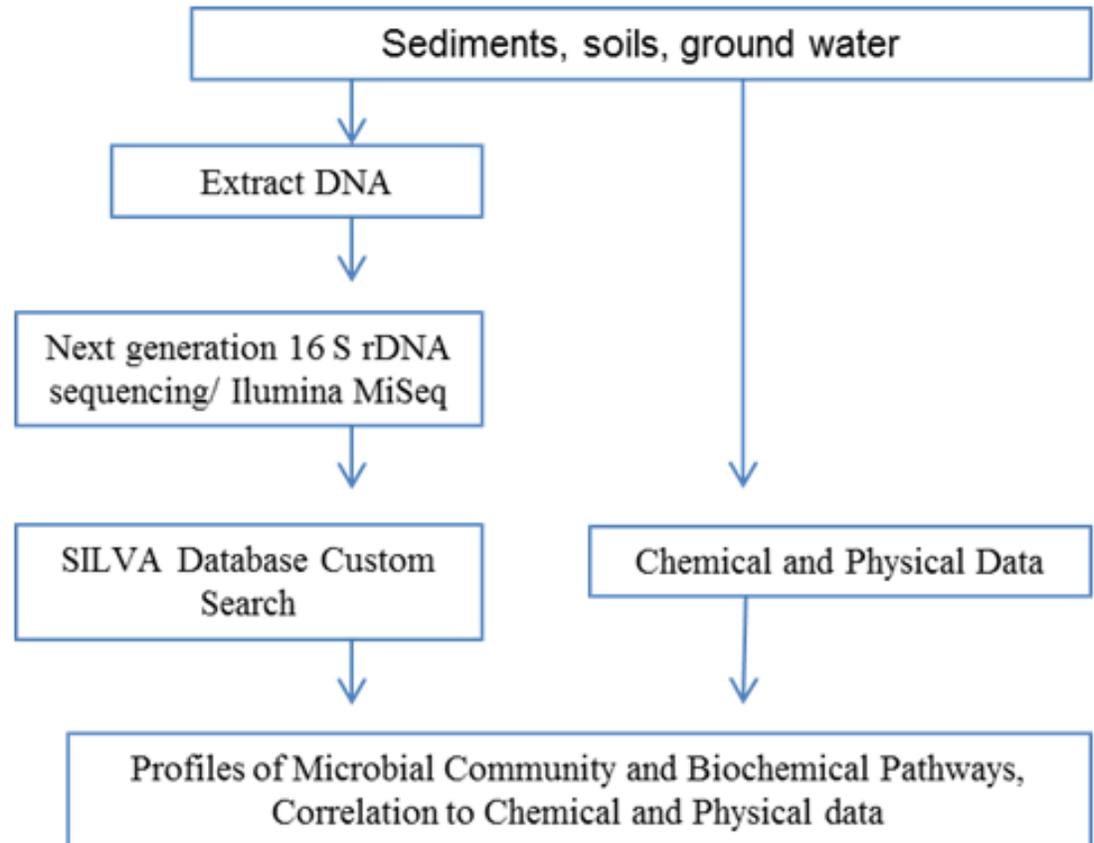
*Carla M. R. Lacerda, and Kenneth F. Reardon Briefings in Functional Genomics and Proteomics 2009;8:75-87*



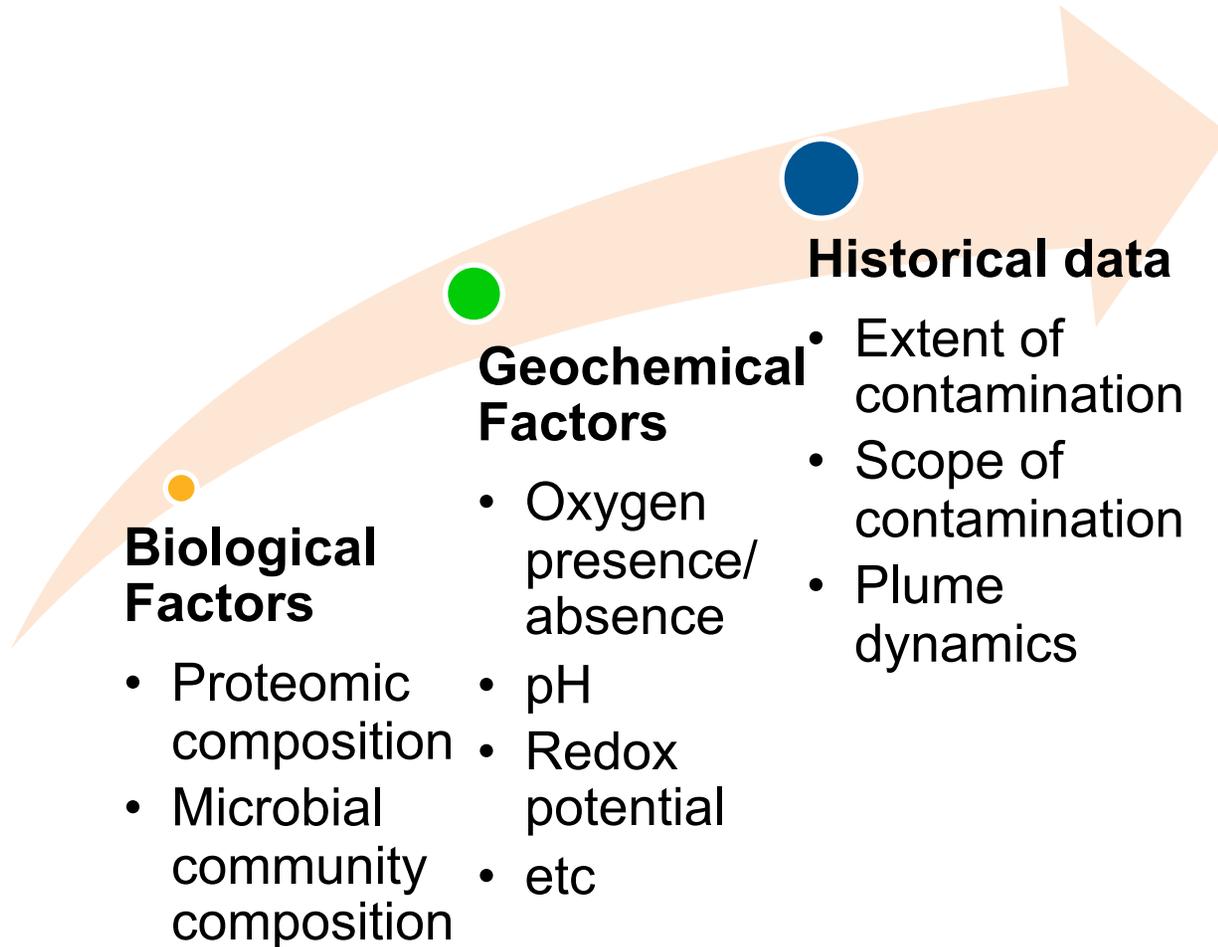
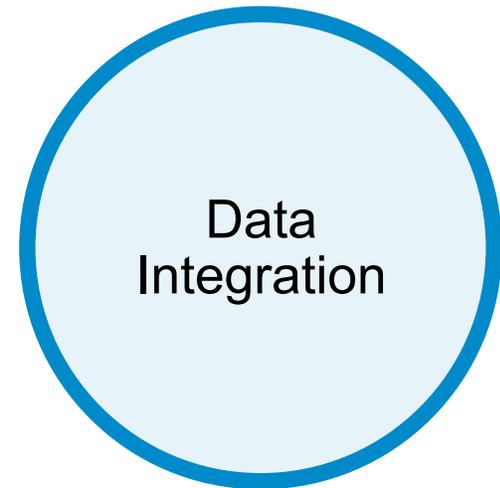
Rapid and direct measurement of microbial activity in the subsurface, which can support ISB diagnostics and optimization as well as transition to passive treatment or long-term monitoring.

# Environmental Metagenomics

- Extensive evaluation of microbial communities with depth previously unattainable
- Identification of microbes involved with:
  - Ecosystem Health
  - Remediation
  - Corrosion and Fouling
- Integration into existing evaluation technologies



# Data Integration



- Peptide markers
- Most common bacterial species
- Crucial geochemical factors

# Project Objectives and Goals

## Develop Quantitative Proteomic (qProteomic) Approach to Target Degradation Biomarkers of Chlorinated Compounds

- Targeted qProteomics has a potential to complement qPCR in scenarios where qPCR does not aid remedial actions.
- qProteomics is a true measure of active biodegradation of contaminants and may support RPMs in decision making as well as application of Monitored Natural Attenuation and save significant \$\$ to the project.

# Experimental Setup

## 1. Environmental subsurface water samples of:

- Chlorinated Volatile Organic Compounds (CVOCs)

## 2. Sample cleanup and trypsin digestion in solution

## 3. Discovery(LC/MS-MS) C18 column (Eksigent 3C18-CL-120, 3 $\mu\text{m}$ , 120 $\text{\AA}$ , 0.3 x 150 mm),

- Shotgun proteomics
- Shotgun proteomics with peptide specific inclusion list
- Time gradient (90 and 120 minutes)

## 4. Quantitative Proteomics

- 90 minutes gradient
- Isotopically labelled peptides
- Bioinformatics

## 5. Spectra searched

using ProteinPilot/

UniProt database or

Mascot/ NCBI nr

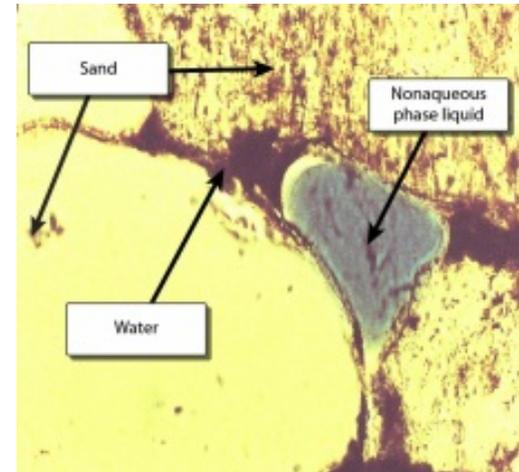
database



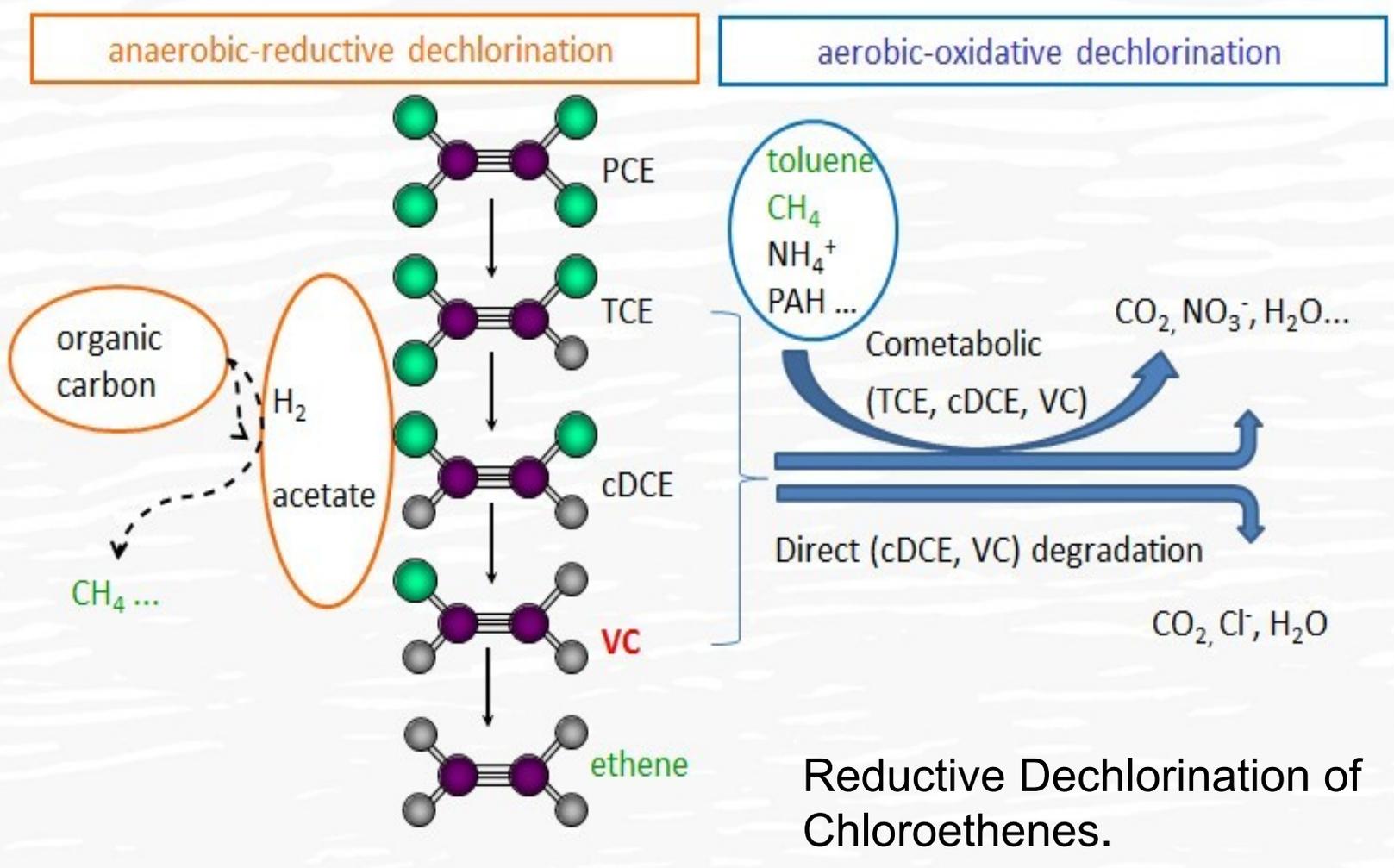
# Environmental Samples

## Chlorinated Volatile Organic Compounds (CVOCs) - Environmental Pollutants

- Environmentally hazardous:
  - Considered toxic and mutagenic
  - Health risk if in groundwater
- Types of contamination:
  - Soil and groundwater
  - DNAPLs in subsurface
  - Managing of DNAPLS, solvents sorbed to solid, volatilized in soil gas and dissolved in water
- Degradation
  - Microbial reductive dehalogenation

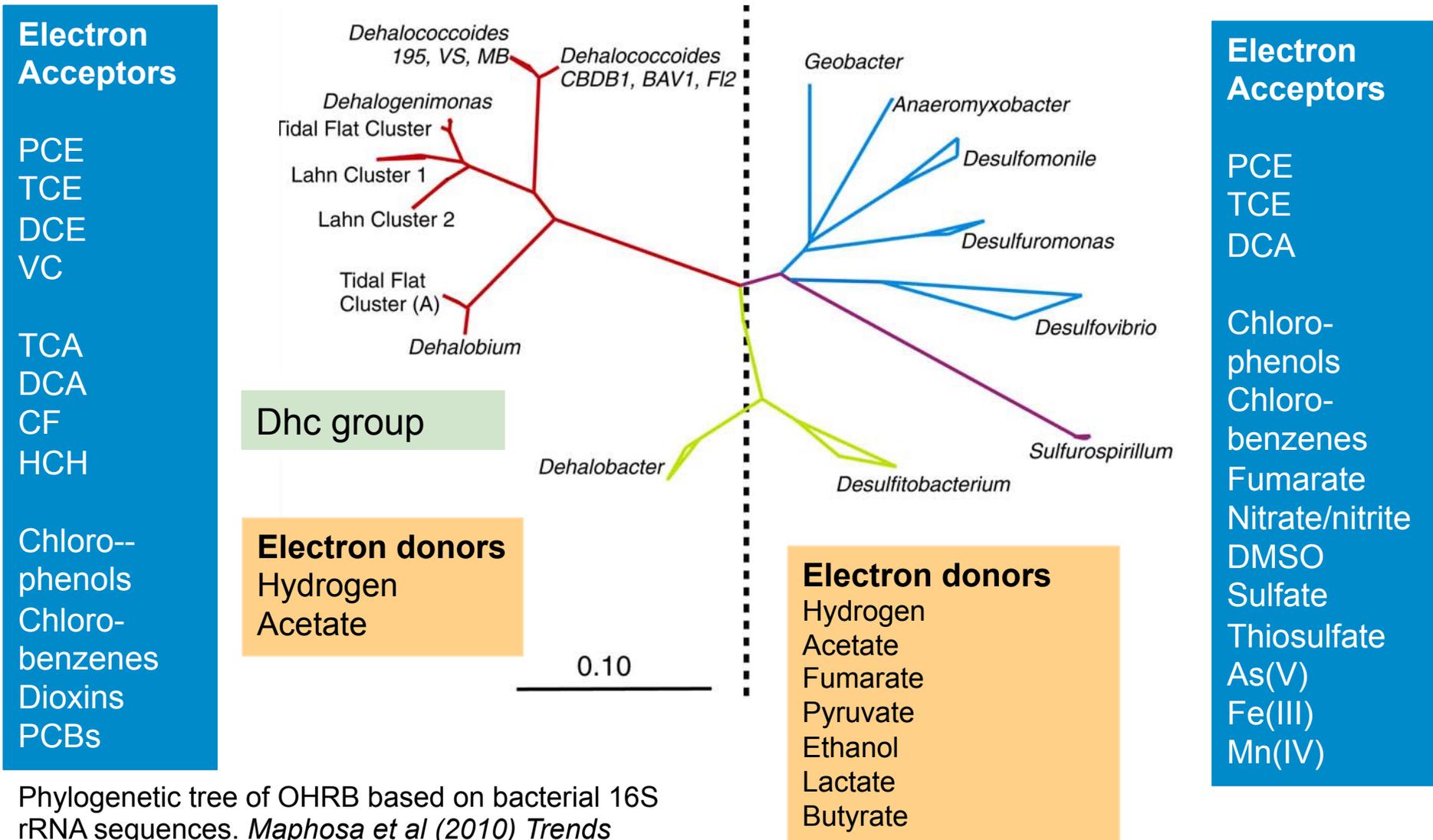


# Microbial Dehalogenation of CVOCs



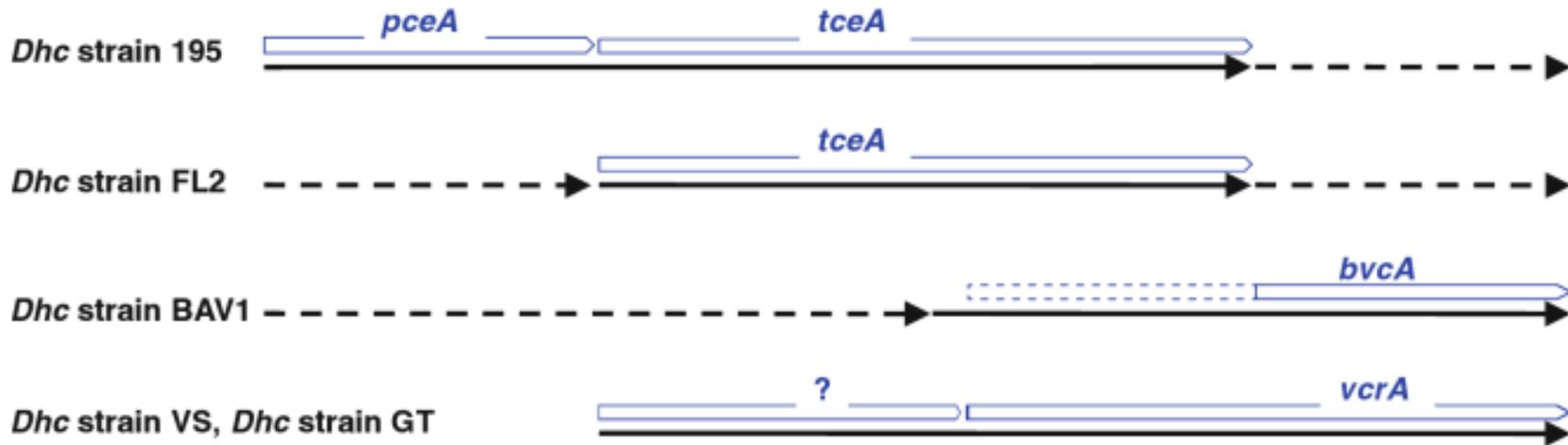
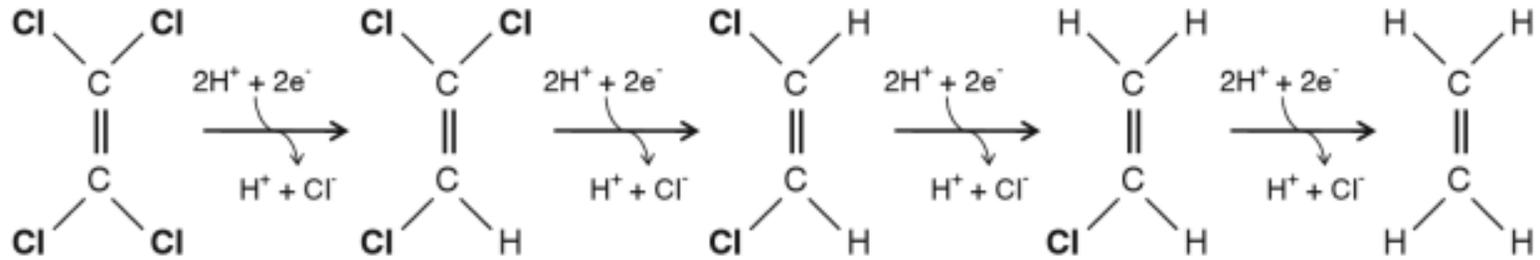
Reductive Dechlorination of Chloroethenes.

# Restricted Metabolism      Versatile Metabolism



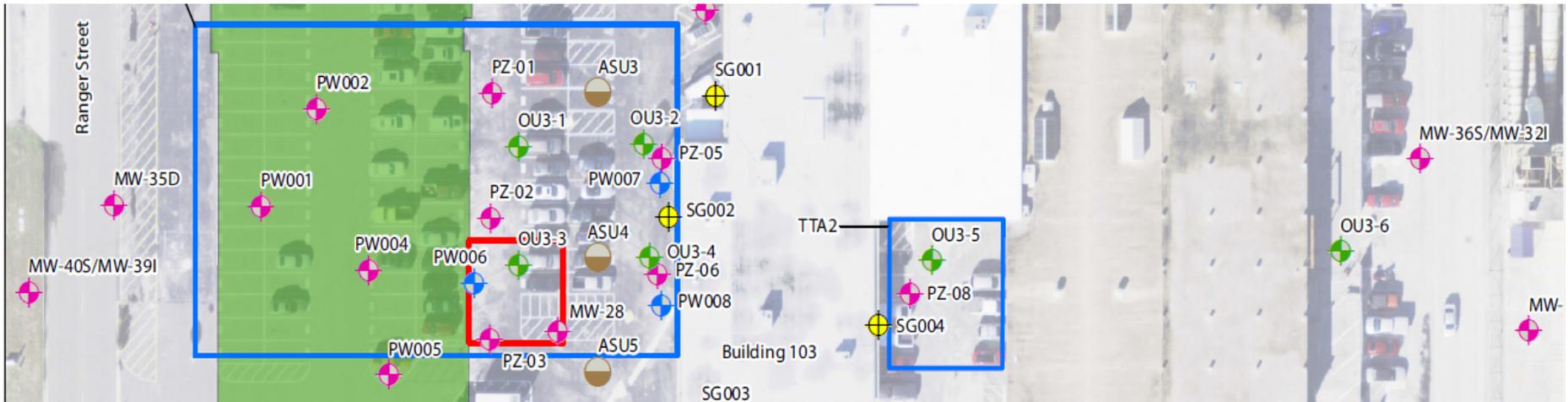
Phylogenetic tree of OHRB based on bacterial 16S rRNA sequences. *Maphosa et al (2010) Trends Biotech.*

# Microbial Dehalogenation of CVOCs



Dhc Rdase genes implicated in reductive dechlorination of chlorinated ethenes.  
*Bioaugmentation for groundwater remediation. (2013) ed. H.Ward .*

# Naval Air Station, Jacksonville, FL



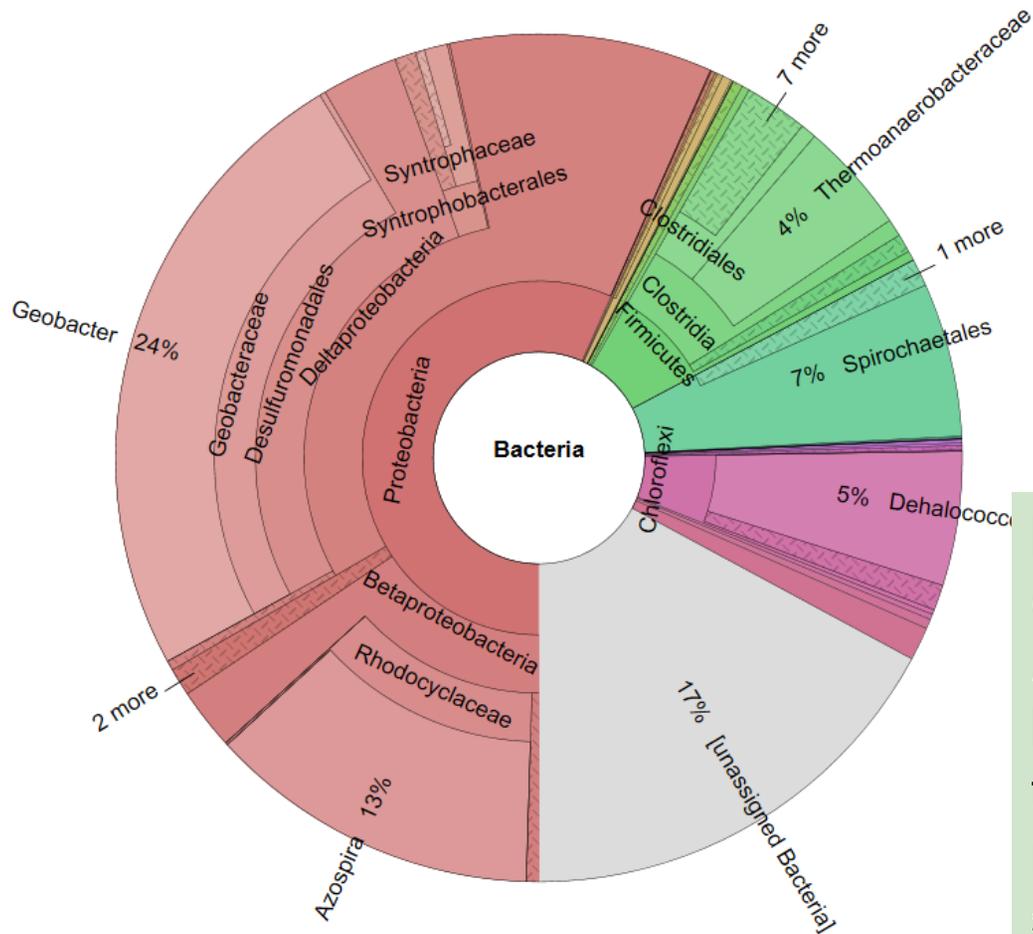
MW-40S - Upgradient, Outside TTAs

**PZ-02 – TTA**

MW-36S - Downgradient, Outside TTAs

PCE (ug/L)	TCE (ug/L)	cDCE (ug/L)	VC (ug/L)	TOC (mg/L)	vcrA (C/L)	pH	ORP (mV)	DO (mg/L)	Methane (ug/L)
<b>PZ-02 (12/11/14) Target Treatment Area</b>									
50	50	3,300	3,300	26	3x10 <sup>7</sup>	6.48	-150.8	0.19	17,000

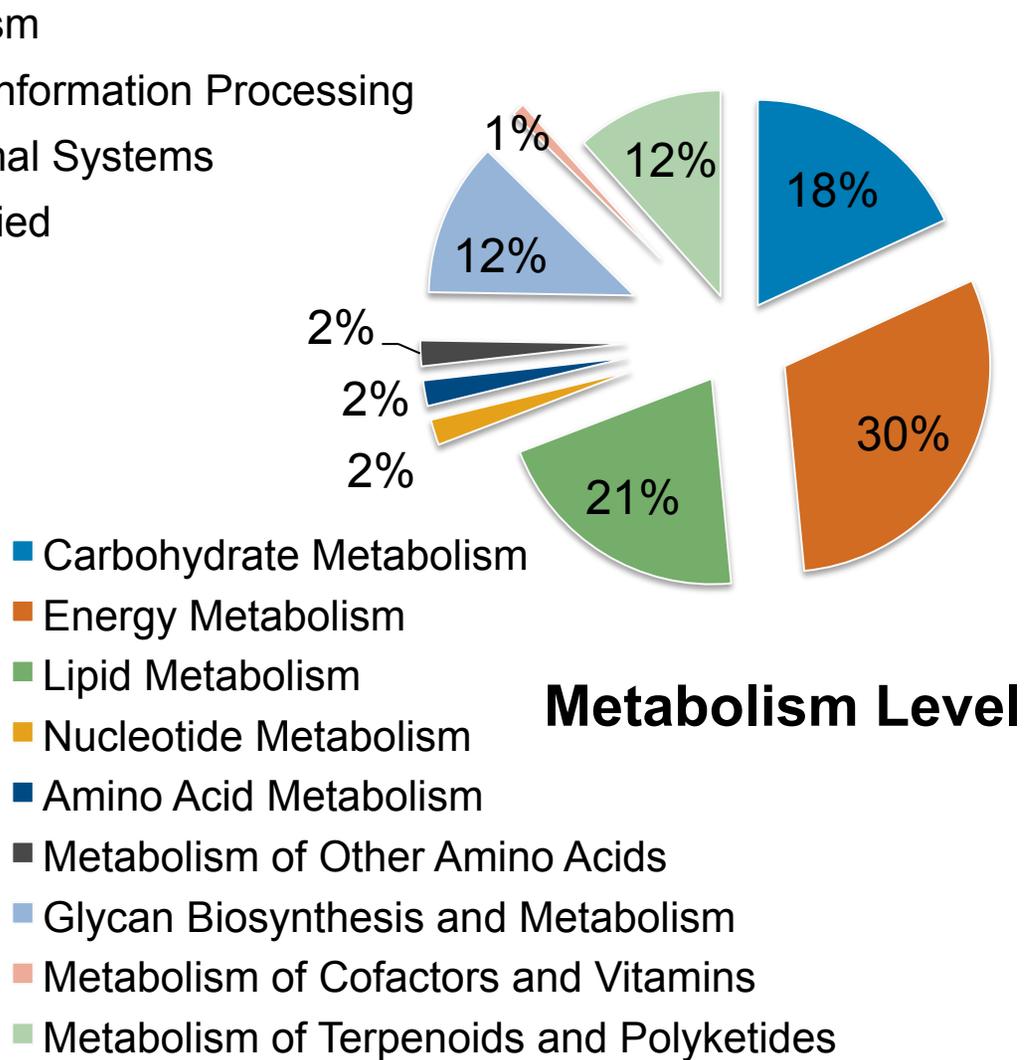
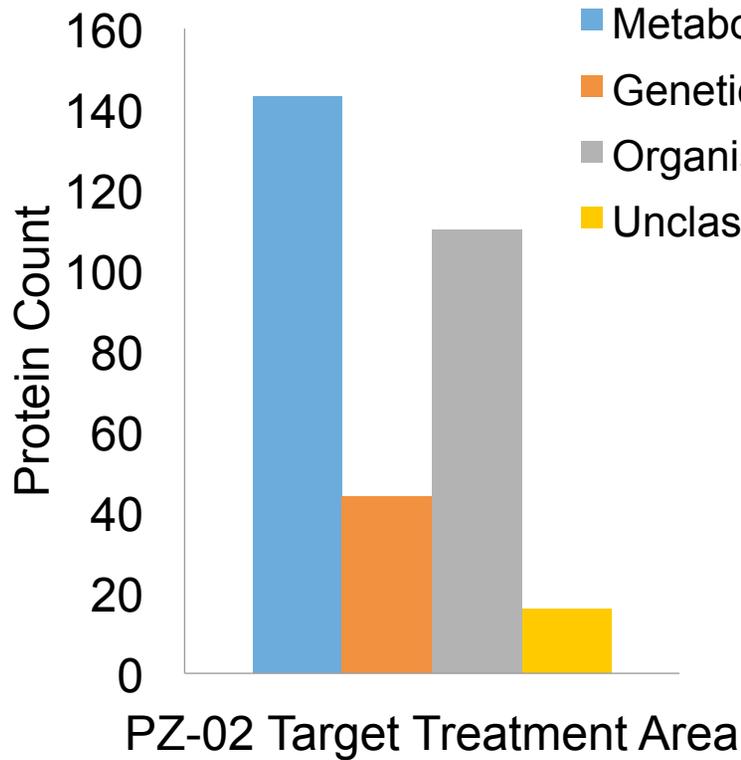
# Discovery – 16S Sequencing



% of Bacteria	
Dehalococcoides	5%
Geobacter	24%
Methylobacter	9%
Desulfuromonadales	27%

High diversity of *Dehalococcoides* (5% of total Bacteria), *Dehalogenimonas* (0.3% of total Bacteria), *Methanogens* (1% of total population) and sulfate reducing bacteria (3% of Bacteria) suggest presence of CVOC degraders.

# Discovery – Shotgun Proteomics



# Discovery – Shotgun Proteomics, Inclusion List - Assembly

- Aligned all protein sequences of:
  - VcrA
  - BvcA
  - TceA
- Selected conservative peptide sequences for each of protein
- Total of 25 conservative peptides identified
- Constructed a library of peptide sequences that LC-MS/MS specifically targets for during the sample analyses

Peptide ID	Peptide Sequence	m/z
VcrA	YFGAGGVGALNLADPK	775.4
VcrA	VPDHAVPINFK	618.8
VcrA	EADYSYYNDAEWVIPTK	1032.4
VcrA	TGAAIHWK	442.2
BvcA	SLNNFPWYVK	631.9
BvcA	STVAATPVFNFFR	772.4
BvcA	SLNNFPWYVK	634.3
BvcA	DFENPTIDIDWSILAR	952.9
BvcA	DEALWFASSTGGIGR	783.8
BvcA	TPVPIWEEVDK	706.3
BvcA	GYNDQK	444.2
BvcA	VANEISPK	429.2
TceA	YHSTVTR	432.2
TceA	LGLAGAGAGALGAAVLAENNLPHFEK	1231.1
TceA	DVDDLLSAGK	516.7
TceA	ALEGDHANK	477.7
TceA	YSGWNNQGAYFLPEDYLSPTYTGR	1400.1

Library of conservative peptides implicated in reductive dechlorination of chlorinated ethenes.

# Discovery – Shotgun Proteomics with Inclusion List

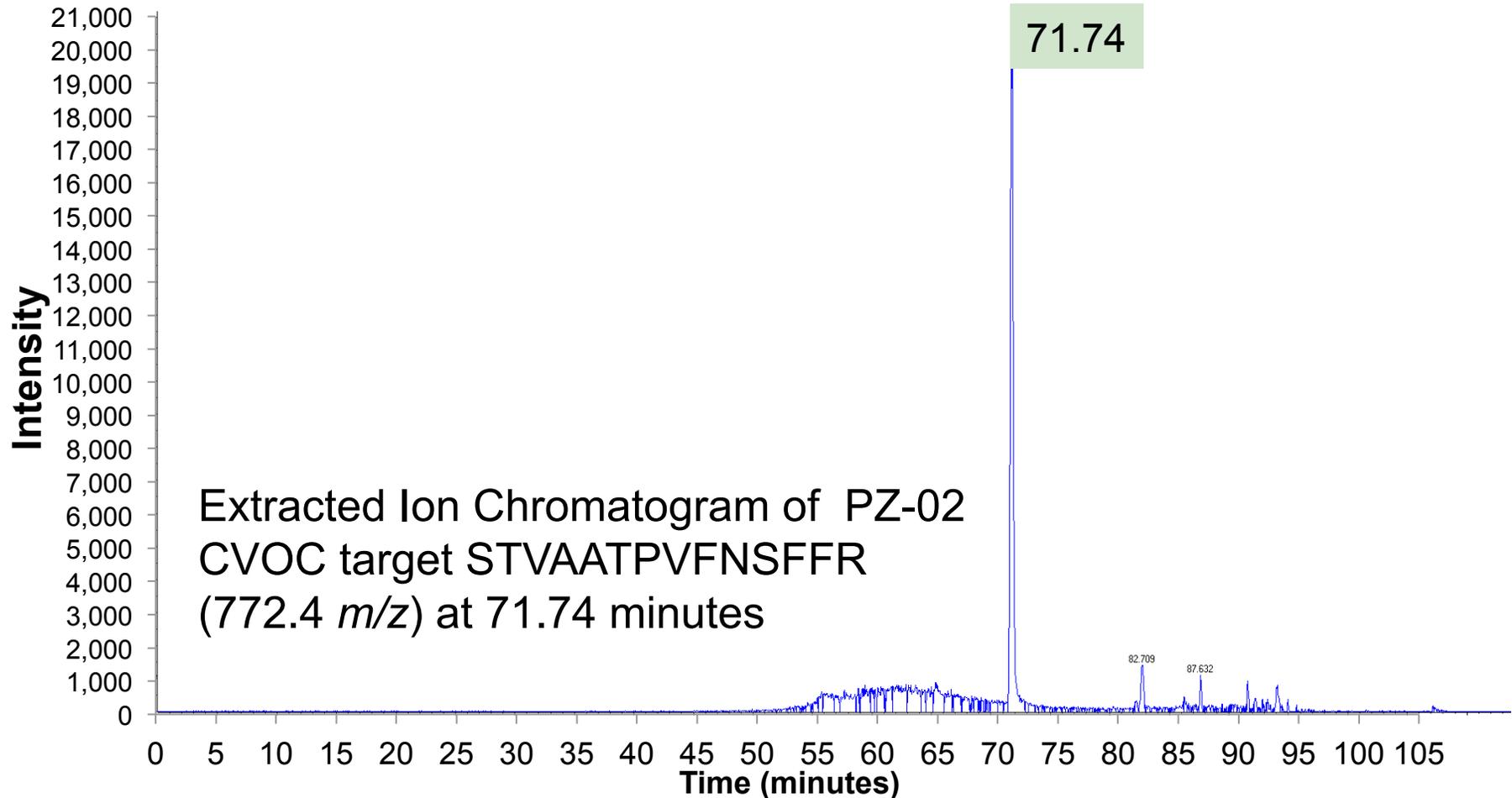
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BvcA	DEALWFASSTGGIGR	783.8
BvcA	TPVPIVWEEVDK	706.3
BvcA	GYNDQK	444.2
BvcA	VANEISPK	429.2
TceA	YHSTVTR	432.2
TceA	LGLAGAGAGALGAAVLAENNLPEFK	1231.1
TceA	DVDDLLSAGK	516.7
TceA	ALEGDHANK	477.7
TceA	YSGWNNQGAYFLPEDYLSPTYTGR	1400.1

Peptide ID	Score	Match	Peptide Sequence
bvcA	478	163	SLNNFPWYVK
	478	163	STVAATPVFNSFFR
	91	41	SLNNFPWYVK
hydA	19	4	LYSTVFK
	19	4	QQQTLIEK
	19	4	MDTHAALYEQGK
	13	7	MGYGQDVTGK
	13	7	AHKPFVVADK
	13	7	SPQQIFGSASK
	13	7	ENGFDIPVLCELK

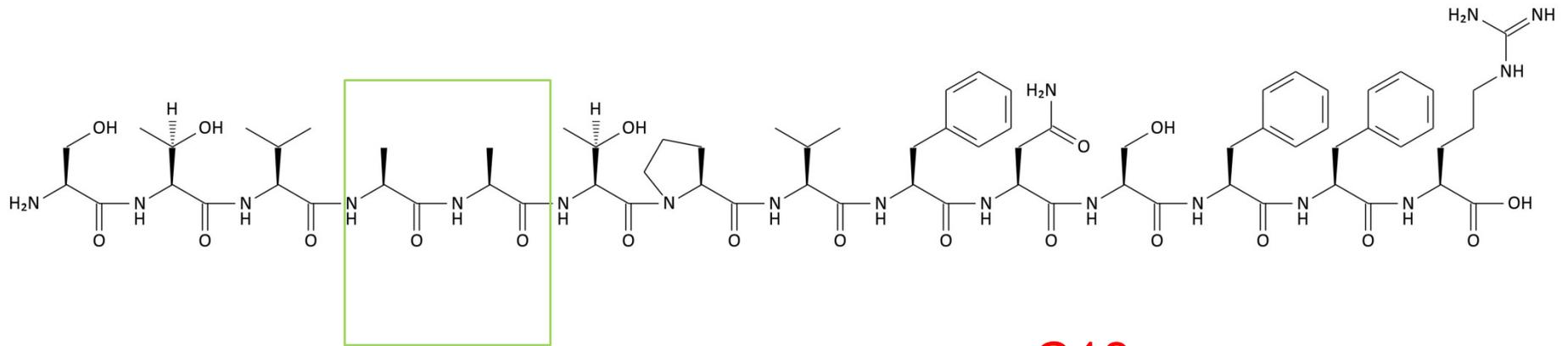
Results of LC/MS-MS proteomics with peptide inclusion list for sample PZ-02.

Library of conservative peptides implicated in reductive dechlorination of chlorinated ethenes.

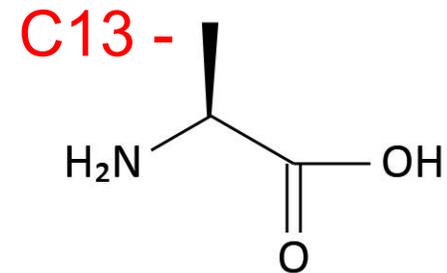
# Discovery – Shotgun Proteomics with Inclusion List



# Discovery – qProteomics with Isotopically Labelled Peptides

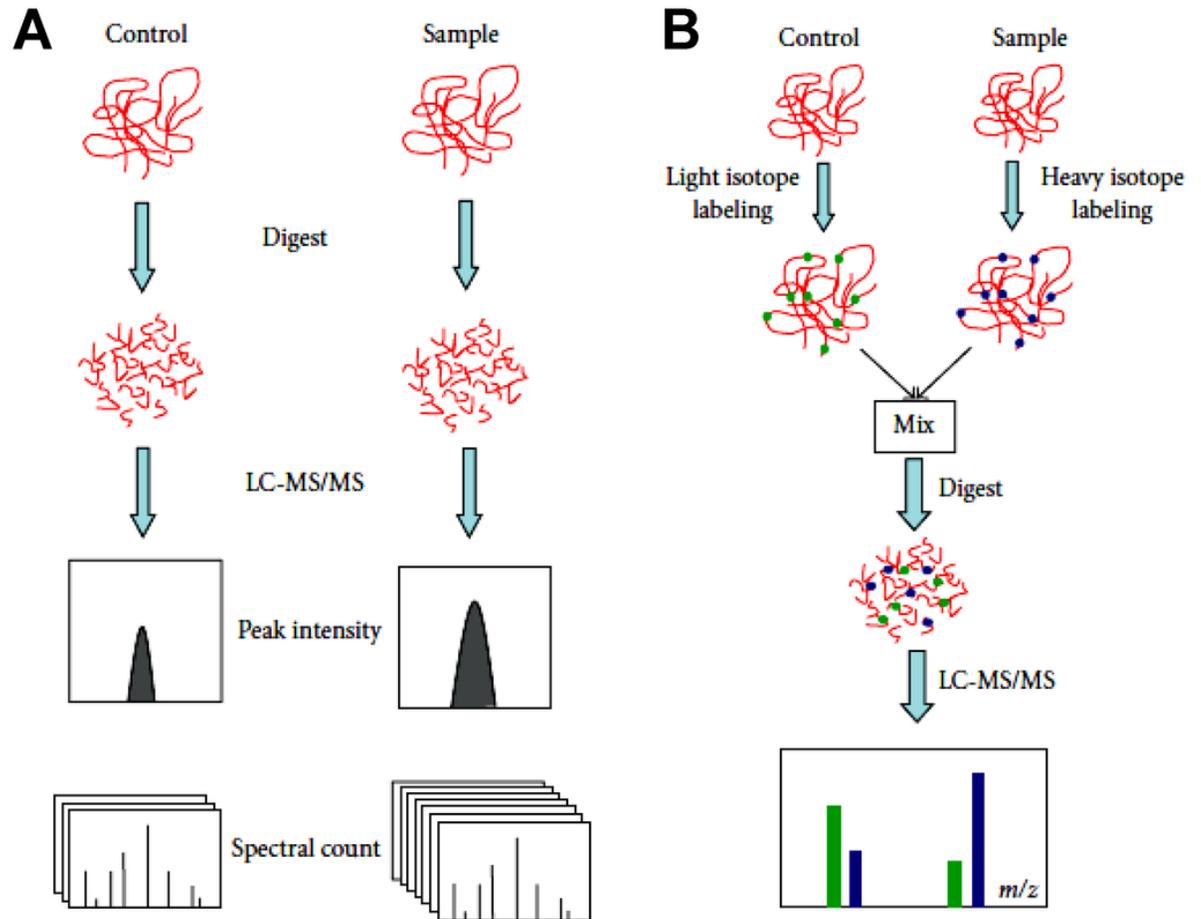


Target STVAATPVFNSFFR (772.4  $m/z$ ) protein  
at 71.74 minutes, labelled at Alanine sites  
( $^{13}C_6$ ;  $^{15}N_2$ ).

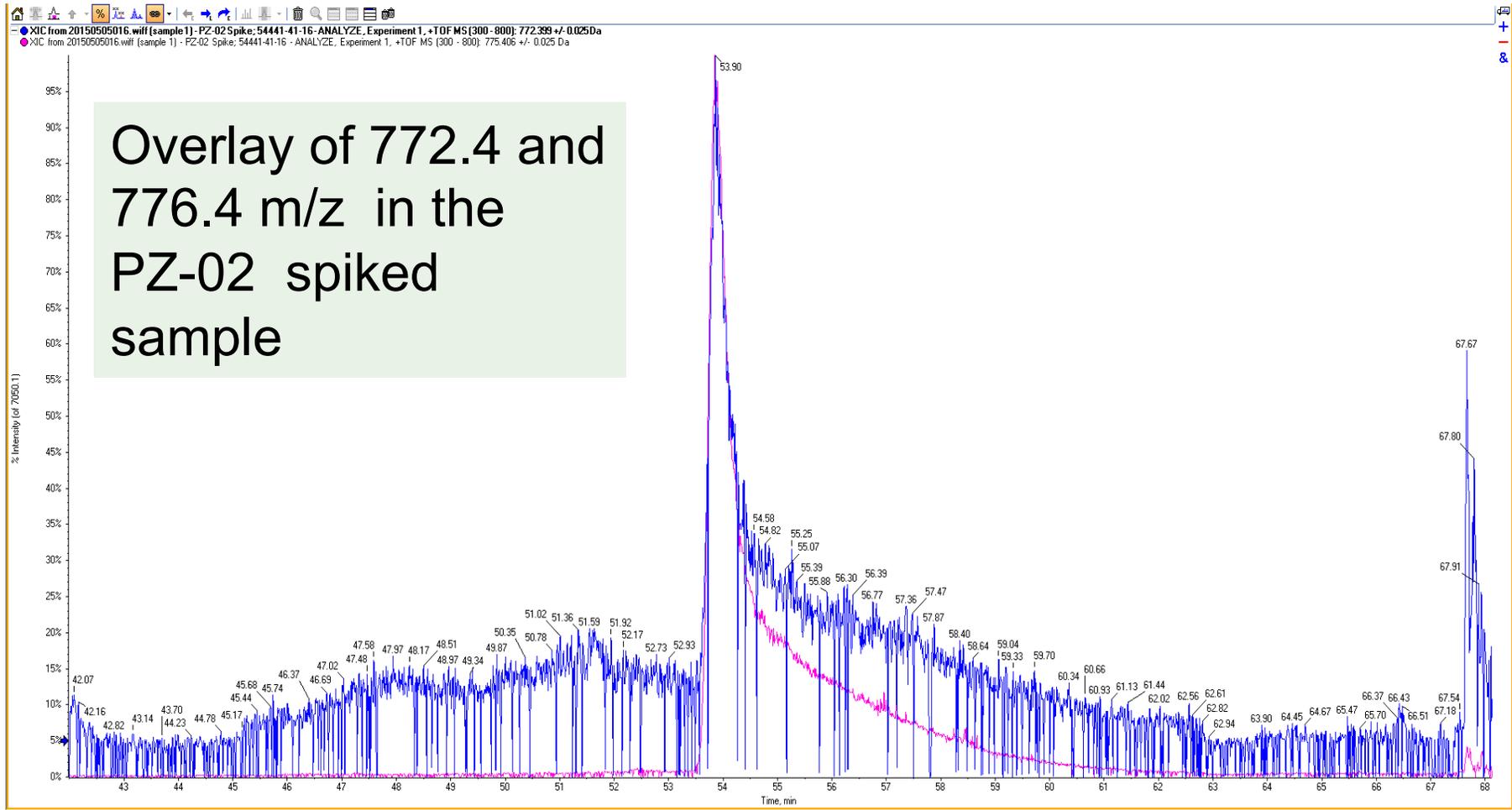


# Discovery – qProteomics with Isotopically Labelled Peptides

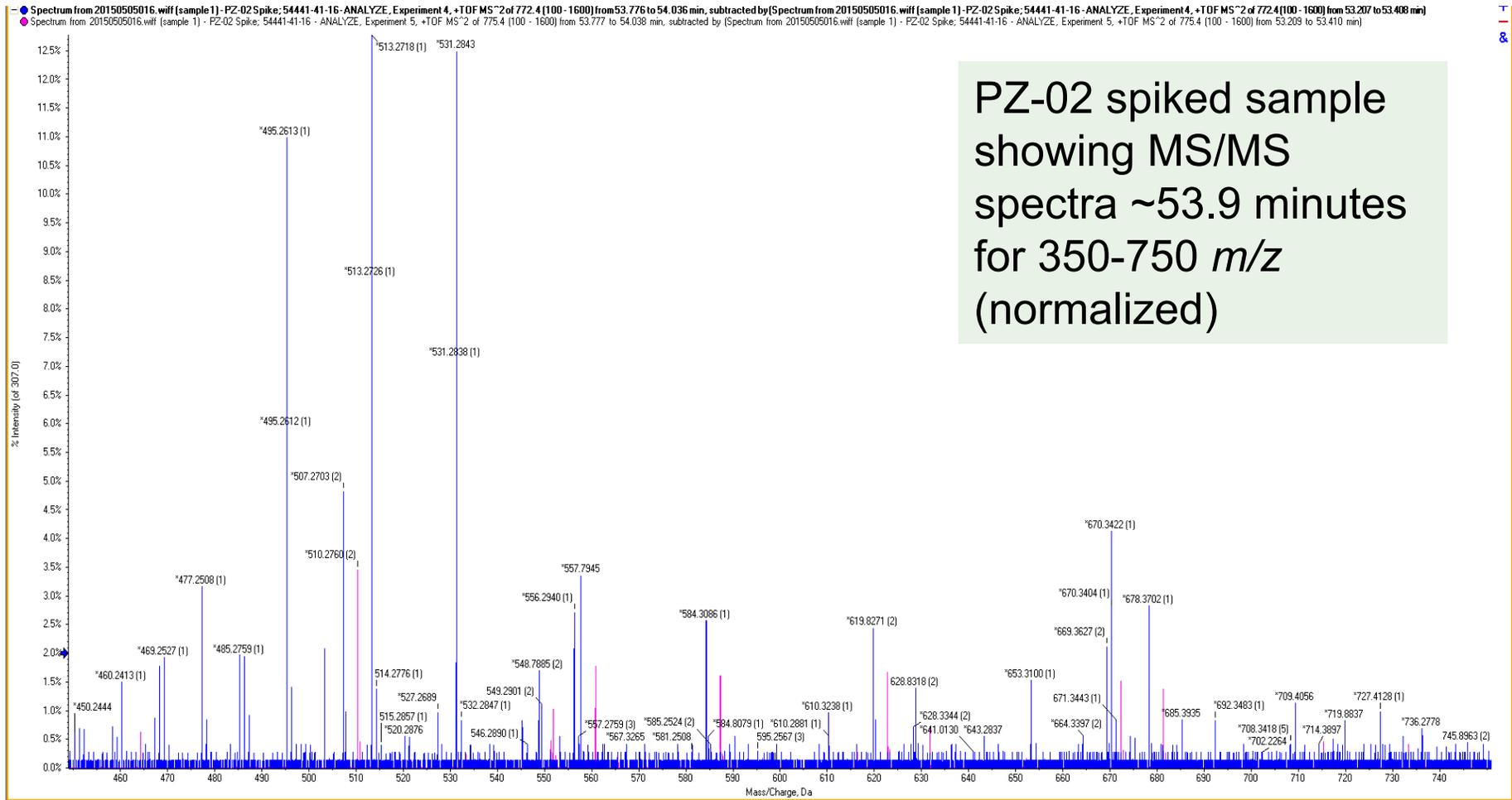
- Detection of specific peptides with improved experimental matrix
- Direct comparison of isotope-labelled peptide pairs. LC-MS/MS, and quantification of peptides of interest.



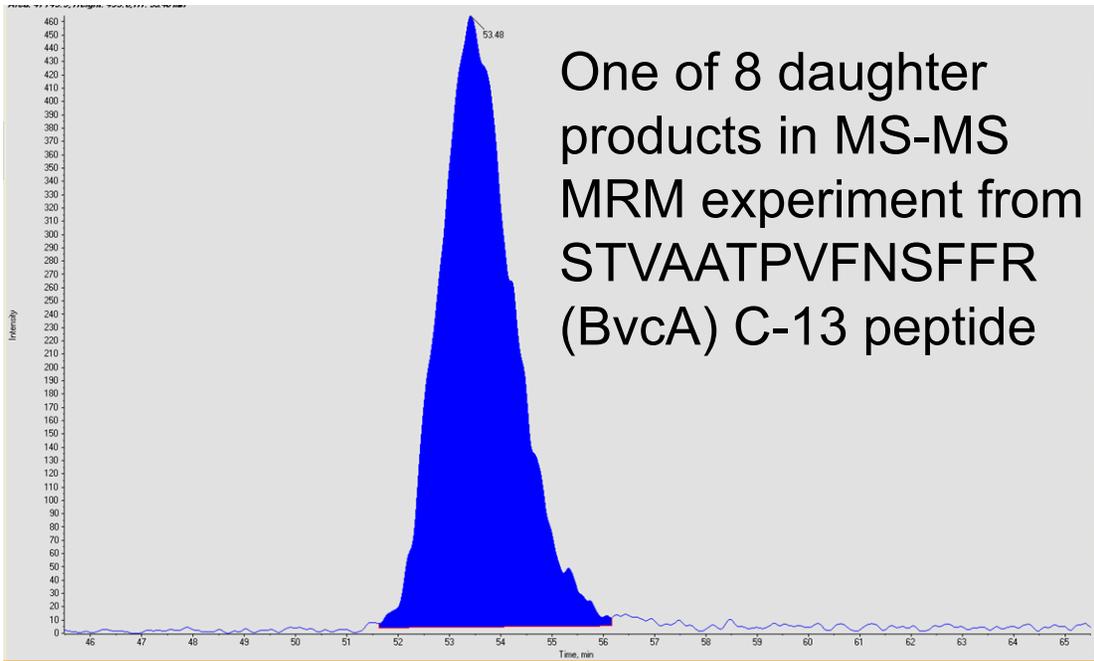
# Discovery – qProteomics with Isotopically Labelled Peptides



# Discovery – qProteomics with Isotopically Labelled Peptides



# Discovery – qProteomics with Isotopically Labelled Peptides



	VcrA (1)	Labeled VcrA (1)
PI 1	692.38	700.39
PI 2	839.44	847.46
PI 3	953.49	961.51
PI 4	1067.54	1075.55
PI 5	447.2	447.2
PI 6	201.12	
PI 7	173.13	173.22
PI 8	284.14	284.14

x = 51.88 fmol on column (80 $\mu$ L injection volume, 0.65fmol/ $\mu$ L)

# Summary

- Shotgun analysis of PZ-02 sample showed diversity of peptides involved in metabolic processes
- Identification of specific peptides to be incorporated into the inclusion list
- Isotopically labelled peptides as an internal standard
- Successful quantification of bvcA peptide concentrations in sample

# Acknowledgements

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- Craig Bartling, PhD
  - Larry Mullins
- Don Stoeckel, PhD

## **The Department of Navy**

- Mike Singletary

Thank you!  
Questions:  
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