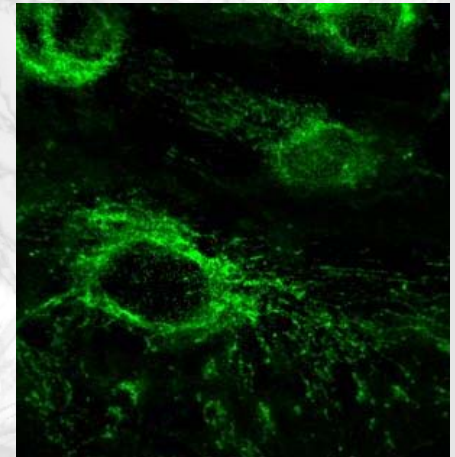
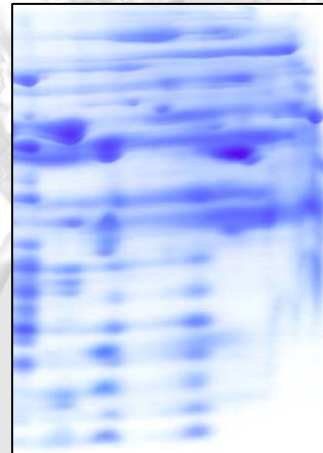
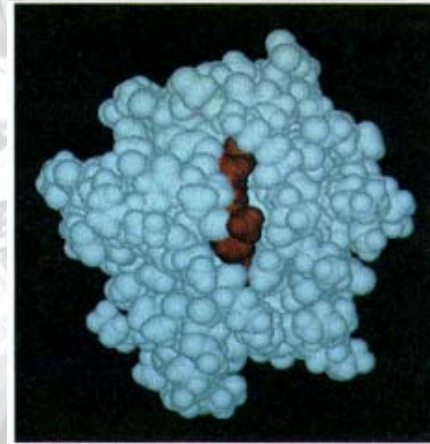
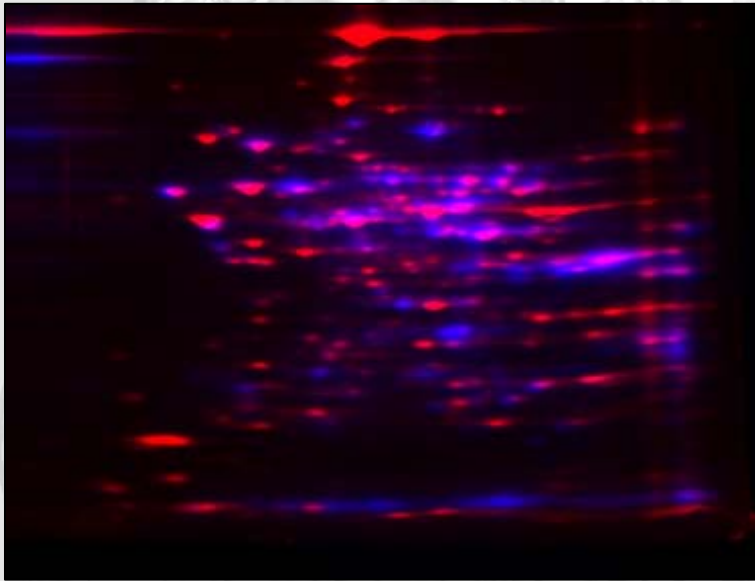
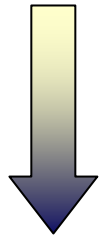


Proteomics and Thiol Modification

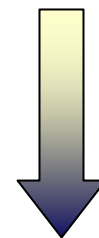


Aimee Landar, Ph.D.

Proteomics is the study of a protein complement in response to a stimulus

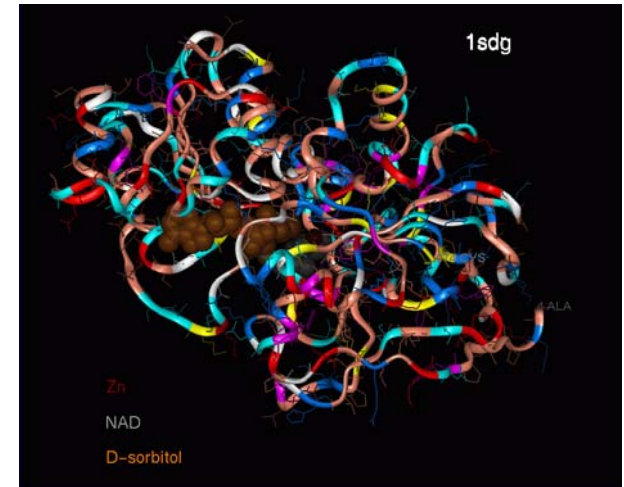
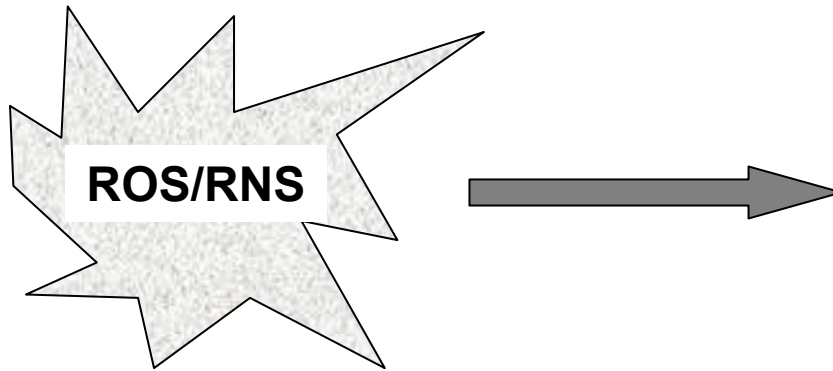


Potential for biomarkers



Defining mechanisms

Increased protein modification in response to oxidative stress

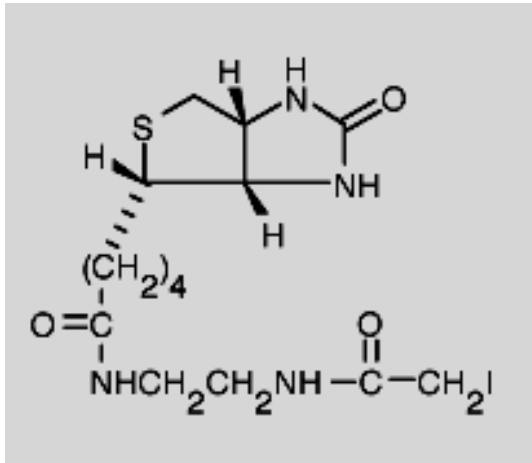


nitrotyrosine
thiol modification
carbonyl formation

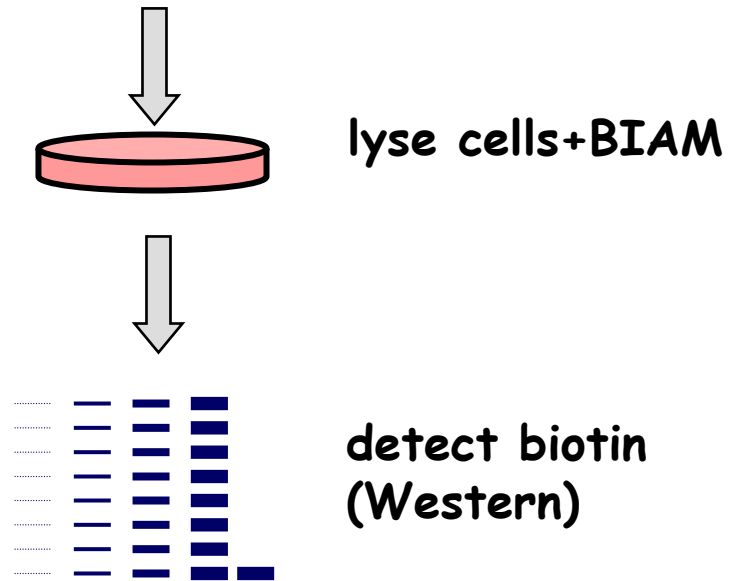
Modified proteins
(altered function)

Role of thiols in protein function and cell signaling

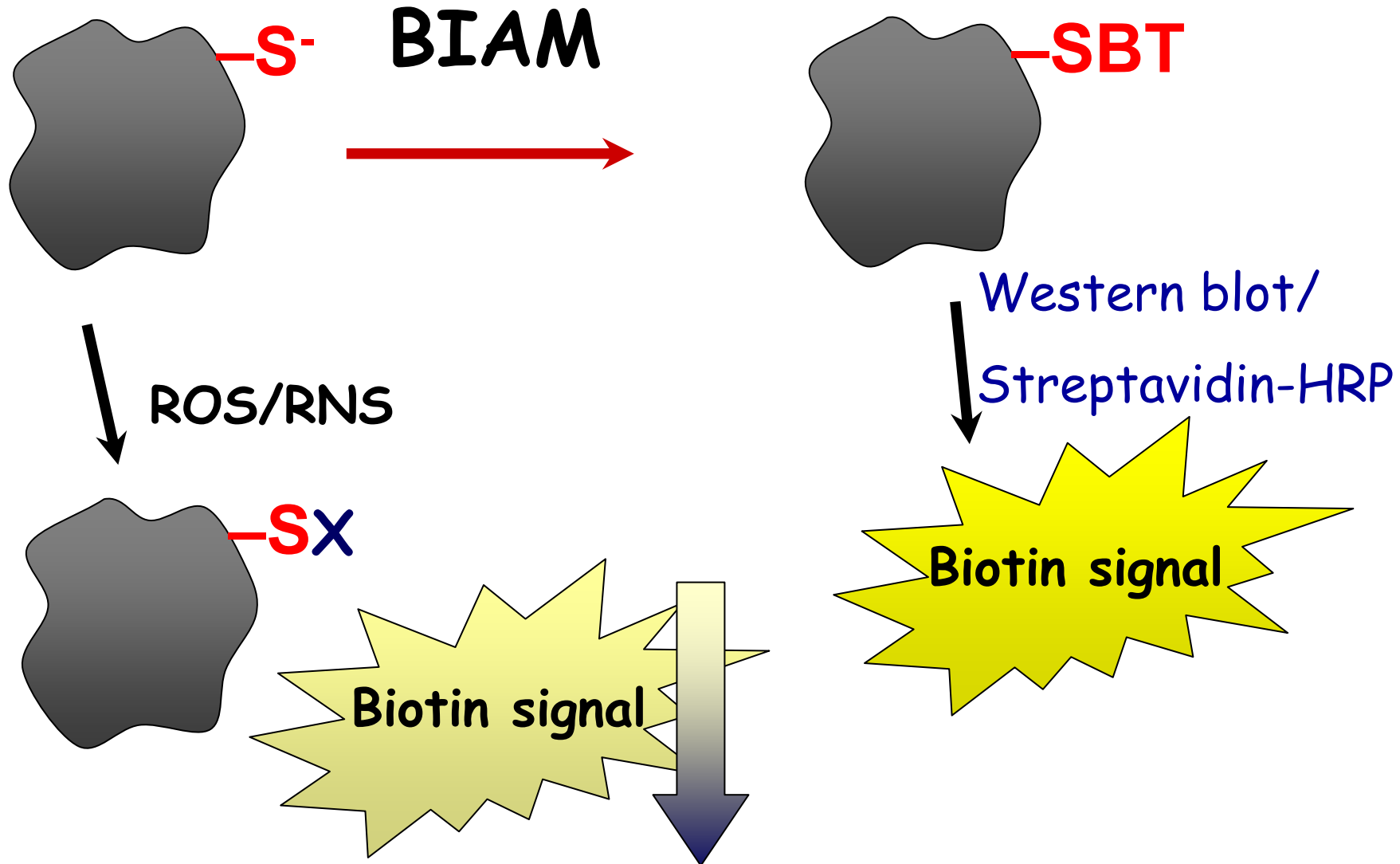
Detection of thiol modifications- BIAM



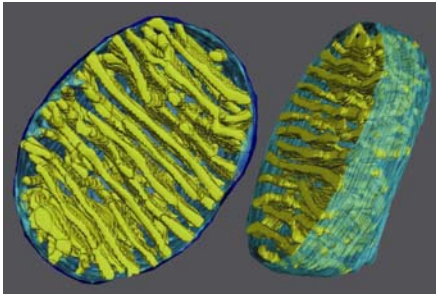
N-(biotinoyl)-N'-(iodoacetyl)-
ethylenediamine
(BIAM)



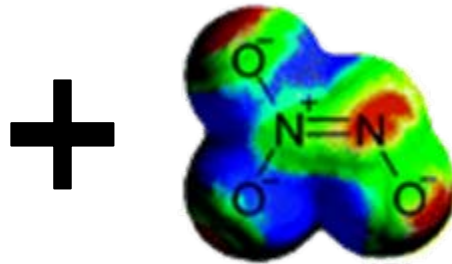
Assessment of Protein Modification:



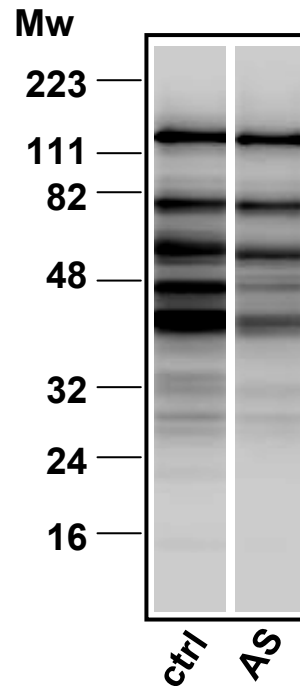
liver
mitochondria



Angeli's Salt



thiol
modification

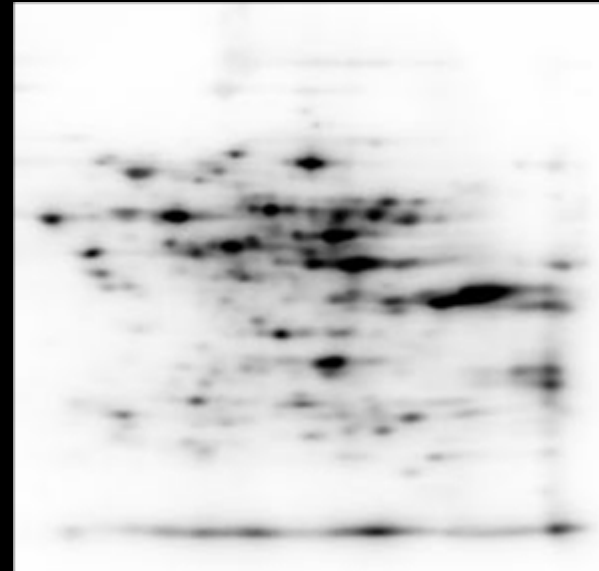
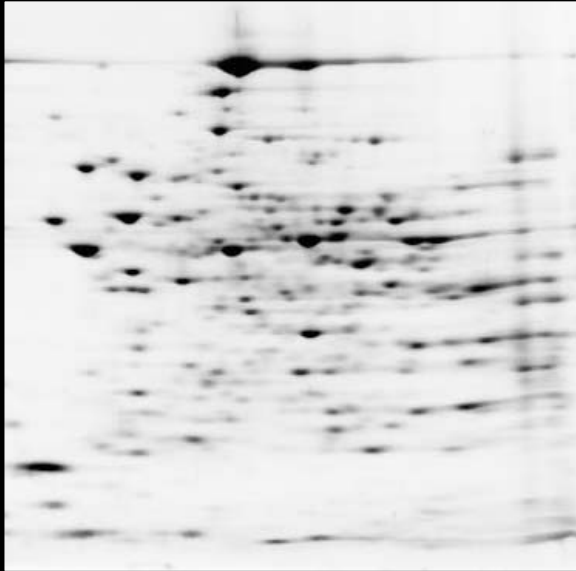


Streptavidin-HRP blot (biotin)

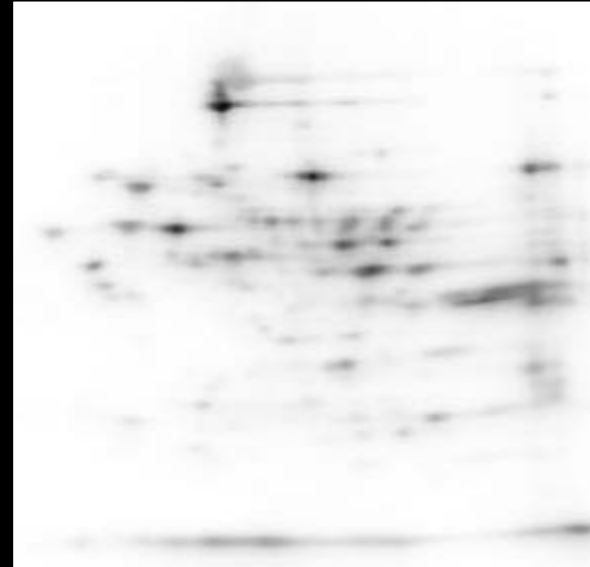
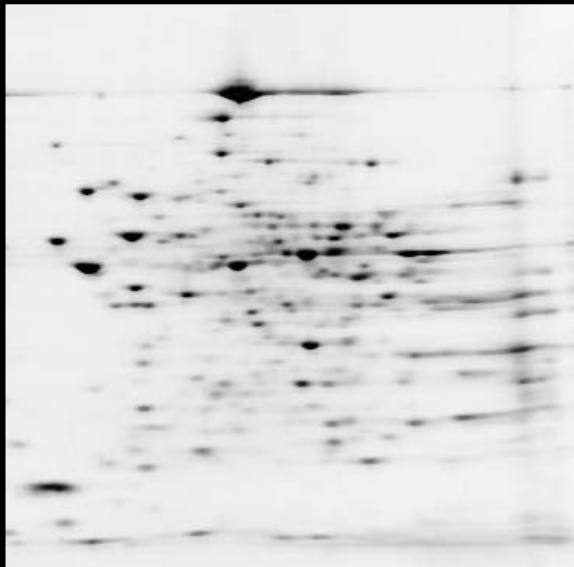
Sypro stain

Streptavidin-HRP blot (biotin)

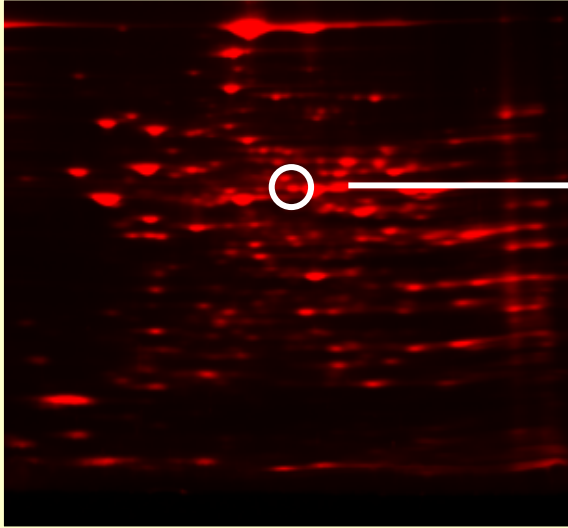
veh.
control



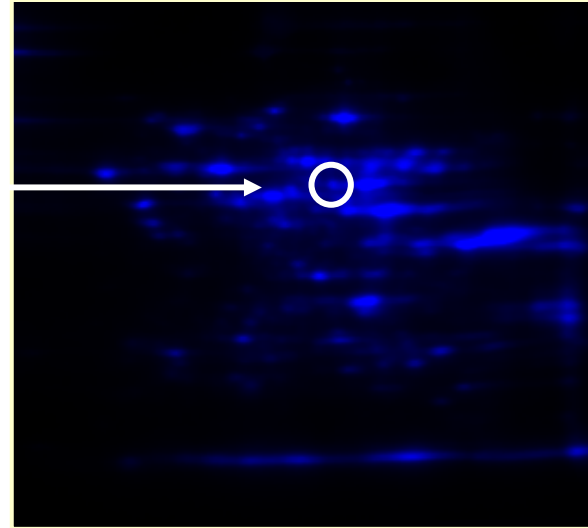
AS



protein stain



biotin blot

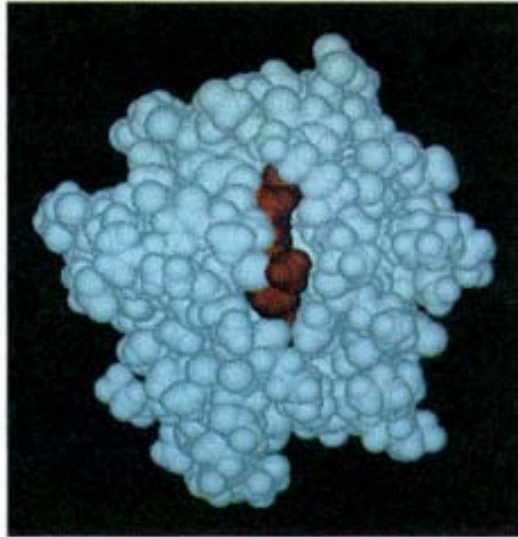


Challenges:

Quantitation of protein and thiols

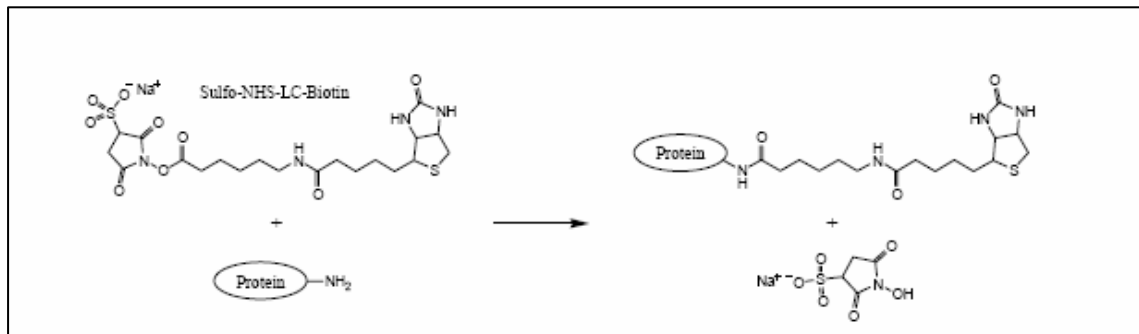
Correlating thiol status with protein function

Cytochrome c as an internal standard for protein and Biotin

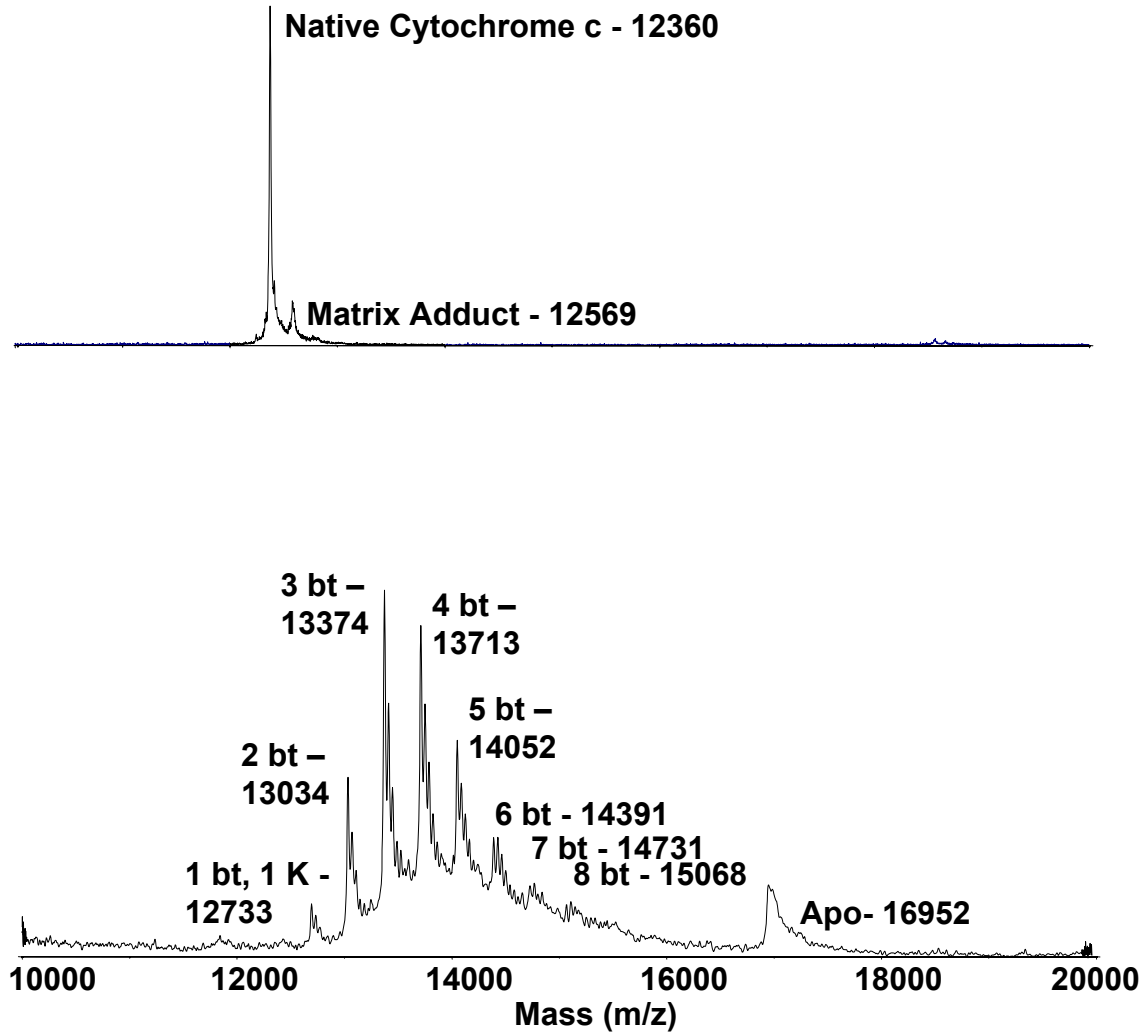


Cytochrome c: small (12,000 kDa), water soluble, multiple surface lysine residues.

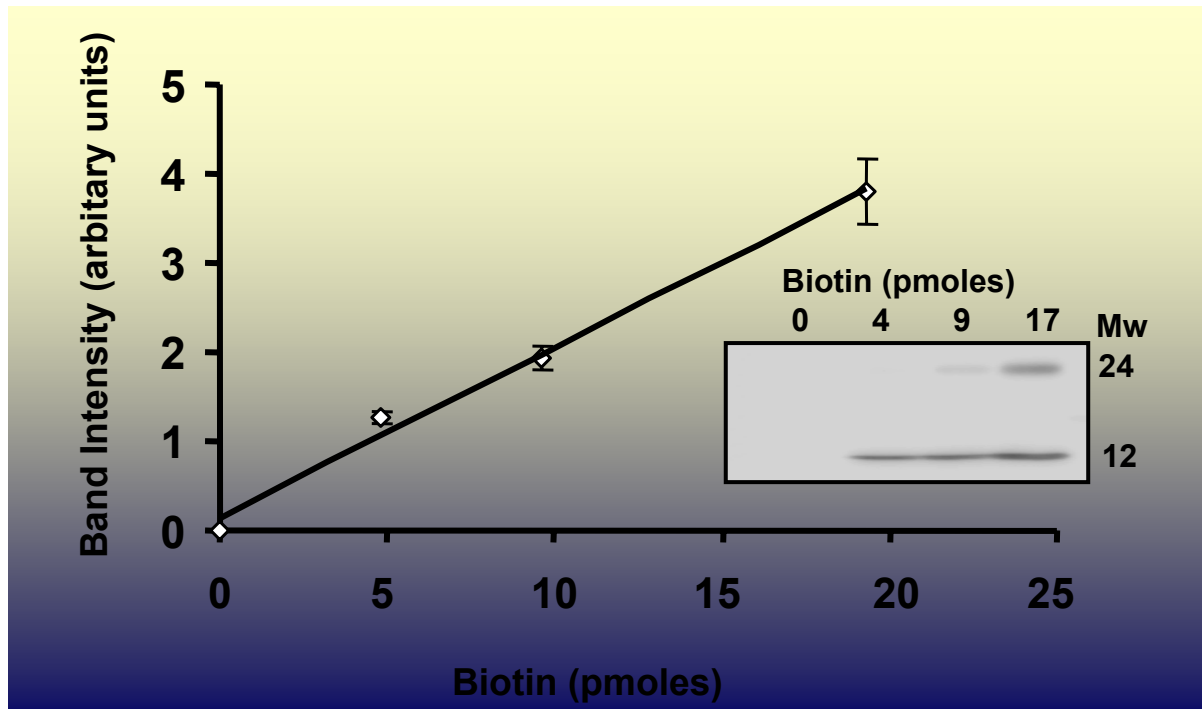
Biotin Tagging through Lysine:



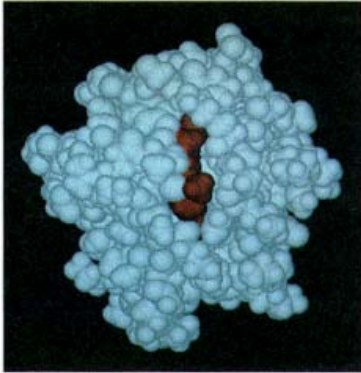
MALDI-TOF characterization of bt-cyt c



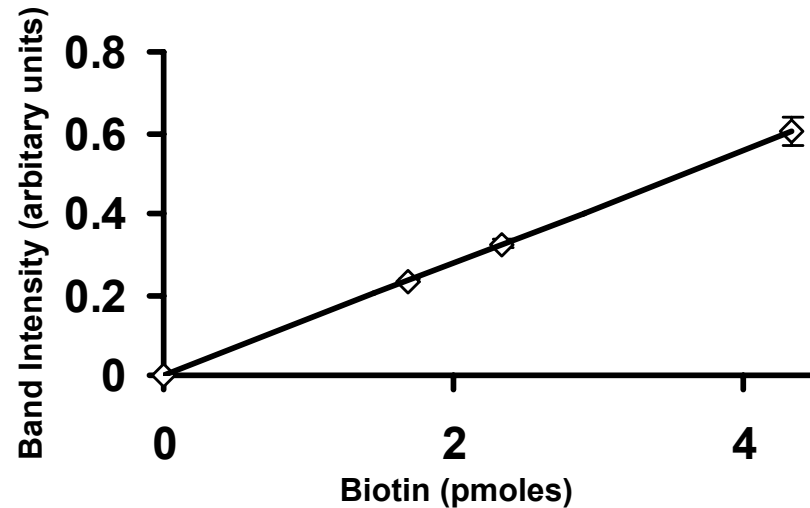
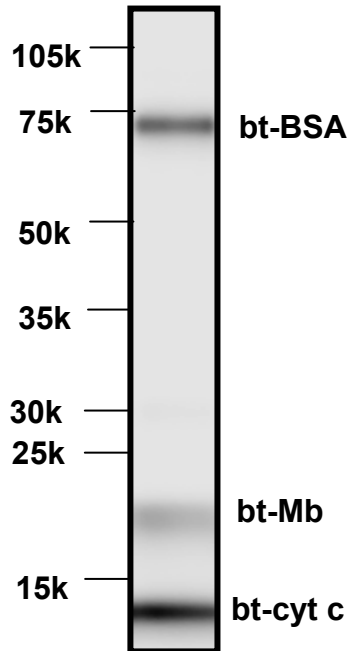
Sensitivity of bt-cyt c on western blot



Internal standards for protein and Biotin



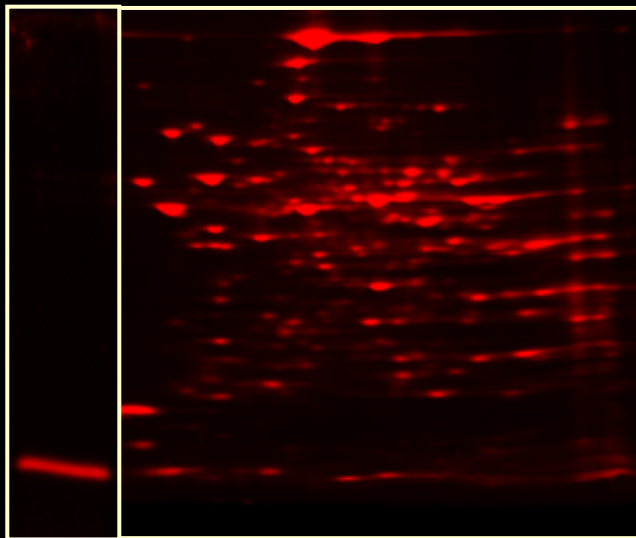
Biotinylated Cytochrome c
Biotinylated Bovine serum albumin
Biotinylated Myoglobin



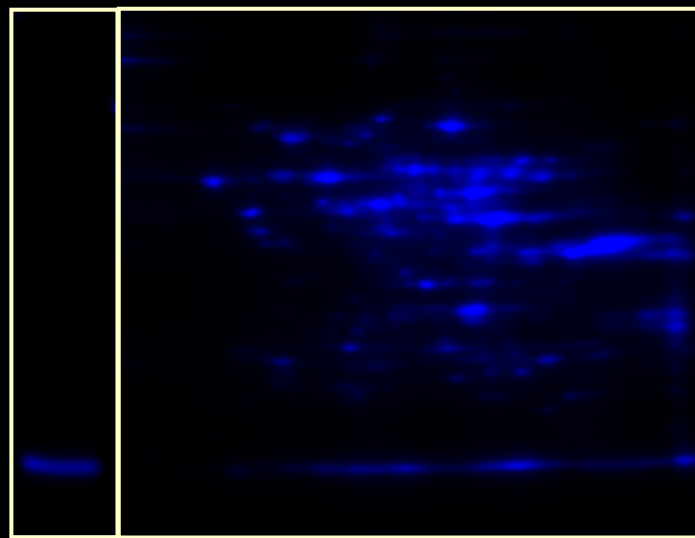
How do we calculate the amount of biotin and protein?

Application to a 2D-Proteomic Format

Sypro Ruby stain

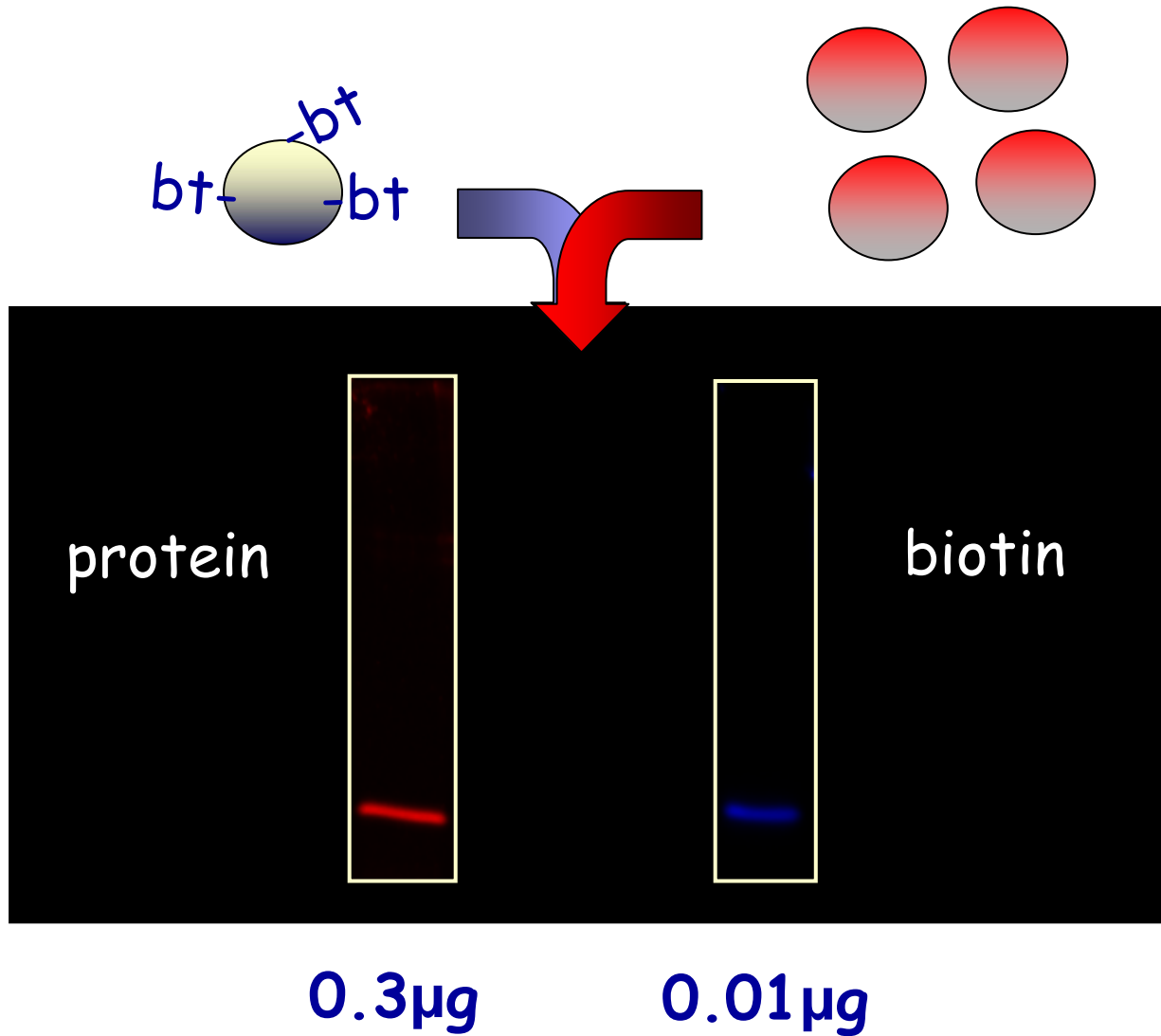


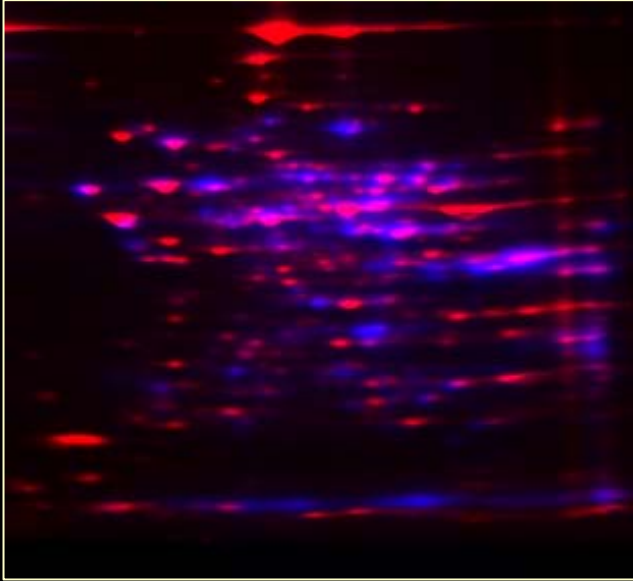
biotin blot



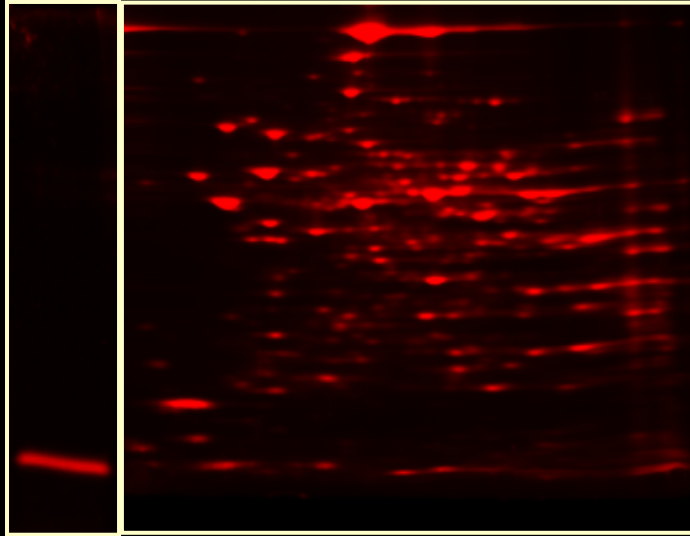
unlabeled cyt c as protein standard

bt-cyt c as biotin standard

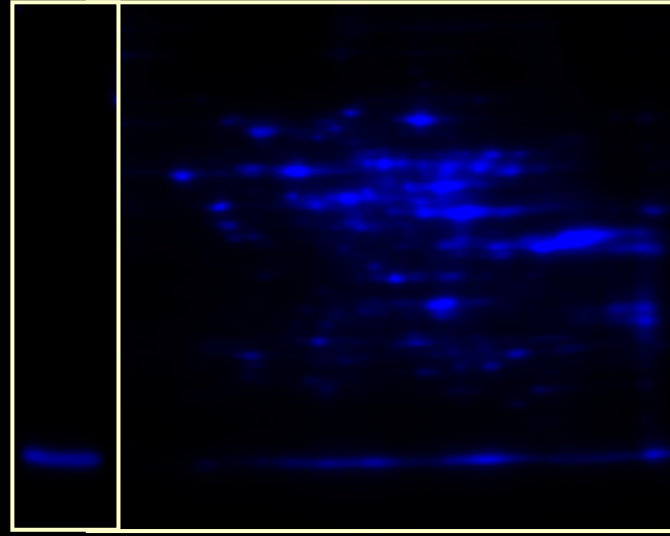




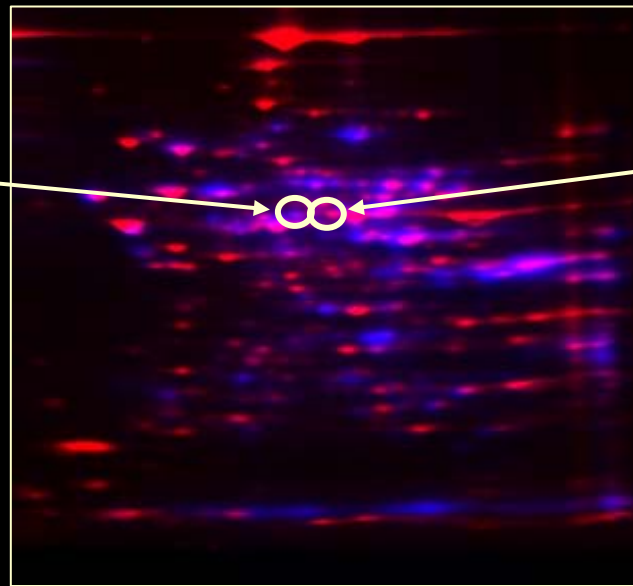
protein stain



biotin blot



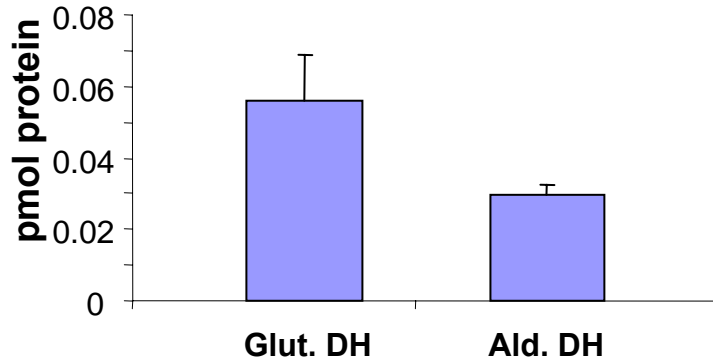
aldehyde
dehydrogenase



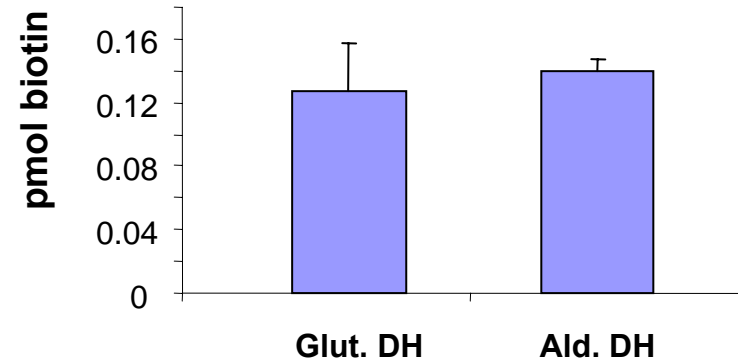
glutamate
dehydrogenase

Quantitation

amount protein



amount biotin

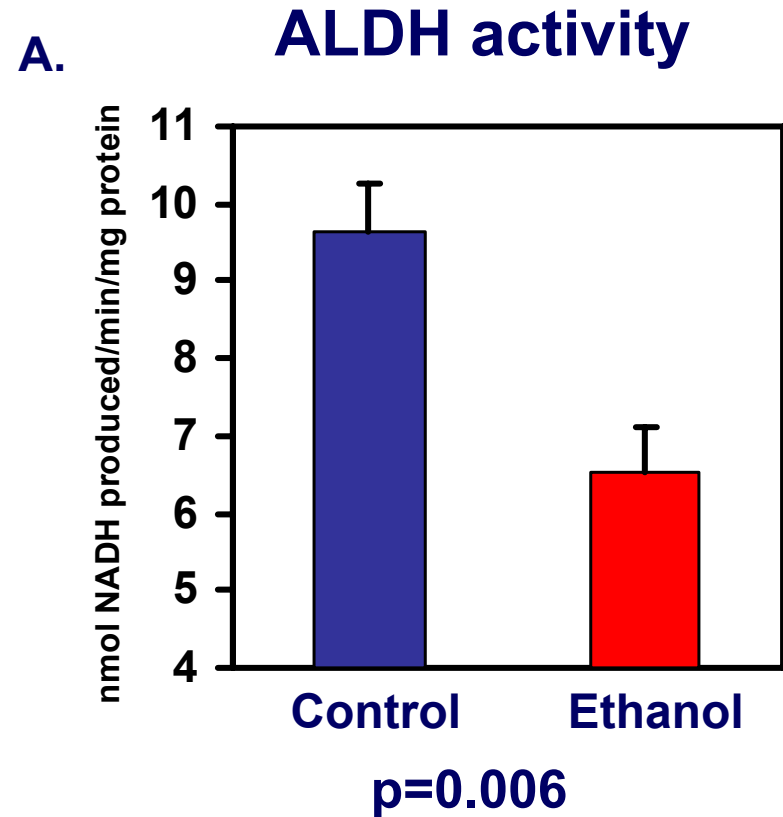
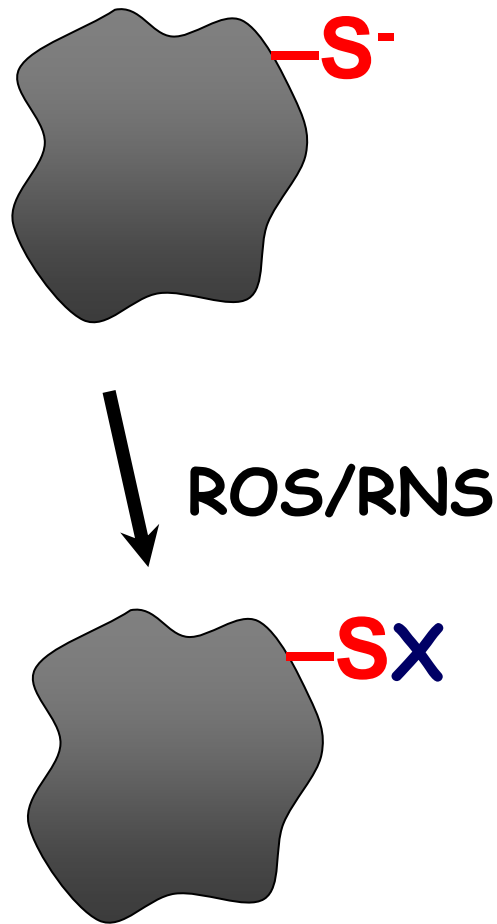


| | mol biotin/mol protein | theoretical maximum |
|--|------------------------|---------------------|
|--|------------------------|---------------------|

| | | |
|-------------------------|-----|---|
| Glutamate dehydrogenase | 2.3 | 6 |
|-------------------------|-----|---|

| | | |
|------------------------|-----|----|
| Aldehyde dehydrogenase | 4.7 | 10 |
|------------------------|-----|----|

Relating Extent of Modification by ROS/RNS to Functional Changes



Thiol Quantitation and Proteomics

Applications:

Direct thiol measurement

Mitochondria

Other subcellular fractions

Cell lysates

Tissue homogenates

Quantitation of different kinds of thiol modifications (biotin switch)

Reversible (-SNO, -SOH, -SSG) vs.

Irreversible (SO_3H , SC_x)

The Lab

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Karina Ricart
Anne Diers
Elena Ulasova
Nobuo Watanabe
Jessica Gutierrez
Brian Dranka
Ashlee Preston
Jeffrey Dubuisson

Collaborators

Stephen Barnes
Amanda Isom

The Proteomic Core Facility
Helen Kim, Director
Landon Wilson
Todd Sanderson