2-Dimensional Proteomic Approach to Dissect the Interplay between Alcohol Consumption, Hepatits C virus infection, and CuZn Superoxide Dismutase deficiency in liver

By

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9-26-2011

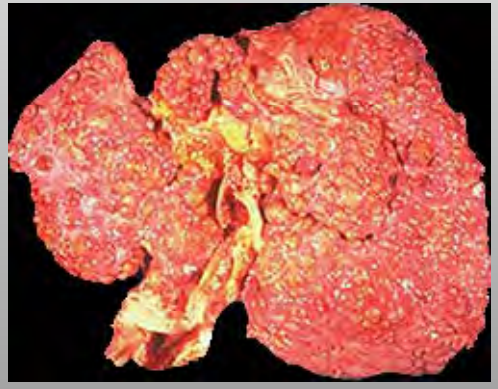
Presented at the 2011 Stanford Mass Spectrometry Users' Meeting

Introduction

- Disruptions of the homeostatic redox state by oxidative stress lead to genetic, neuronal, cardiovascular, metabolic, and hepatic diseases.
- Hepatocellular carcinoma (HCC) is the primary liver tumor often resulted from chronic liver cirrhosis.

Healthy

Cirrhosis



Carcinoma

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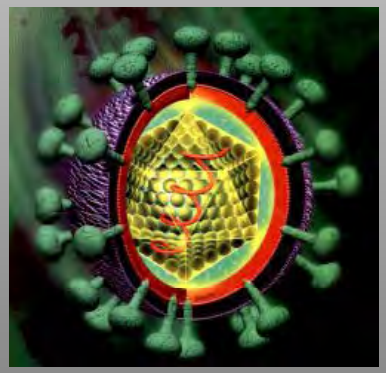

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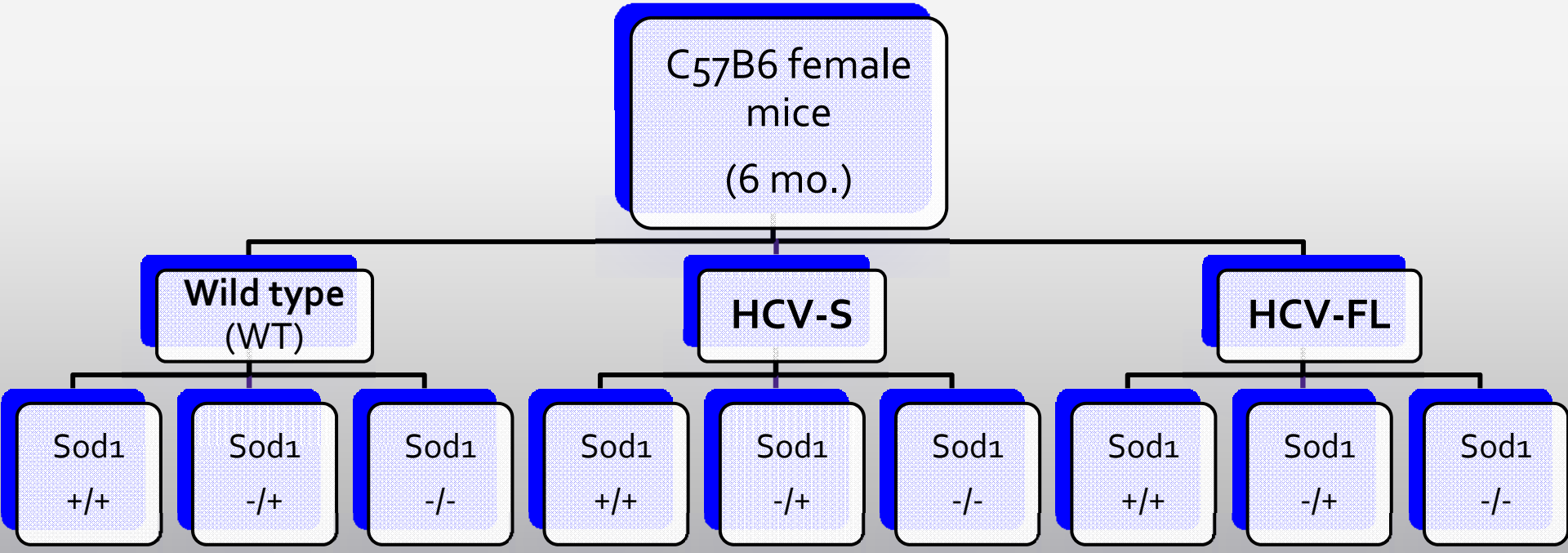
- Alcohol and HCV → Reactive oxygen species (ROS) → Oxidative stress → liver damages.
- Superoxide dismutase 1 (Sod1) is a major "**antioxidant**" enzyme to protect liver.

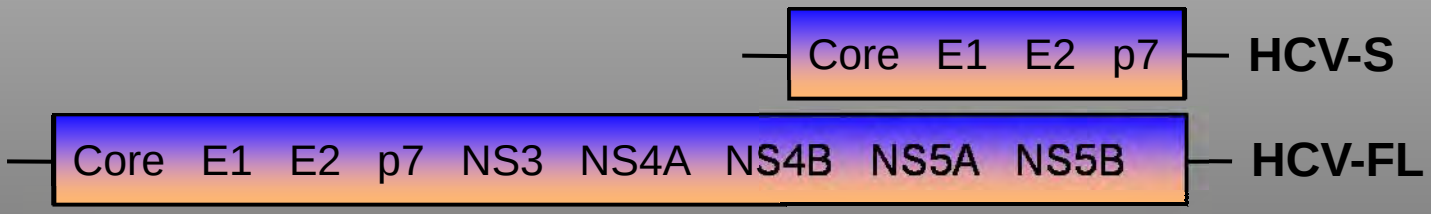
Research objectives

1. To understand the interaction between and the consequences of chronic alcohol exposure and HCV infection under conditions of reduced anti-oxidant capacity.

2. To identify redox sensitive proteins, under the experimental conditions, that may contribute to liver damage and carcinogenic process.

Experimental groups

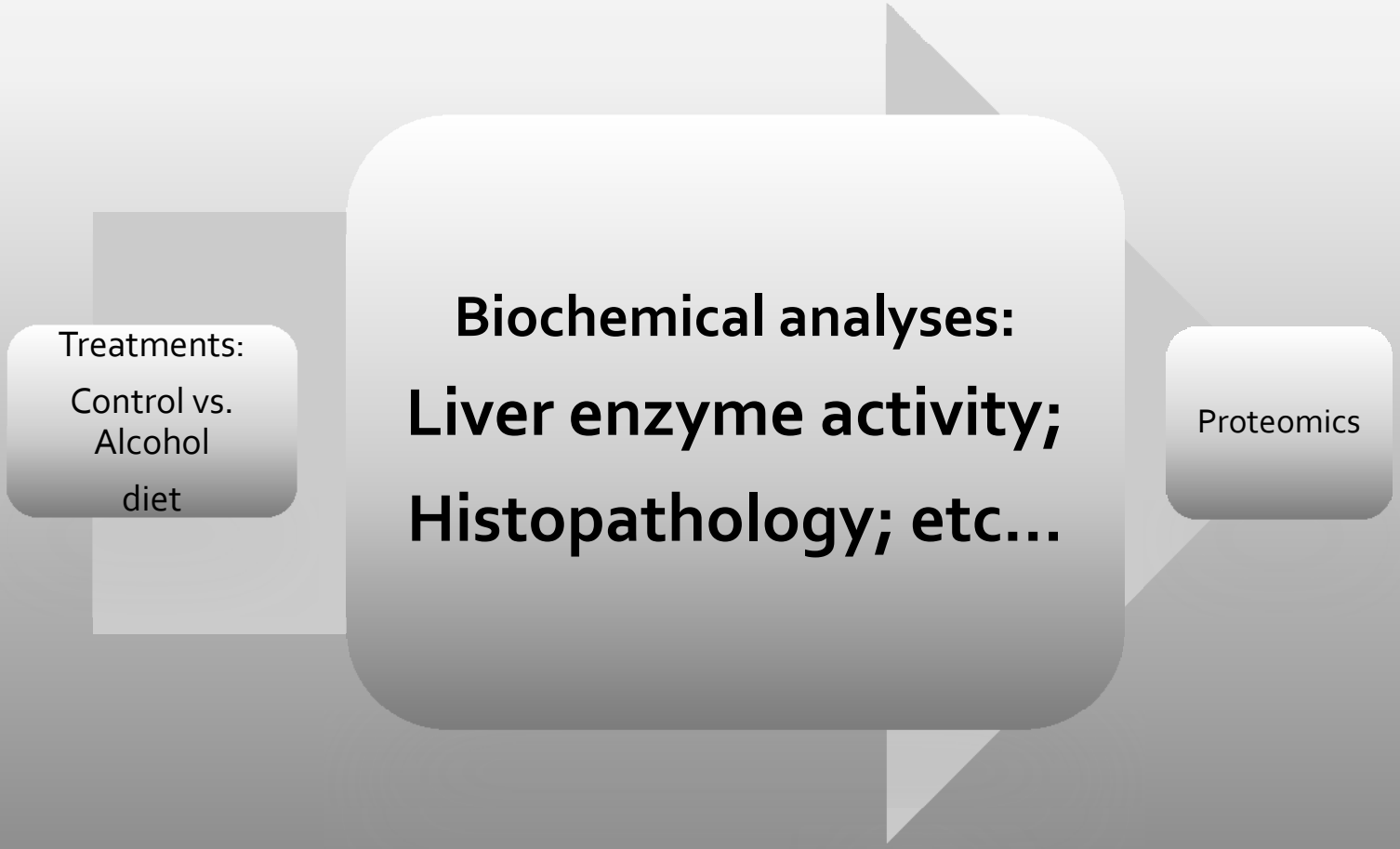




Procedures


Treatments:
Control vs. Alcohol diets
Biochemical analyses
Proteomics

Procedures


Treatments:
Control vs. Alcohol diet
Biochemical analyses:
Liver enzyme activity;
Histopathology; etc…
Proteomics

Procedures

Treatments:
Control vs. Alcohol diet

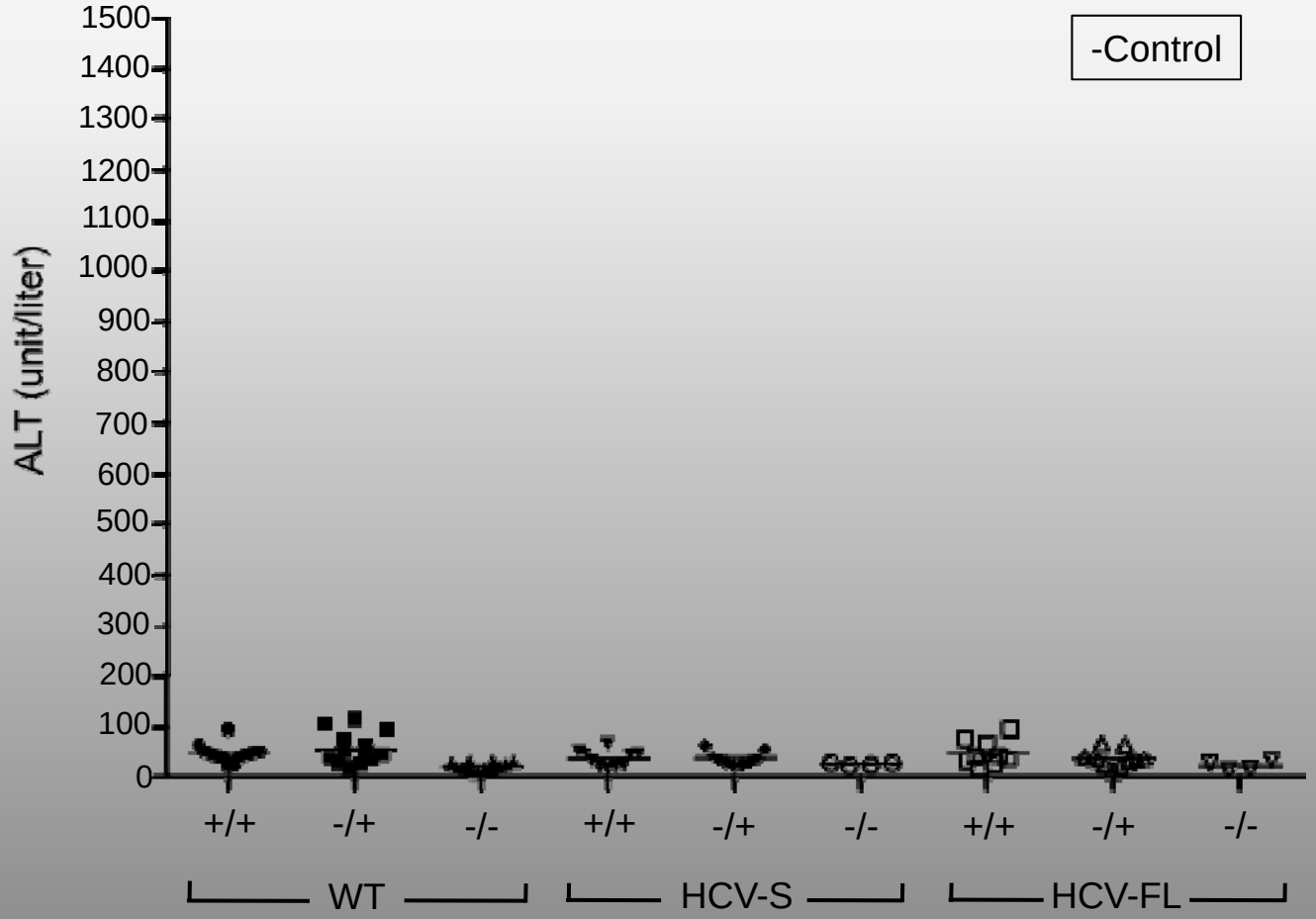
Biochemical analyses:
Liver enzyme activity;
Histopathology

Proteomics:

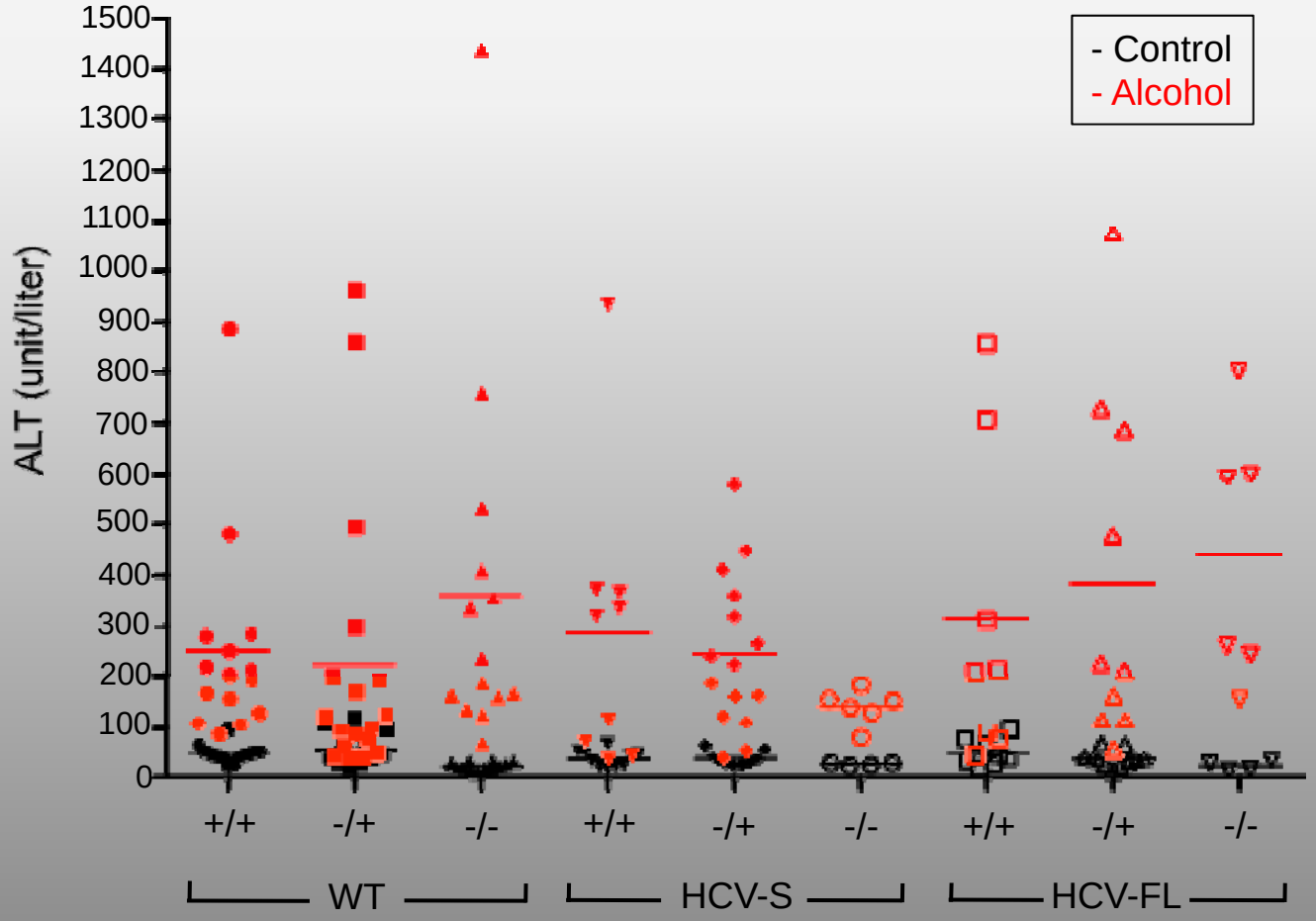
Detecting protein modification via bisANS or disulfide labeling;

2D electrophoresis; Mass Spectrometry

Serum ALT assay measures liver function

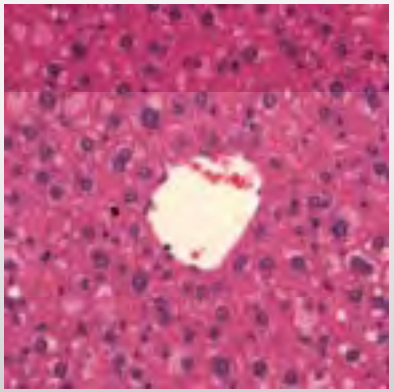


Alcohol treatment causes an elevated serum ALT level, suggesting liver dysfunction

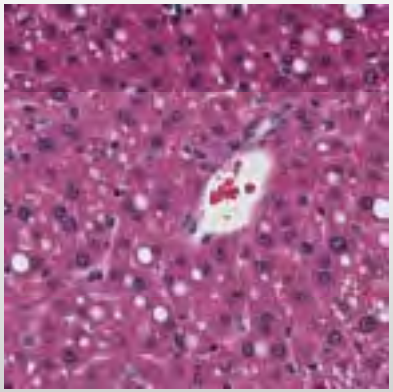


Histopathology:

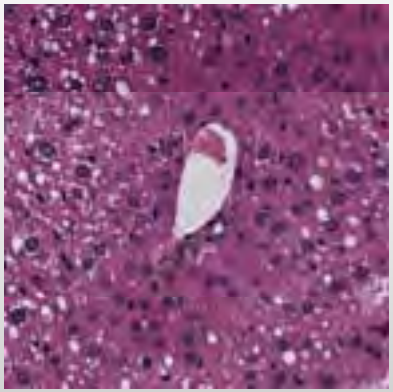
Normal liver



Sod1 +/+



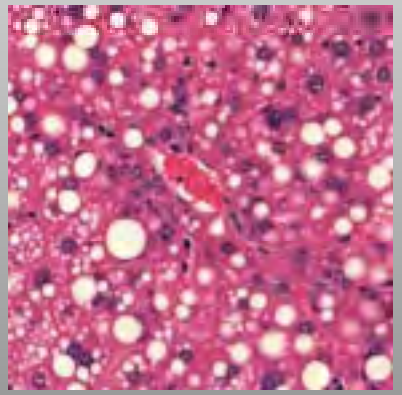
Sod1 -/+



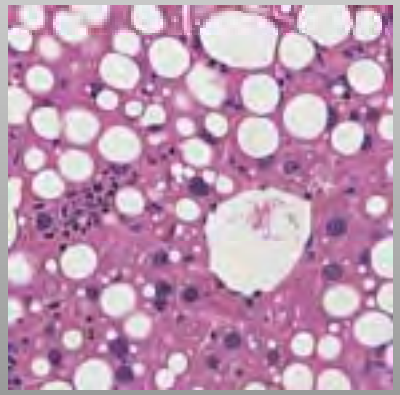
Sod1 -/-

Control

Fatty liver



Sod1 +/+



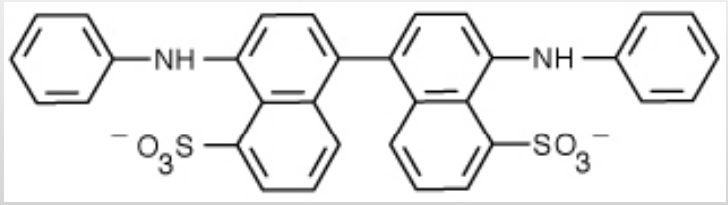
Sod1 -/+

Sod1 -/-

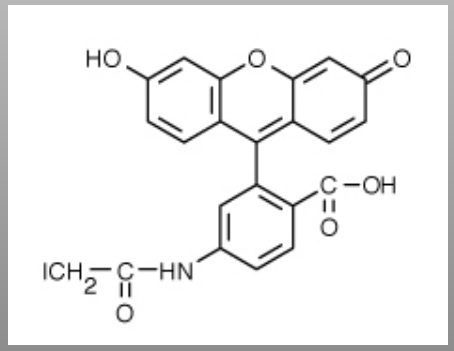
Alcohol

Proteomics

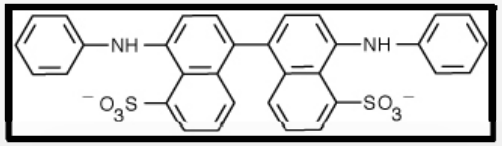
1. BisANS for protein conformational changes



1. 6-IAF for protein modification



BisANS detects protein conformational changes

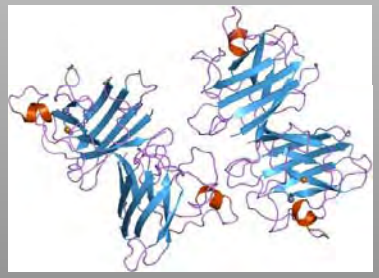


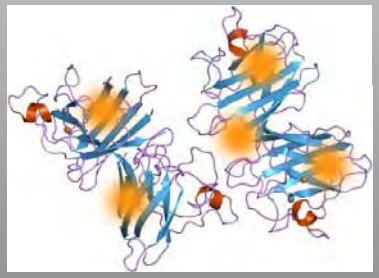
Protein extraction

Add bisANS

UV irradiation
& cross link

2-D gel electrophoresis

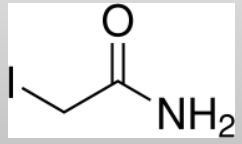


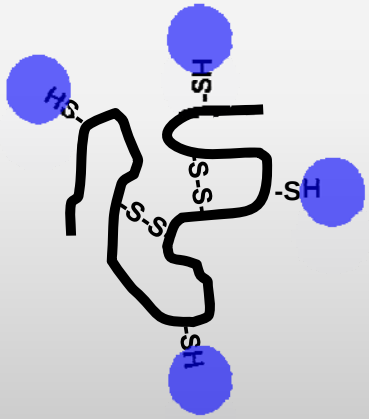


6-IAF for protein modifications

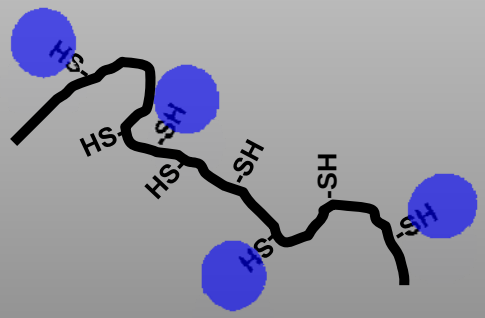
Protein extraction

Add **IAA**

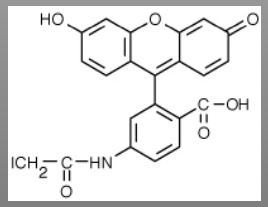


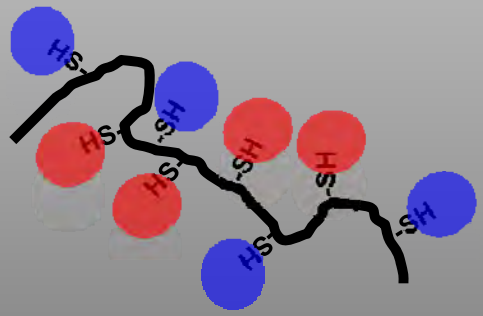


Reducing agent



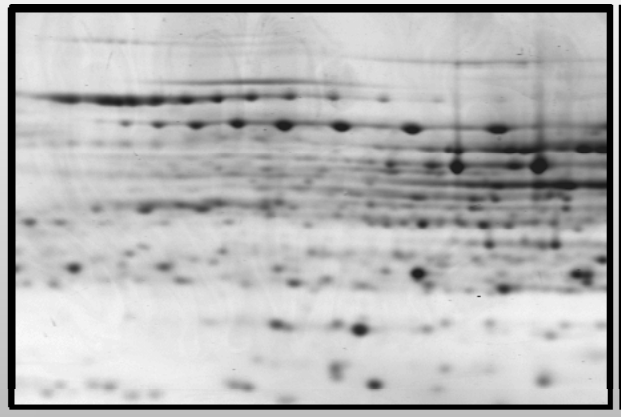
Add **6-IAF**



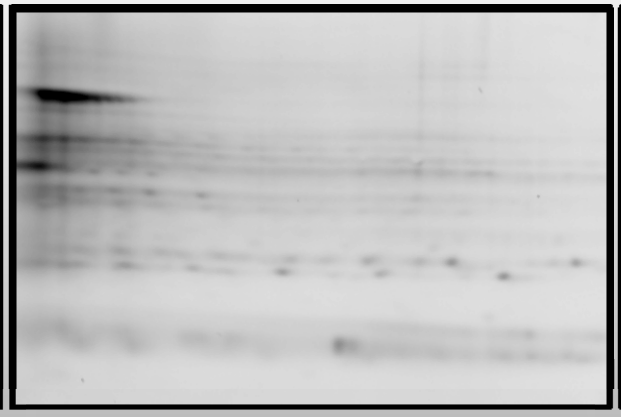


2-D gel electrophoresis

2-dimensional gel electrophoresis



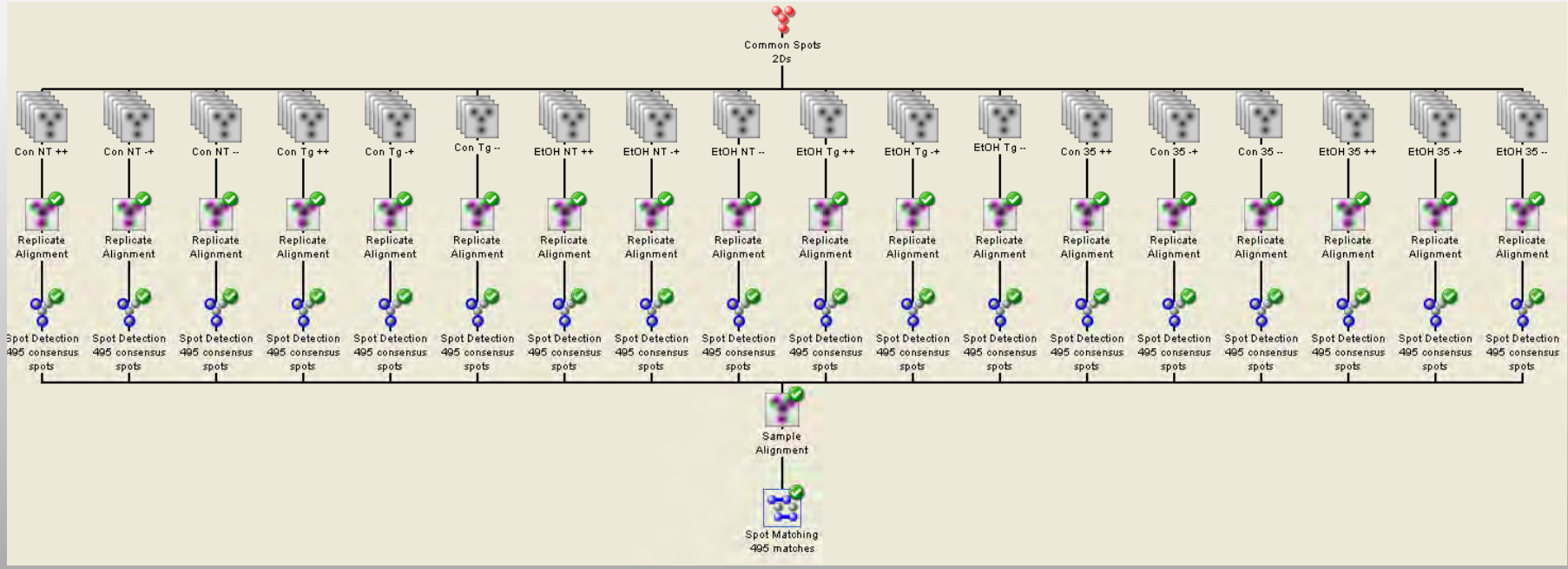
Total protein
via
Coomassie blue



Hydrophobicity
via
bisANS

Disulfide bond
via
6-IAF

2-dimensional analysis by Dymension



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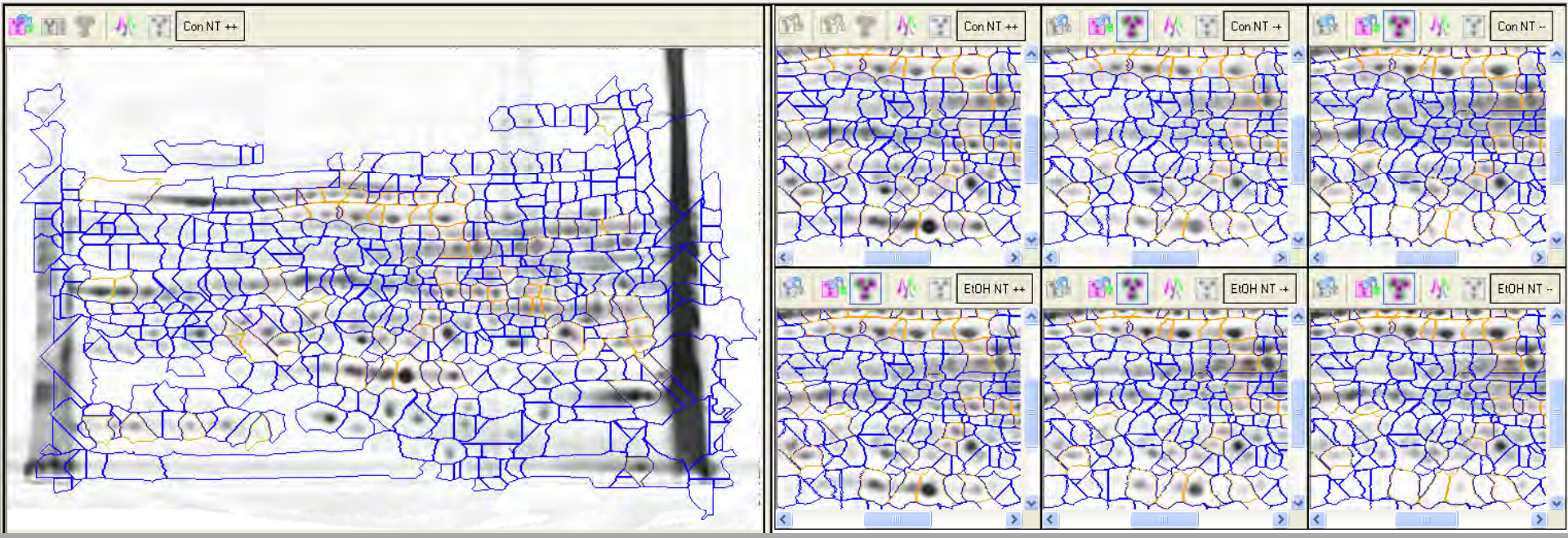
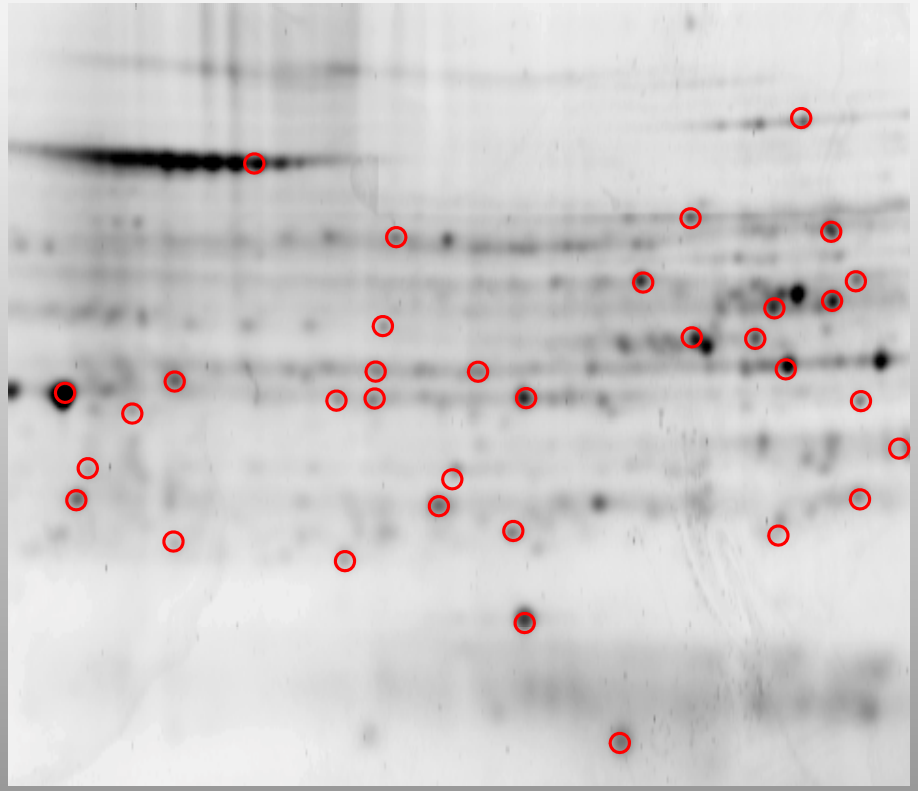


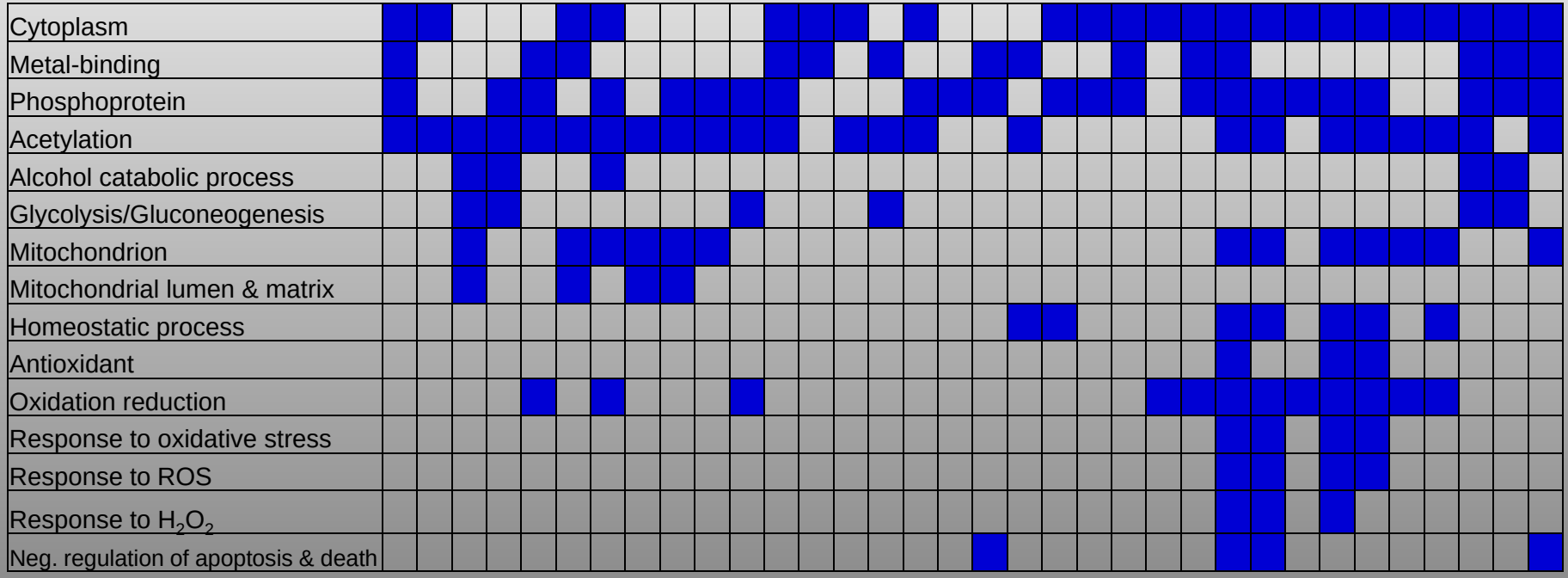
2D gel
Spot picking



Mass
Spectrometry

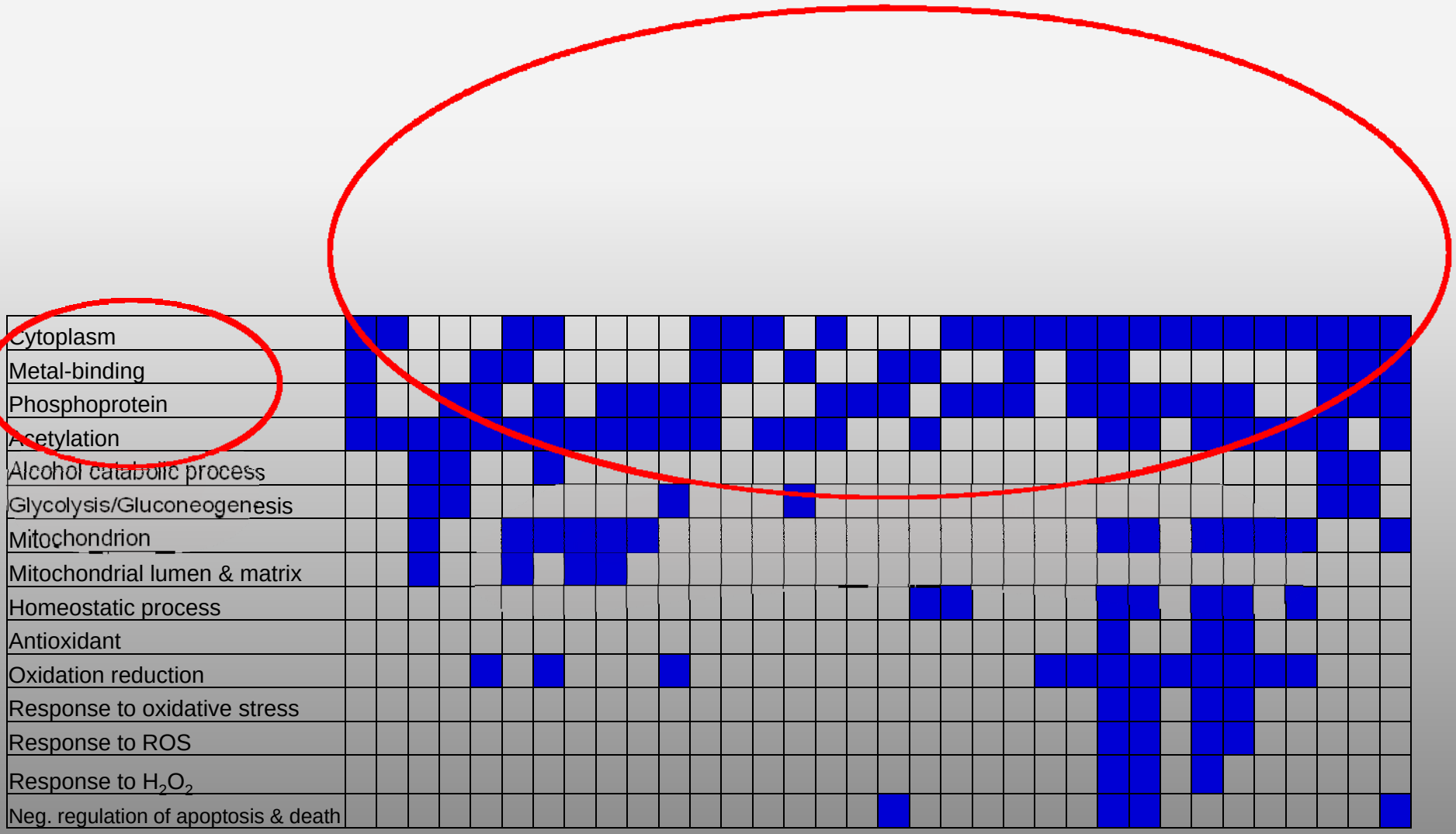
LC/MSD Trap XCT Ultra, Agilent Technologies

Functional annotation



DAVID Bioinformatics Resources, NIAID

Functional annotation



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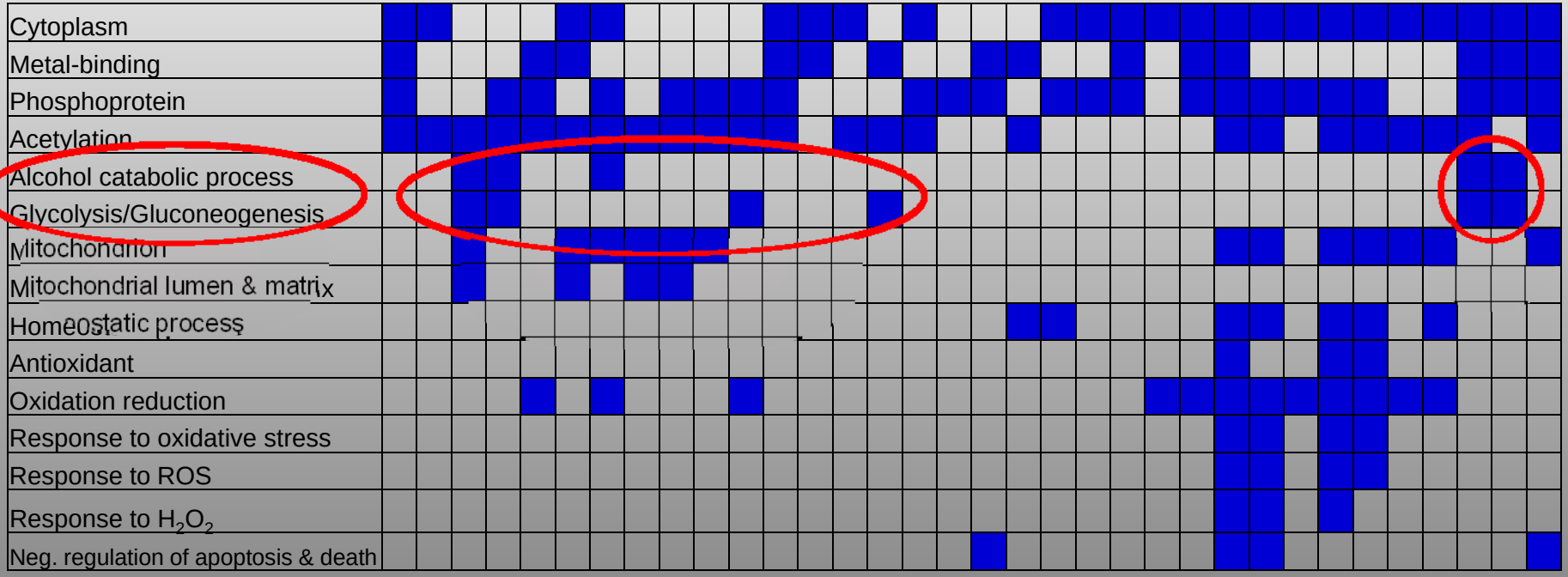

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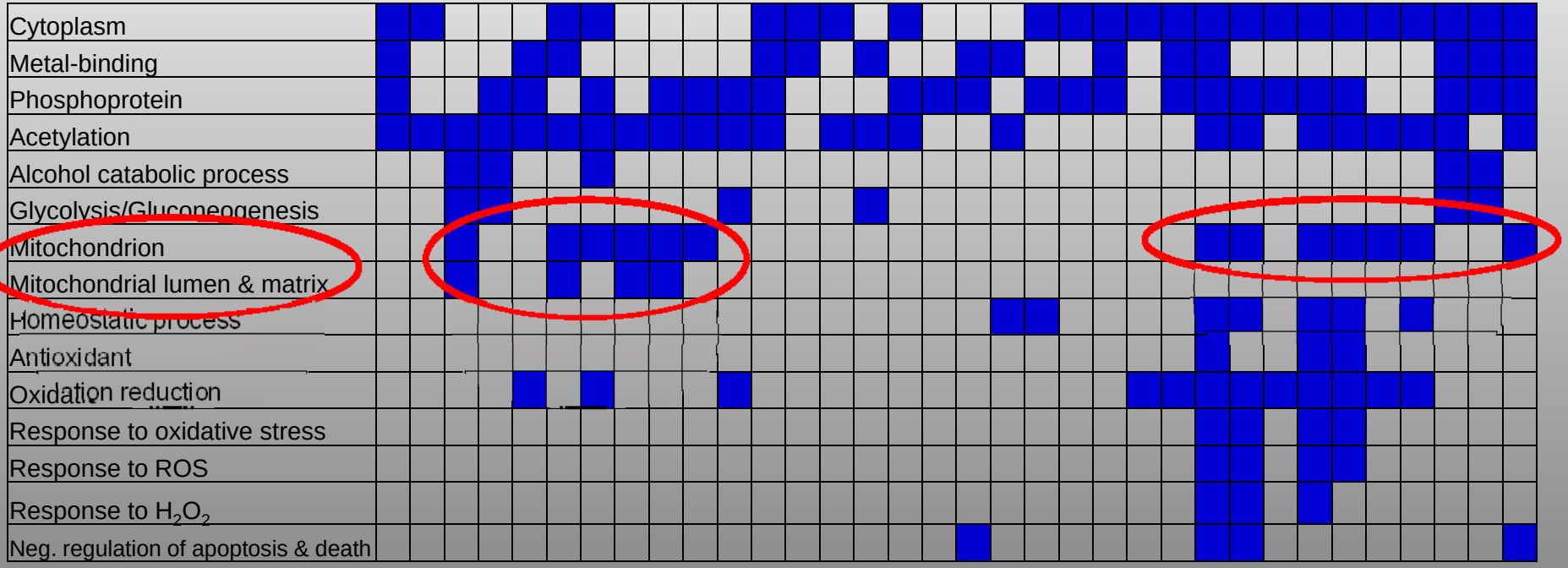

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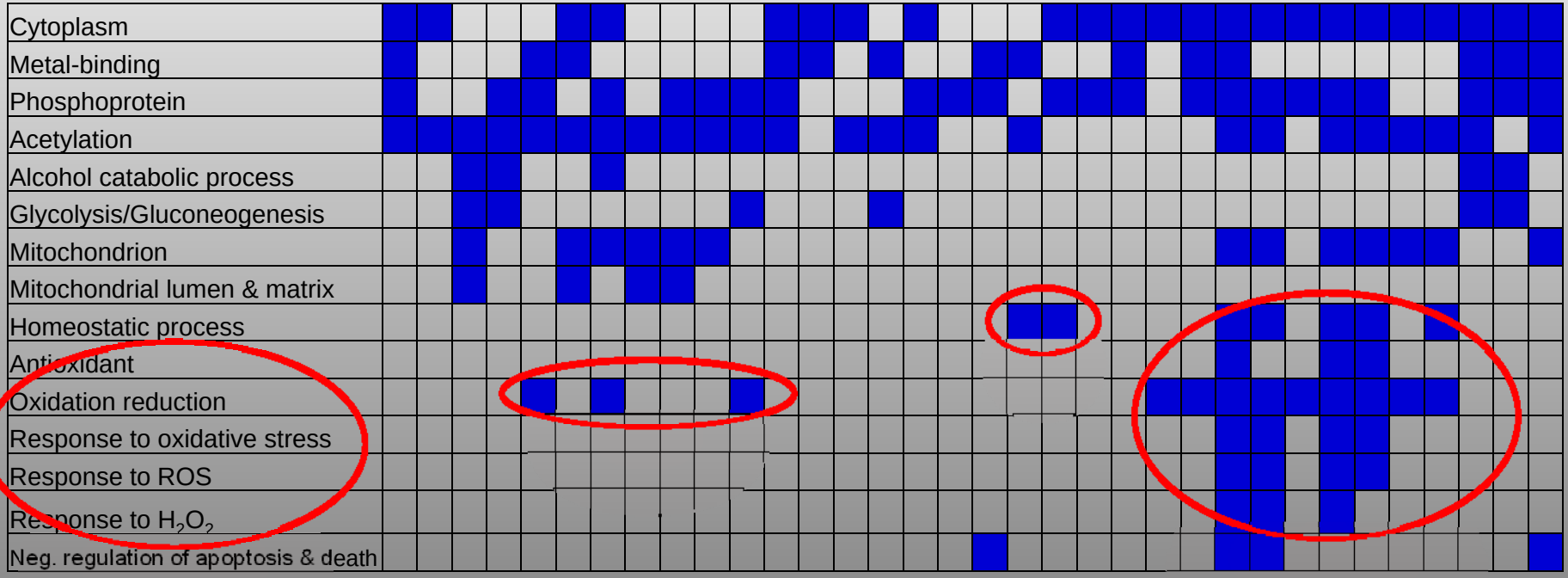

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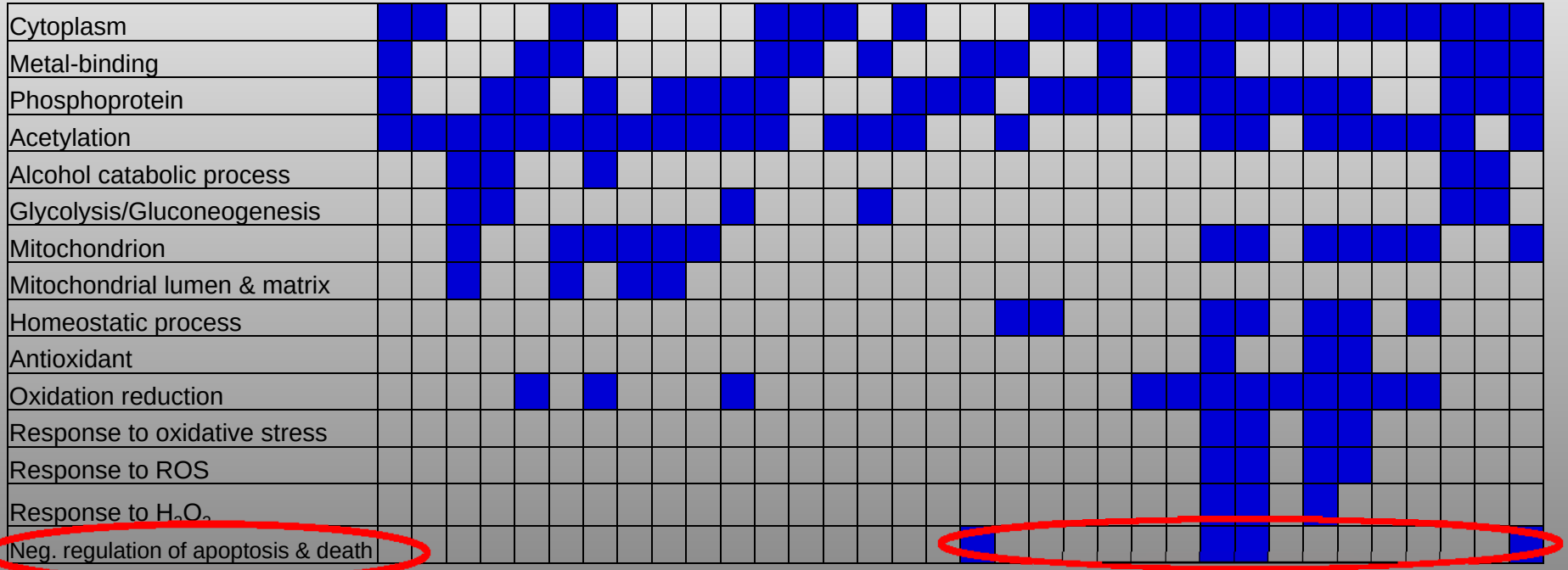

DAVID Bioinformatics Resources, NIAID

Summary & future directions

- Oxidative stress and reduced antioxidant capacity increase live pathology. There is an additive effect of HCV transgenes and SOD1 deficiency on alcohol-mediated liver damages.
- Our mouse model is useful to investigate the interaction between alcohol and chronic HCV infection and the long term effect on liver carcinogenic development.
- BisANS and disulfide based fluorescent labels are useful tools to detect protein conformational changes and modifications from oxidative stress.
- LC coupled Ion-Trap Mass Spectrometry enables efficient identification of redox sensitive proteins , and further analyses have been undertaken to reveal their functional roles and regulations.

Acknowledgement

Ting-Ting Huang, *Stanford University, VAPAHCS*

Asish Chaudhuri, *UTHSC, San Antonio*

Anson Pierce, *UTHSC, San Antonio*

Raymond A. Sobel, *Stanford Univeristy, VAPAHCS*

Sayanti Bhattacharjee, *Stanford University, VAPAHCS*

XinLi Guo, *VAPAHCS*

Jiahui Lin, *Weill Cornell Medical College*

Dongjia Chen, *UC San Diego*

Orr Sharpe, *Stanford/VA Mass Spectrometry Core, VAPAHCS*

Funding agencies:

National Cancer Institute,

VA Palo Alto Health Care System (VAPAHCS)

Digestive Disease Center (DDC), *Stanford University*