Proteomics Approaches in Applied microbiology

Hundreds of microbes have been sequenced

- These sequences encode many valuable biological phenomena
  - Global elemental cycling
  - Novel pathways to convert cellulose to simple sugars
  - Novel pathways to convert sugars to bio-fuels
  - Pathways to degrade pesticides
  - Create engineered plants
  - Antibiotic resistance
    - etc

http://genome.jgi-psf.org/mic_home.html

Mattozzi, M., et al. 2006. Appl. Environ. Microbiol. 72:6699-6706.
Dien, B. S., et al. 2003. Appl Microbiol Biotechnol 63:258-66.
Demain, A. L., ey al. 2005. Microbiol Mol Biol Rev 69:124-54

Other reasons to study microbial physiology.

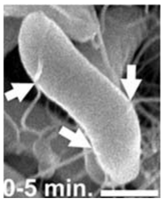
- Biofouling
- Activation of toxic metals
- Pathogenesis
- Virulence and crop disease

Heavy Metal Waste

- Radioactive and heavy metal waste is a problem
  - Expected cleanup costs in US alone total $300 billion with current techniques
  - Metal reducing bacteria can precipitate heavy metals, and thus halt their movement through the environment. Therefore, bioremediation is of interest.
- *Desulfovibrio vulgaris* has the ability to reduce several heavy metals including uranium, chromium, and iron.

Wall, J. D., and L. R. Krumholz. 2006. Annu Rev Microbiol 60:149-66.

Desulfovibrio vulgaris Hildenborough

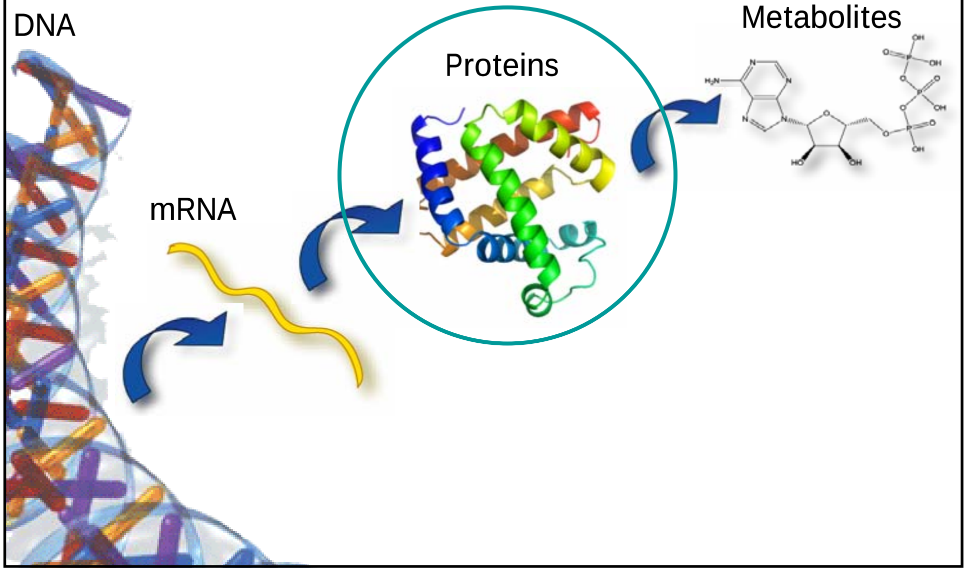


- Sulfate reducing bacterium
- Anaerobic organism
- Genome sequence available

Goal:

Develop better cellular models to understand bioremediation potential

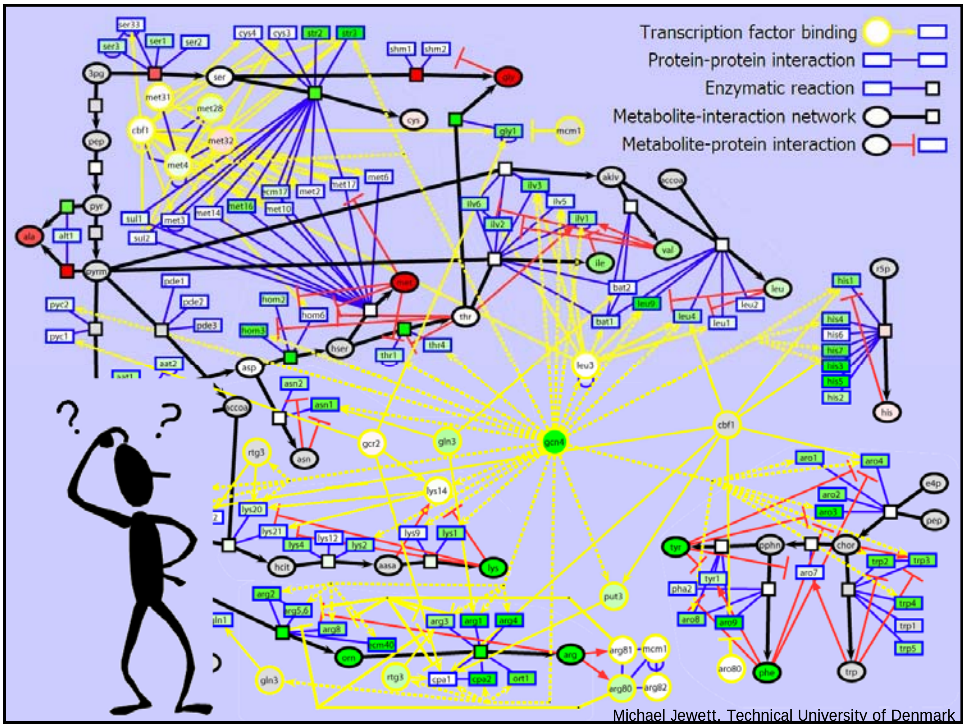
Transfer of Cellular Information


DNA

mRNA

Proteins

Metabolites



Michael Jewett, Technical University of Denmark

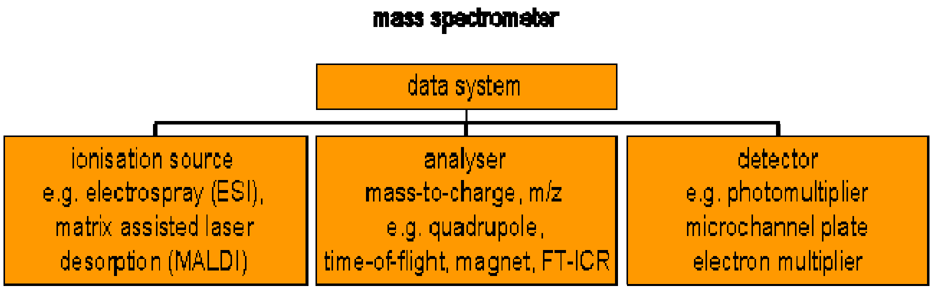
A high throughput method to analyze proteins

In 2002 John Fenn and Koichi Tanaka won the Nobel prize.

“”*for their development of soft desorption ionisation methods for mass spectrometric analyses of biological macromolecules”*

Today almost all proteomics tools rely on mass spectrometry

Mass Spectrometry



mass spectrometer

data system

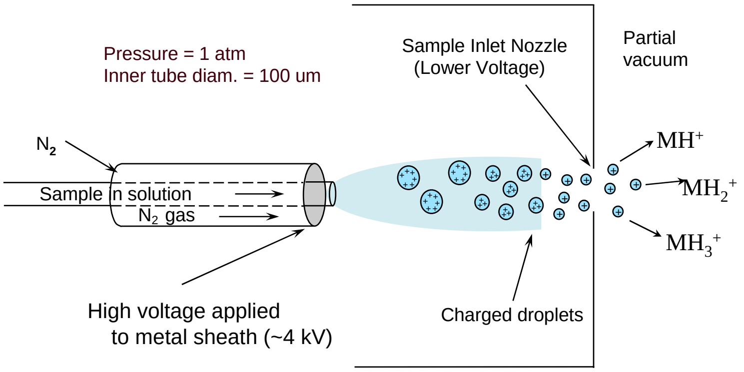
ionisation source
e.g. electrospray (ESI),
matrix assisted laser
desorption (MALDI)

analyser
mass-to-charge, m/z
e.g. quadrupole,
time-of-flight, magnet, FT-ICR

detector
e.g. photomultiplier
microchannel plate
electron multiplier

Ion Sources make ions from sample molecules

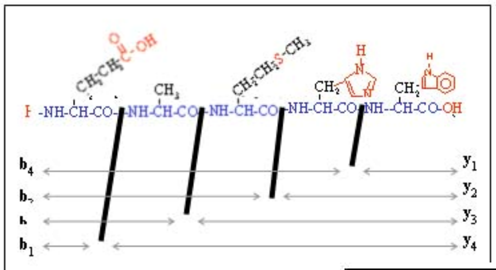
E.g: Electrospray ionization:



Mass analyzers separate ions based on their mass-to-charge ratio (m/z)

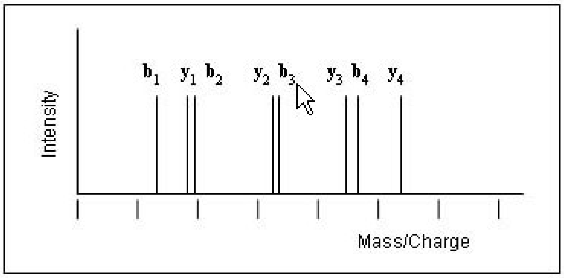
- Operate under high vacuum (keeps ions from bumping into gas molecules)
- Actually measure mass-to-charge ratio of ions (m/z)
- Key specifications are resolution, mass measurement accuracy, and sensitivity.
- Several kinds exist: for bioanalysis, quadrupole, time-of-flight and ion traps are most used.

Peptide parent ions can be fragmented for MS/MS data

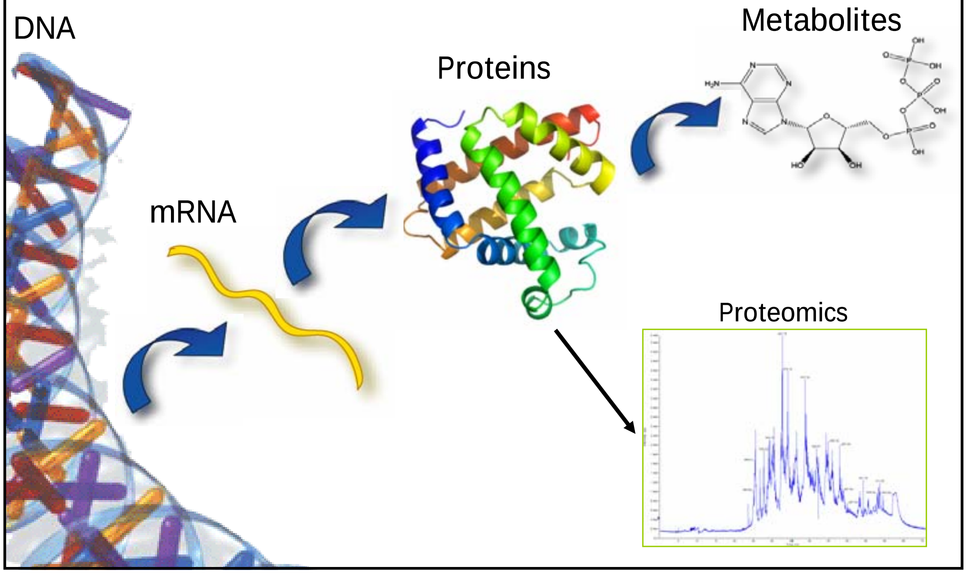


Peptides produce unique ion series that can be matched to theoretically predicted ion series for a given sequence

Peptide sequences are used for protein identification.



Transfer of Cellular Information



DNA

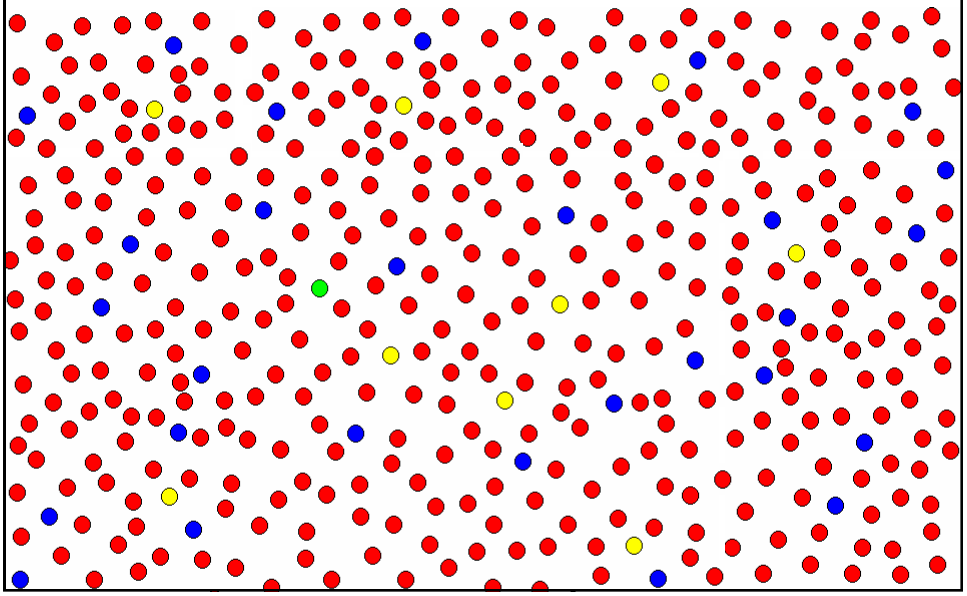
mRNA

Proteins

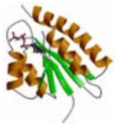
Metabolites

Proteomics

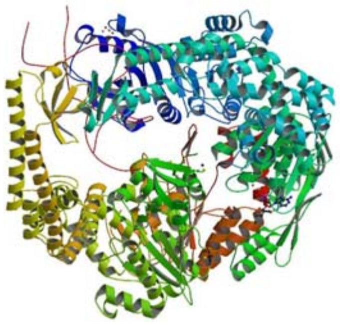
A Vast Dynamic Range



Not All Proteins Are Created Equal



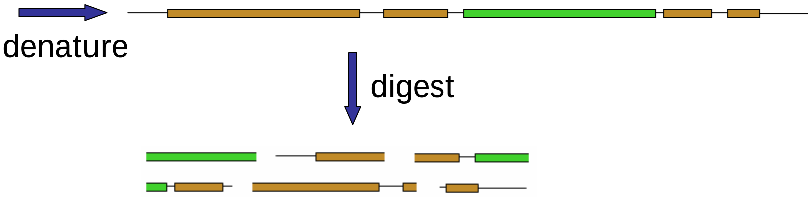
162 amino acids



853 amino acids

- Size
- Abundance
- Physical properties
- Associated with membrane

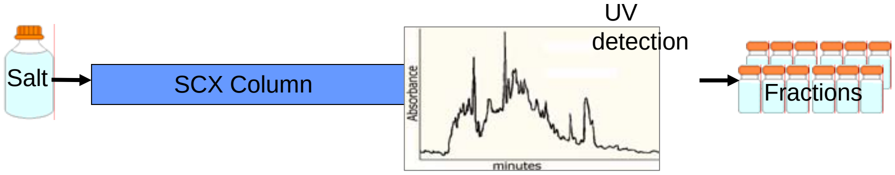
Protein Digestion



~4000 proteins × 5-10 peptides / protein (at least) = ~40,000 unique peptides

2-D Liquid Chromatography

Strong Cation Exchange (SCX) Separation

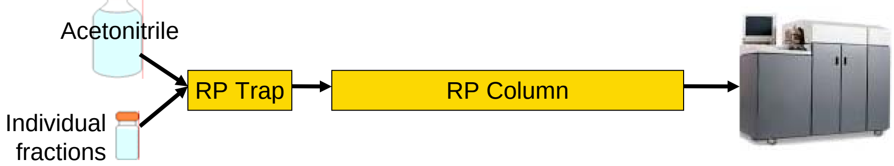

Salt

SCX Column

UV detection

Fractions

Reverse Phase (RP) Separation


Acetonitrile

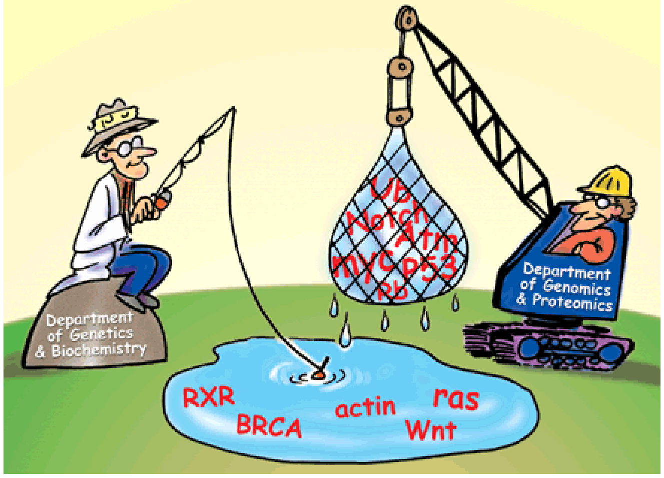
Individual fractions

RP Trap

RP Column

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

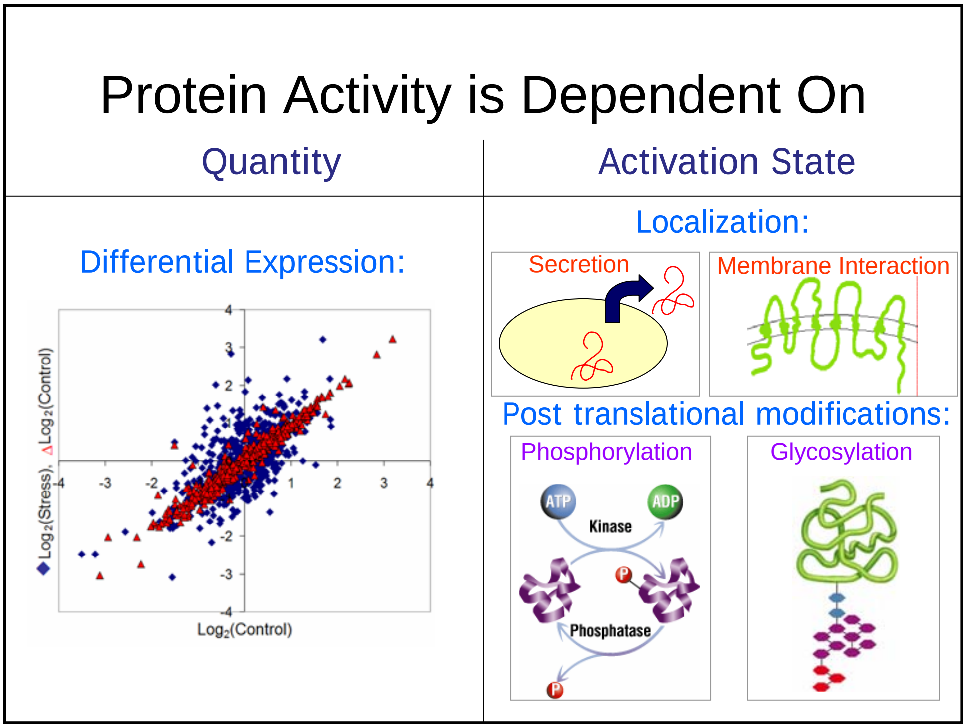
Proteomics



Fields, S. 2001. PROTEOMICS: Proteomics in Genomeland Science 291:1221-1224.

Proteomics

- Most high throughput proteomics requires availability of a genome sequence
- Predicted list of proteins and corresponding amino acid sequences are used to create theoretical databases which are used to analyze MS and MSMS spectra
- However, de-novo sequencing of MS data can allow identification of proteins from un-sequenced bacteria.



Protein Activity is Dependent On

Quantity

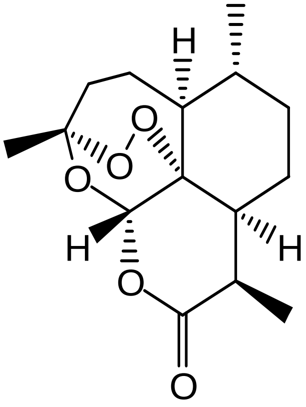
Differential Expression:

Activation State

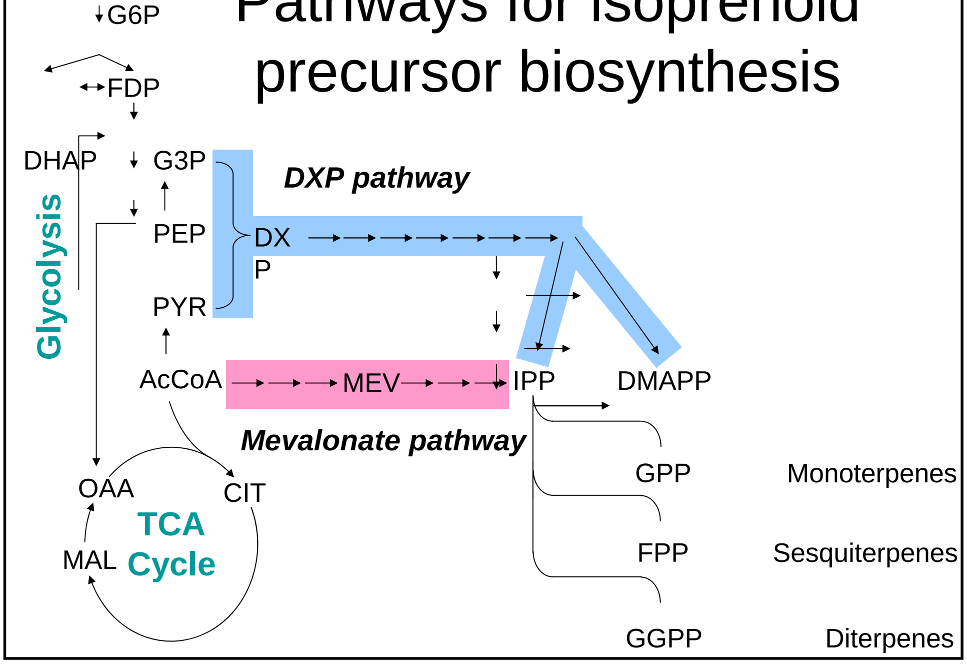
Localization:

Post translational modifications:

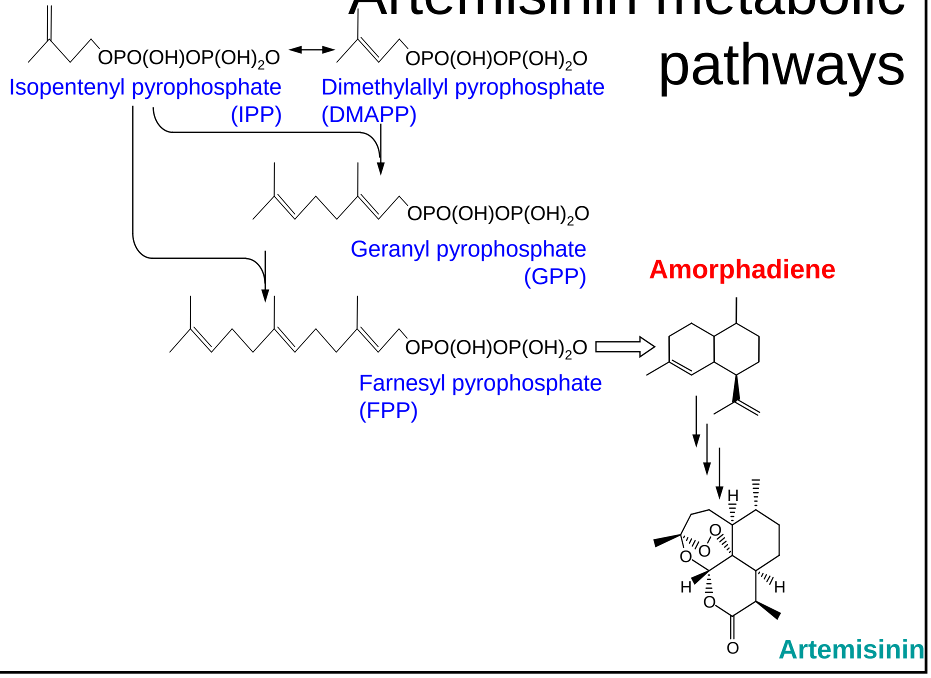
Artemisinin



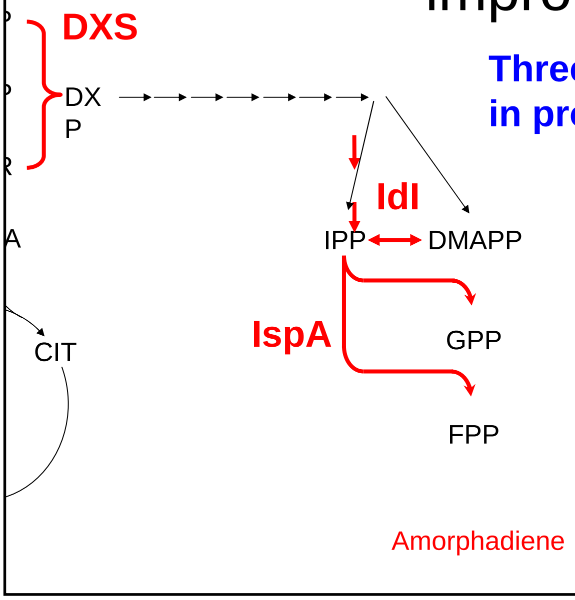
Pathways for isoprenoid precursor biosynthesis



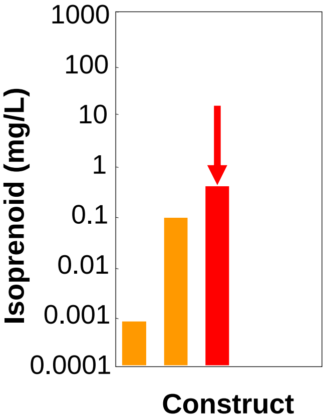
Artemisinin metabolic pathways

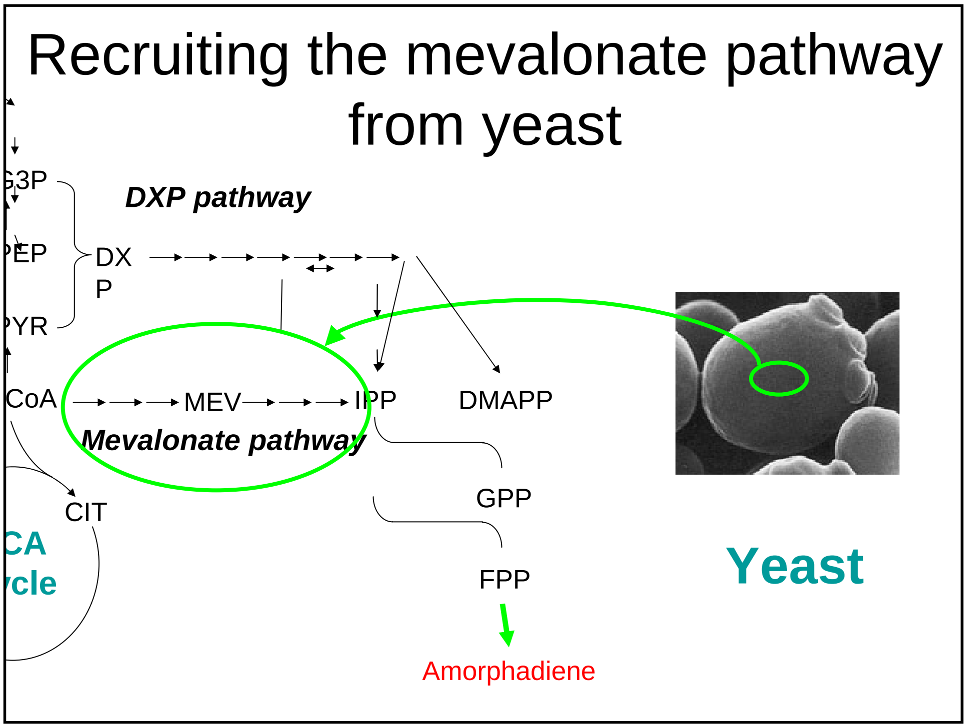


Engineering *E. coli's* native pathway improves production



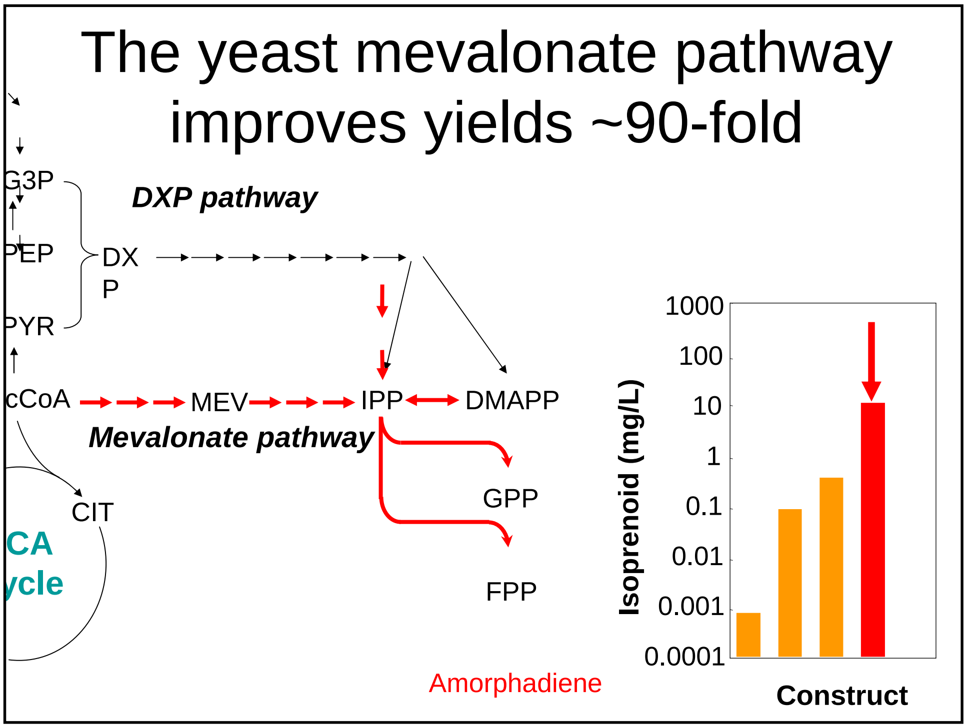
Three-fold improvement in production





Recruiting the mevalonate pathway from yeast

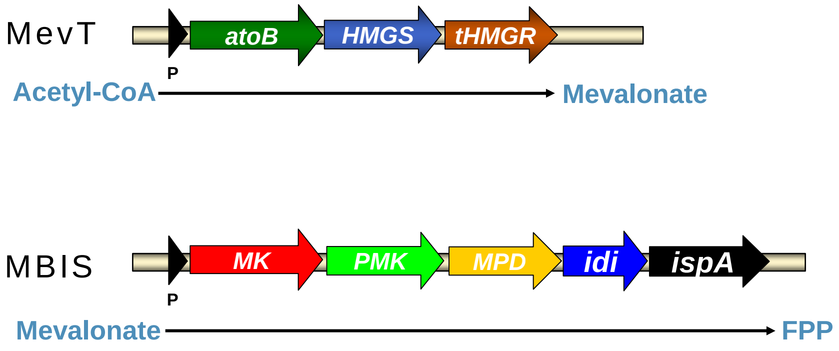
G3P
PEP
PYR
CoA
DXP pathway
DX
P
MEV
IPP
DMAPP
Mevalonate pathway
GPP
FPP
CIT
CA
cle
Amorphadiene
Yeast



The yeast mevalonate pathway improves yields ~90-fold

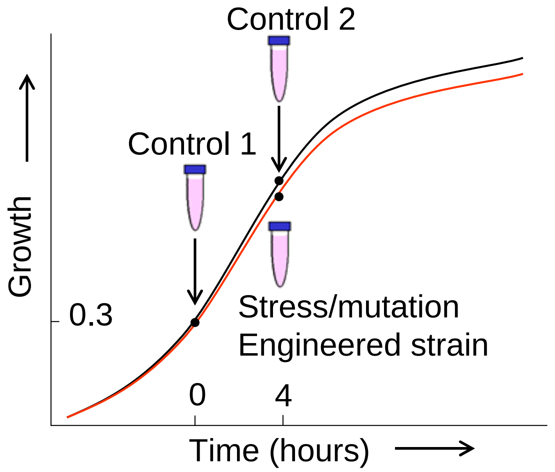
G3P
PEP
PYR
cCoA
DXP pathway
DX
P
MEV
IPP
DMAPP
Mevalonate pathway
GPP
FPP
CIT
CA
ycle
Amorphadiene

Optimizing the bottom part of the mevalonate pathway



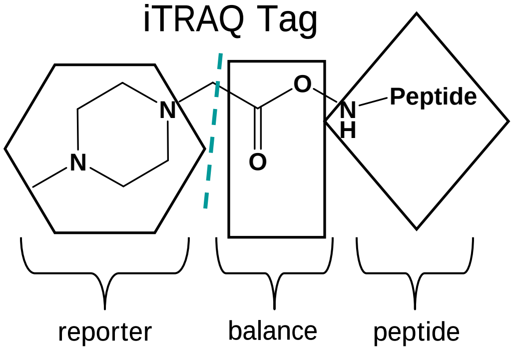
Martin, V.J., Pitera, D.J., et al, 2003.. Nat. Biotechnol. 21, 796–802

Experimental Design

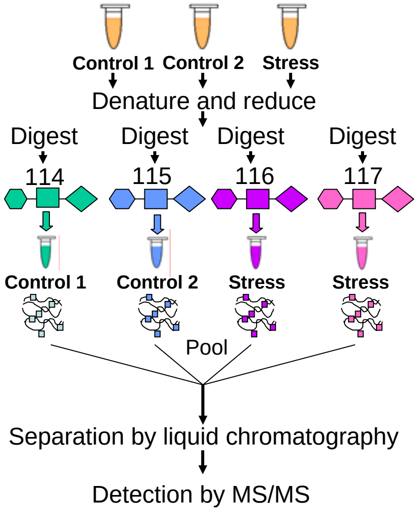


iTRAQ Peptide Labeling Strategy

iTRAQ Tag



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	--



Control 1 Control 2 Stress

Denature and reduce

Digest Digest Digest Digest

114 115 116 117

Control 1 Control 2 Stress Stress

Pool

Separation by liquid chromatography

Detection by MS/MS

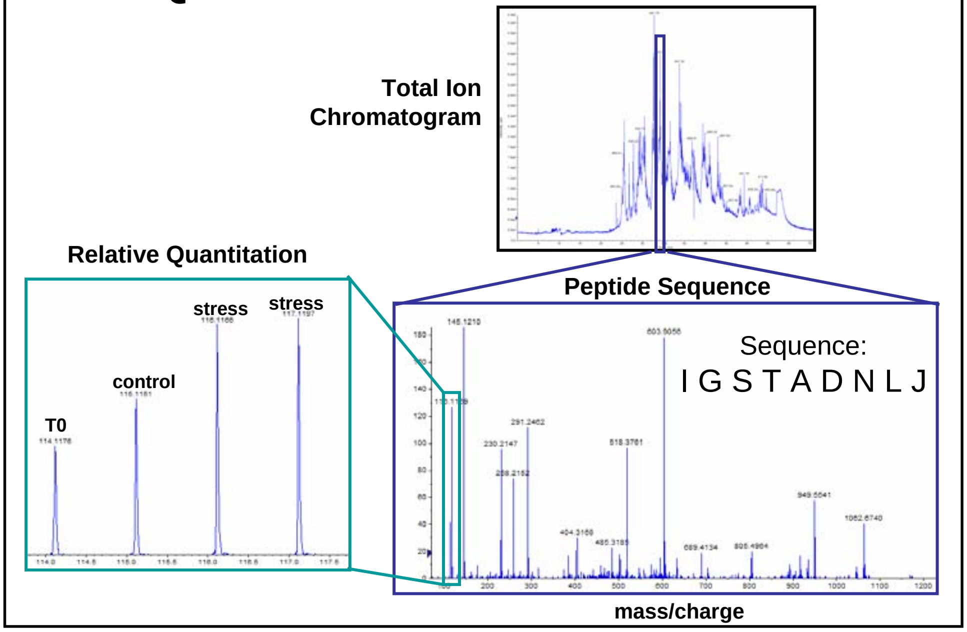
Ross PL, et al., Mol Cell Proteomics, 2004; 3(12):1154-69

Quantitative Proteomics

Total Ion Chromatogram

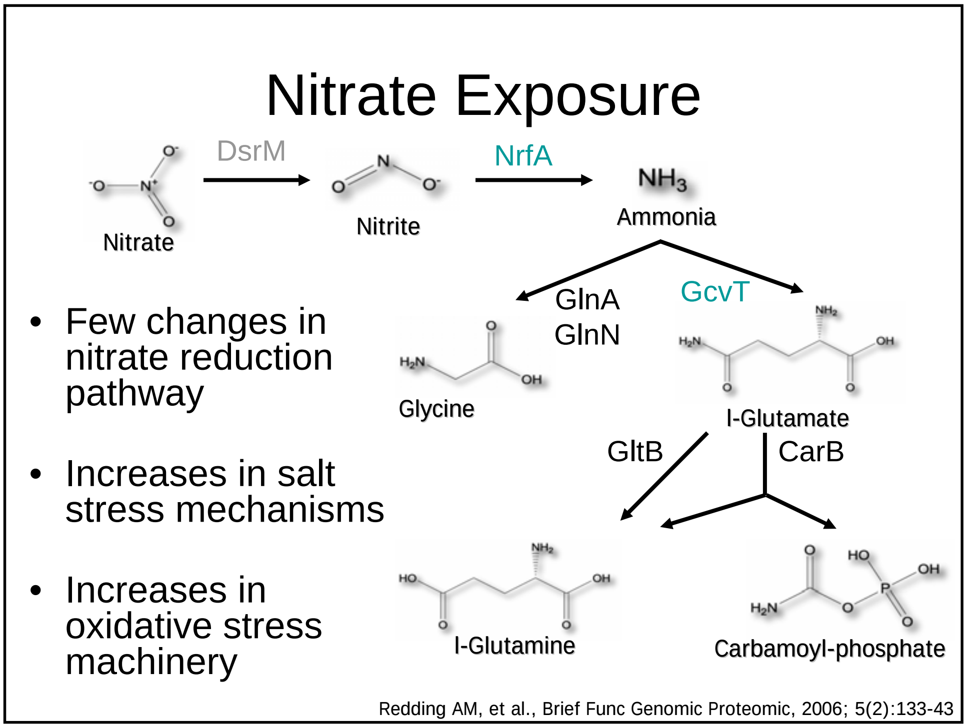
Relative Quantitation

Peptide Sequence



Sequence:
I G S T A D N L J

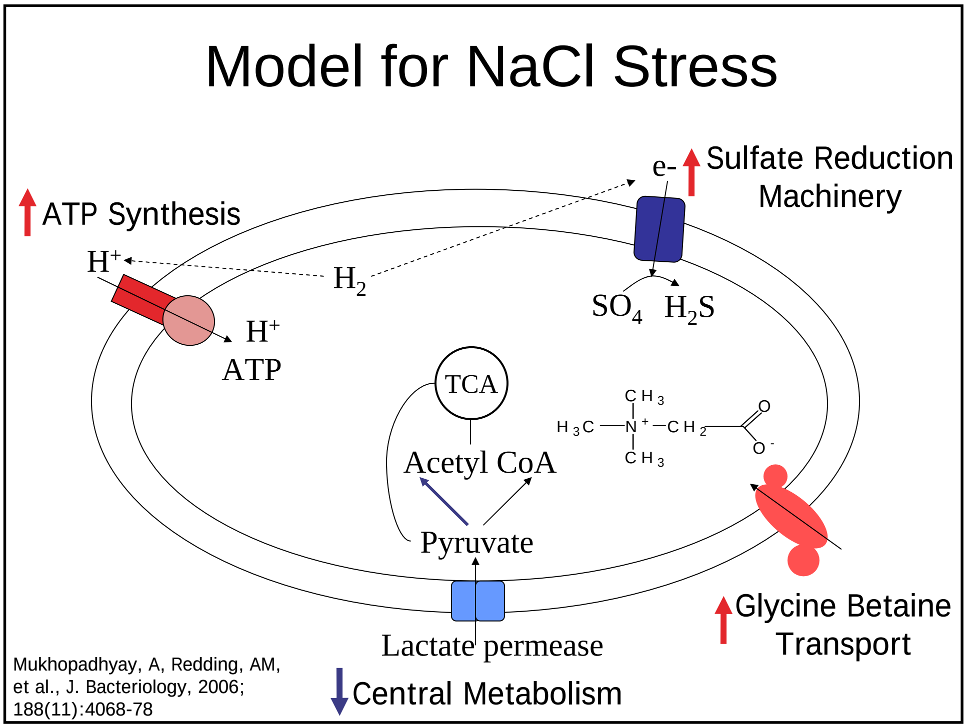
mass/charge



Nitrate Exposure

- Few changes in nitrate reduction pathway
- Increases in salt stress mechanisms
- Increases in oxidative stress machinery

Redding AM, et al., Brief Func Genomic Proteomic, 2006; 5(2):133-43



Model for NaCl Stress

Mukhopadhyay, A, Redding, AM, et al., J. Bacteriology, 2006; 188(11):4068-78

Summary

- Proteomics is an important component of cellular study
- Relative quantification of proteins increases our understanding of cellular pathways
- Understanding cellular pathways enables better cellular engineering

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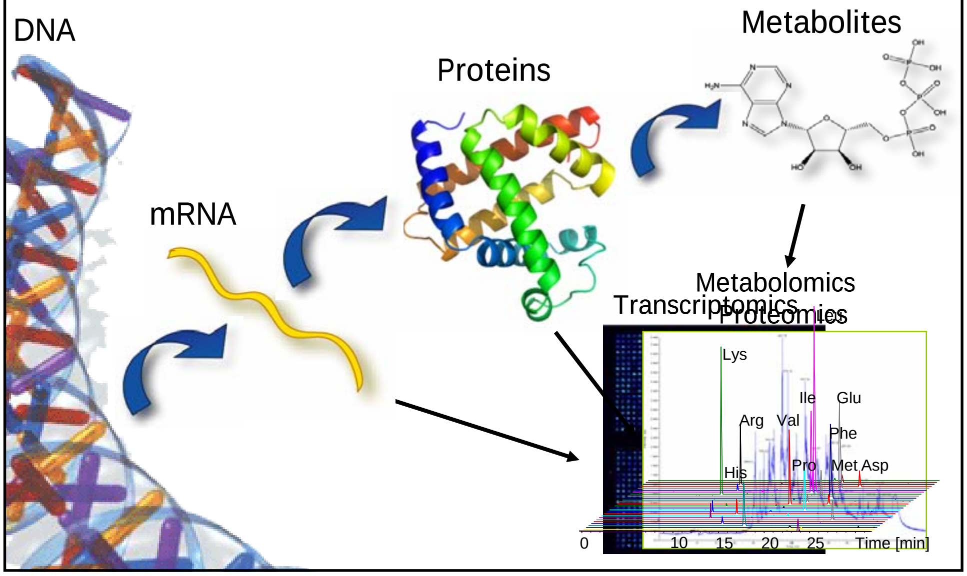
Transfer of Cellular Information



DNA

mRNA

Proteins

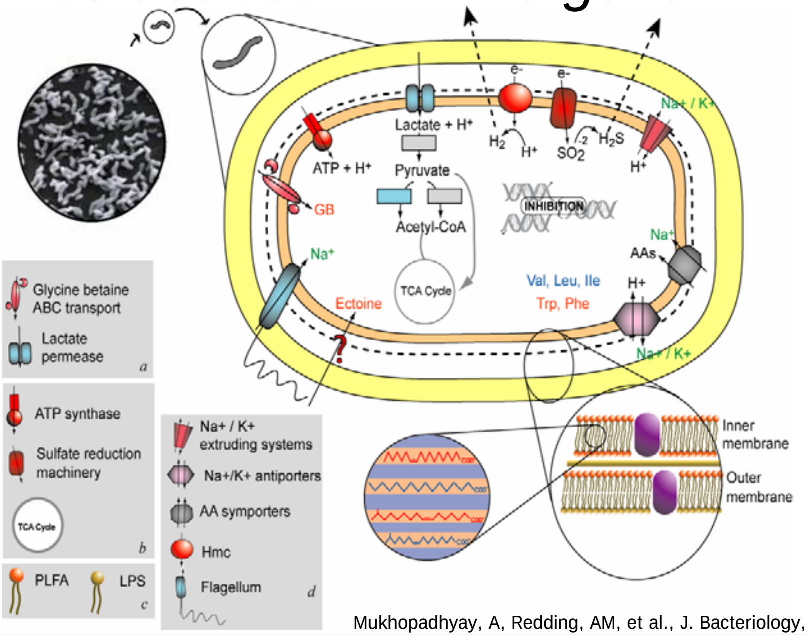
Metabolites

Metabolomics

Transcriptomics

Proteomics

Salt stress in *D. vulgaris*



Mukhopadhyay, A, Redding, AM, et al., J. Bacteriology, 2006

Summary

- Cell wide studies are required to observe non-obvious effects on pathways distant from the engineered system/ stress.
- Integration across multiple experiments is required to develop testable hypothesis
    - Cell wide studies at various levels
    - Comparative analysis of multiple stress
- Omics studies provide the starting point for further analysis.