Properties of Protein Targets of Reactive (Lipid) Species

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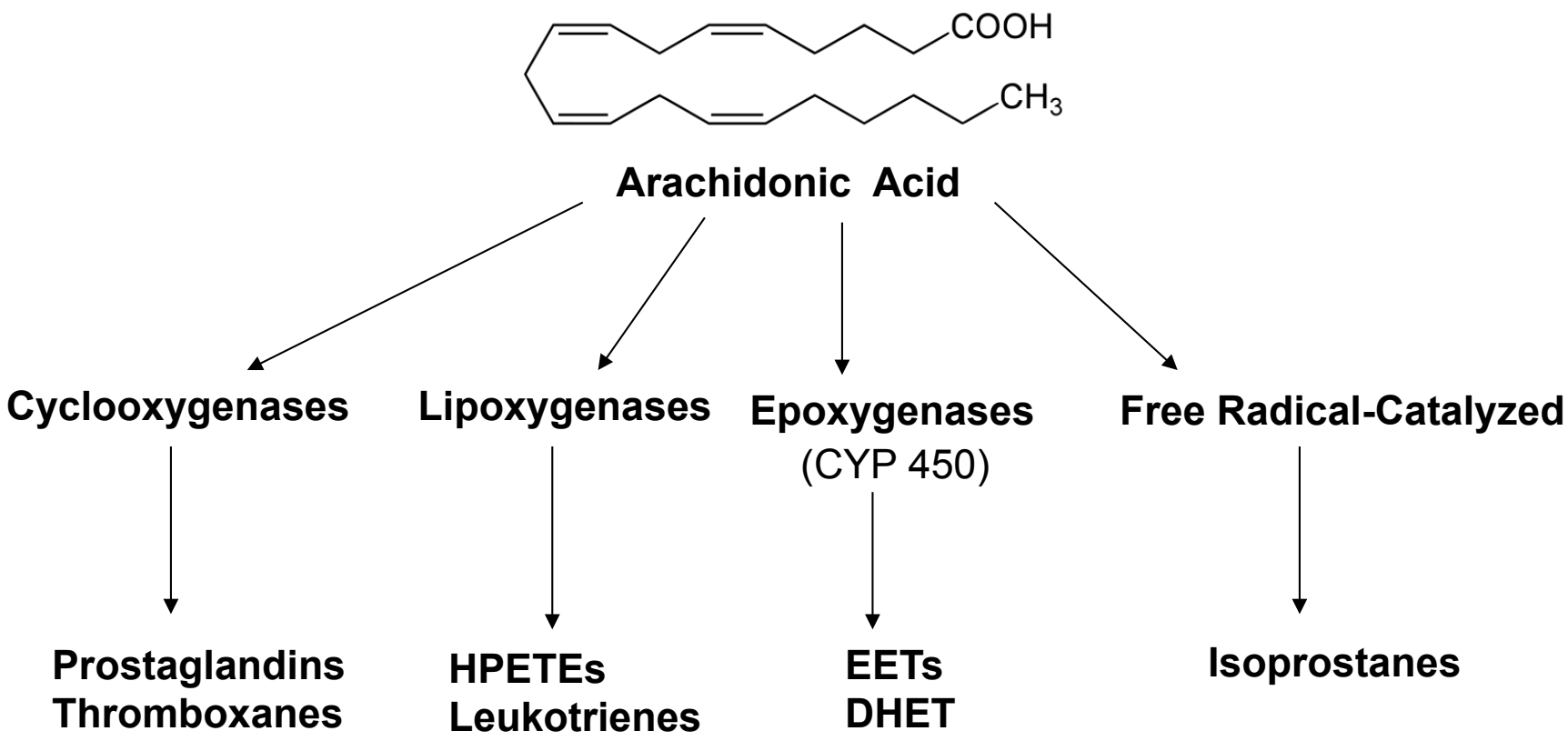
Overview of Presentation

- Sources and characteristics of reactive lipid species (electrophiles)
- Characteristics of protein target residues (nucleophiles)
- Survey of relative rates of electrophile-nucleophile reactions
- Detection and Identification of post-translational protein modifications by lipid electrophiles
- Electrophile-protein modification: functional and structural characterization
- Limitations and challenges

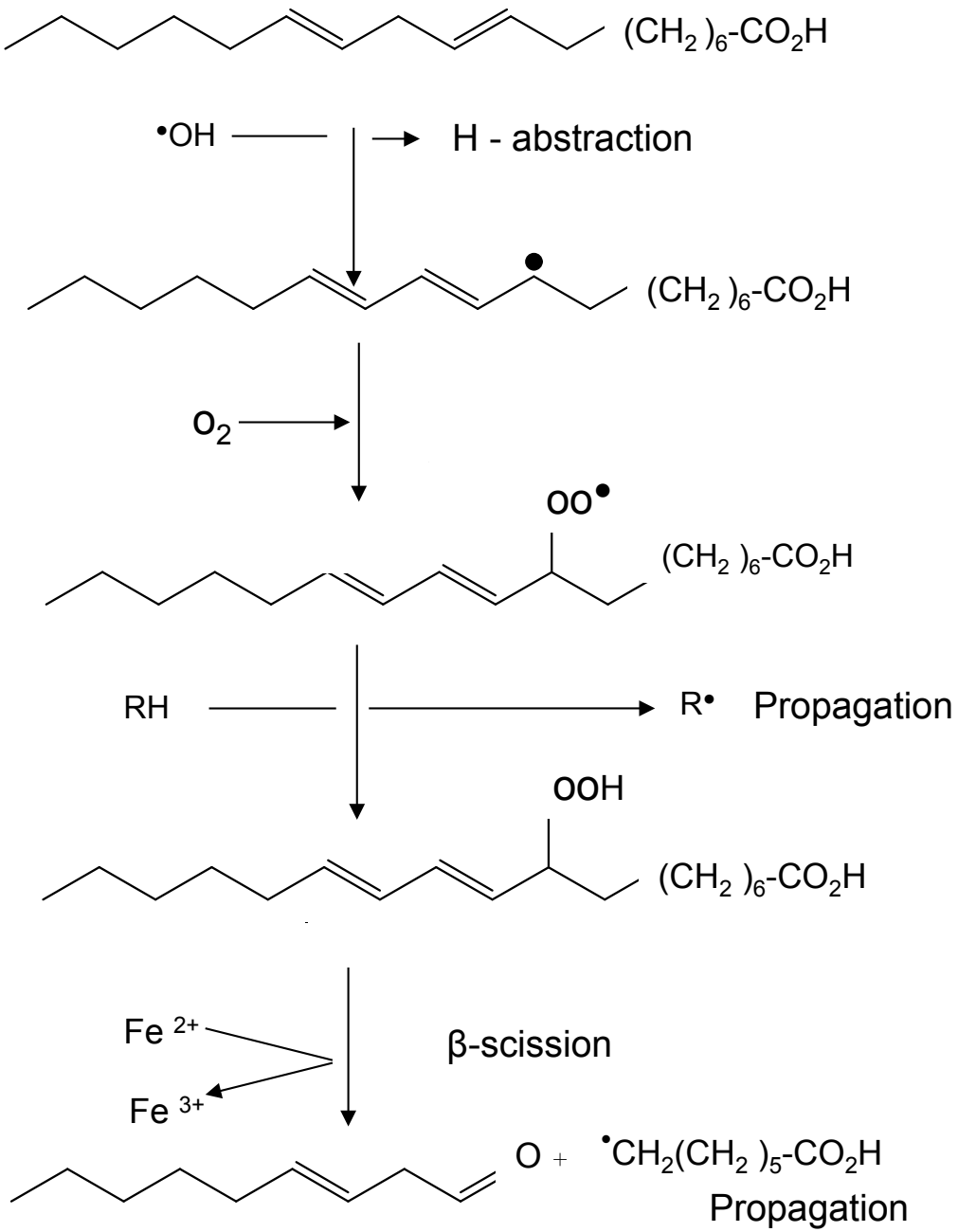
Reactive Lipid Species: The Eicosinoids



Characteristics: Biosynthetic pathways are in response to specific stimuli and are well-regulated

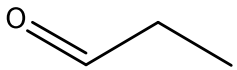
Bioactivities are diverse, pronounced and mediated *via* specific receptors

Endogenous Generation of Lipid Peroxidation Products

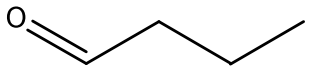


Endogenous Lipid Peroxidation Products

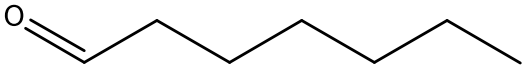
Saturated Alkanals



Propanal

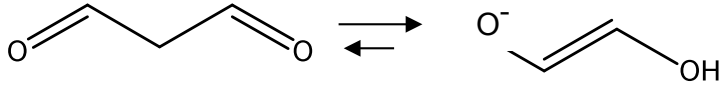


Butanal

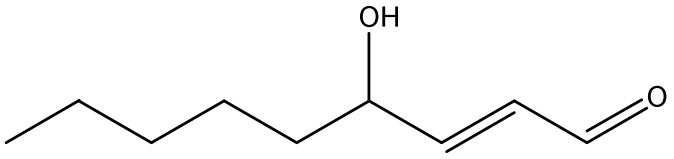


Heptanal

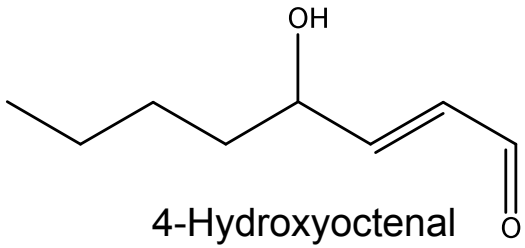
Alkenals and Hydroxyalkenals



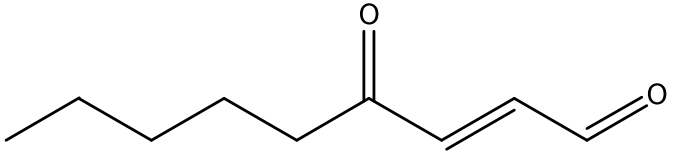
Malondialdehyde



4-Hydroxynonenal



4-Hydroxyoctenal



4-Oxononenal

Esterbauer et al. *Biochem. J* . 208:129, 1982
Poli, et. al. *Biochem. J.* 227:629,1985
Lee & Blair, *Science* 292:2083, 2001

Endogenous Lipid Peroxidation Products (cont.)

Characteristics: Generated endogenously and stimulated by oxidative stress

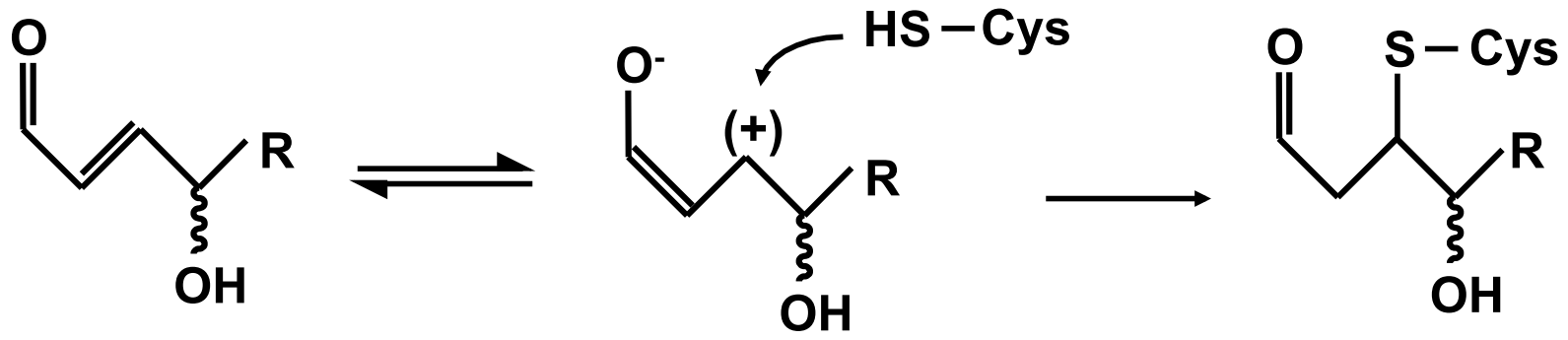
Cascades of generation are non-enzymatic and autocatalytic

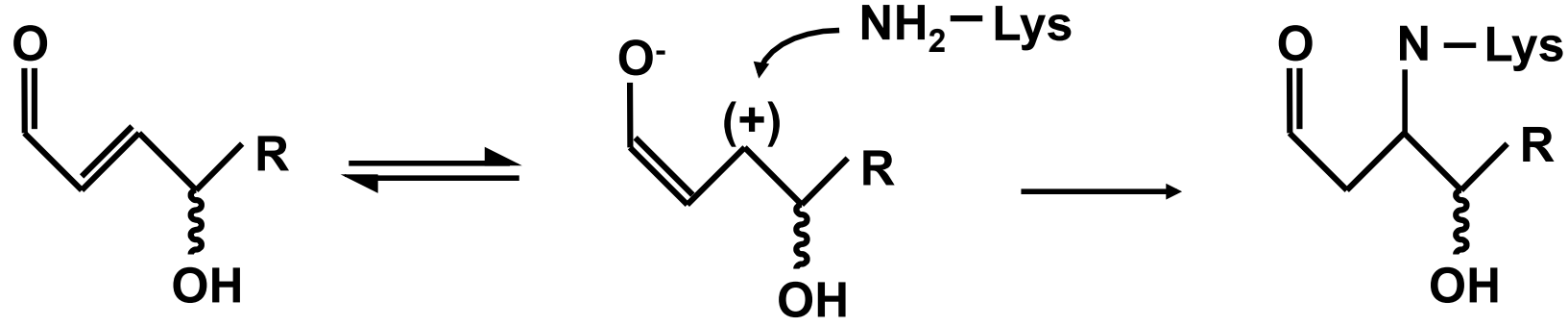
Bioactivities/toxicities are diverse and mediated via interactions with proteins containing highly reactive nucleophilic amino acid residues

The Lipid Peroxidation Product 4-Hydroxynonenal

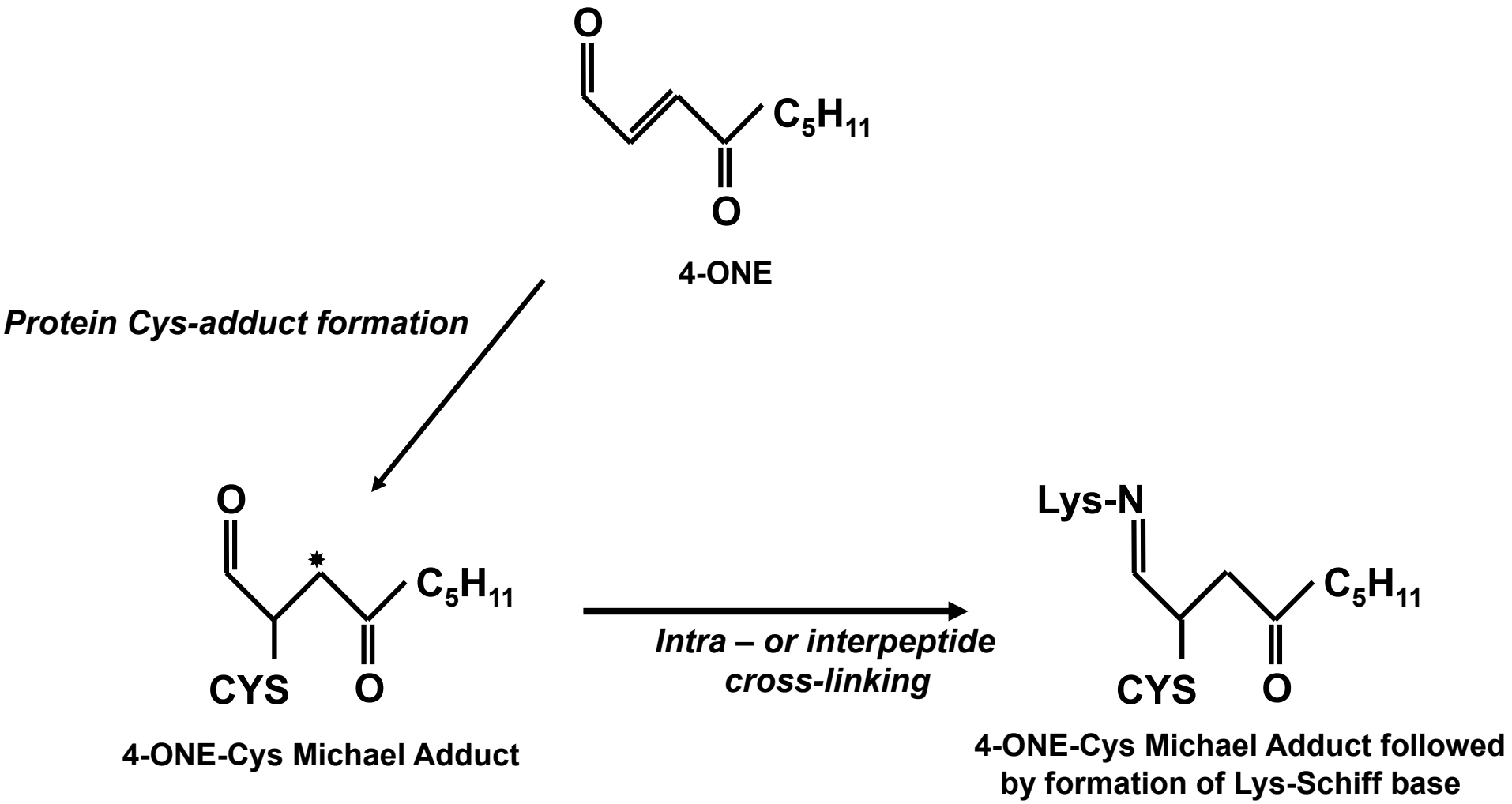
4-HNE as a Michael Acceptor

R: C_5H_{11}





Proposed Interactions of 4-ONE with Proteins



Rate Constants of 4-ONE and 4-HNE with Peptides

Compound	$k(M^{-1}s^{-1})$ 4-ONE	4-HNE	Ratio 4ONE/4HNE
NAC	186 ± 29	1.2 ± 0.03	153
GSH	145 ± 10	1.3 ± 0.05	109
Ac-Lys-amide	7 ± 0.6 $x10^{-3}$	1.3 ± 0.08 $x10^{-3}$	6
Ac-His-amide	2 ± 0.17$x10^{-2}$	2.1 ± 0.30 $x10^{-3}$	10

Overall Reactivity ⟶ Cys >> His > Lys

4-HNE hepatocellular $t_{1/2}$ = ~ 2 min

4-ONE Hepatocellular $t_{1/2}$ = < 1 sec

Doorn and Petersen *Chem Res Tox 15:1445, 2002*
LoPachin et al. *Chem Res Tox 22:1499, 2009*

Hepatocellular Biotransformation of 4-Hydroxynonenal

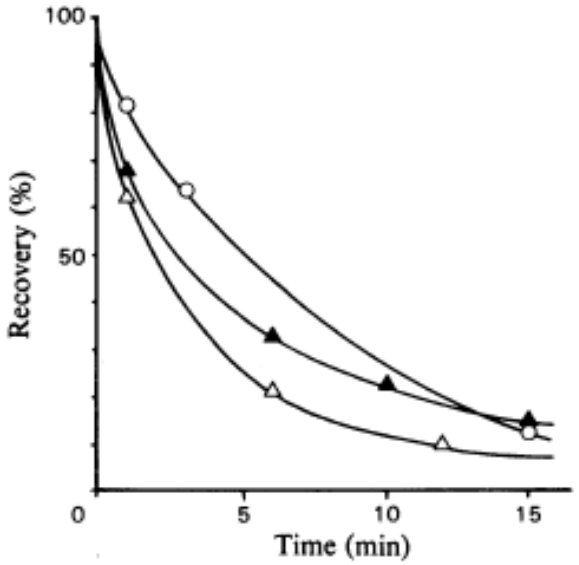


Fig. 4. *Kinetics of the metabolism of 4-hydroxyalkenals by isolated rat hepatocytes*

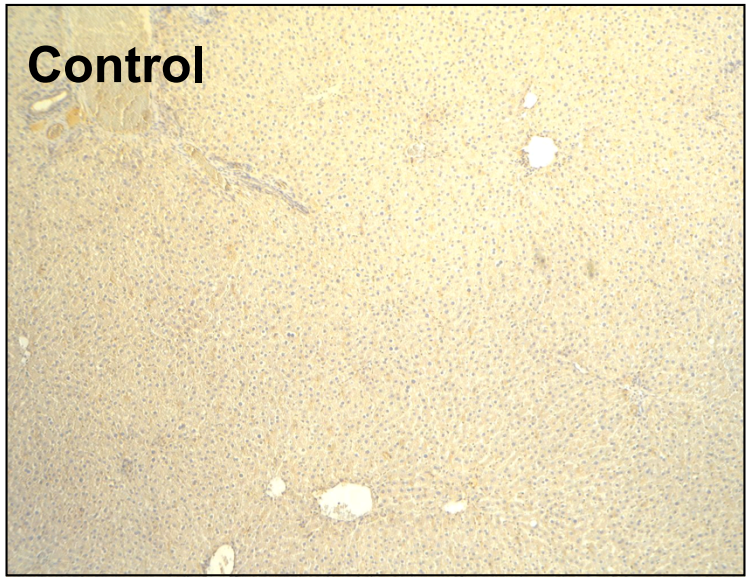
○, 4-Hydroxypent-2-enal; ▲, 4-hydroxyoct-2-enal; △, 4-hydroxynon-2-enal. Hepatocytes (0.2×10^6 cells/ml) were incubated in the presence of each aldehyde (0.1 mM), and the disappearance of the aldehyde was monitored by h.p.l.c.

Poli, et. al. *Biochem. J.* 227:629,1985

Bioconversion Pathways	**Overall Contribution**
Alcohol/aldehyde dehydrogenase	10%
GSH/glutathione transferases	70%
Unidentified	20%

Hartley et al. *Arch Biochem Biophys* 316:197, 1995

4-HNE Protein Adduct Formation In Liver of Rats Chronically Consuming Alcohol



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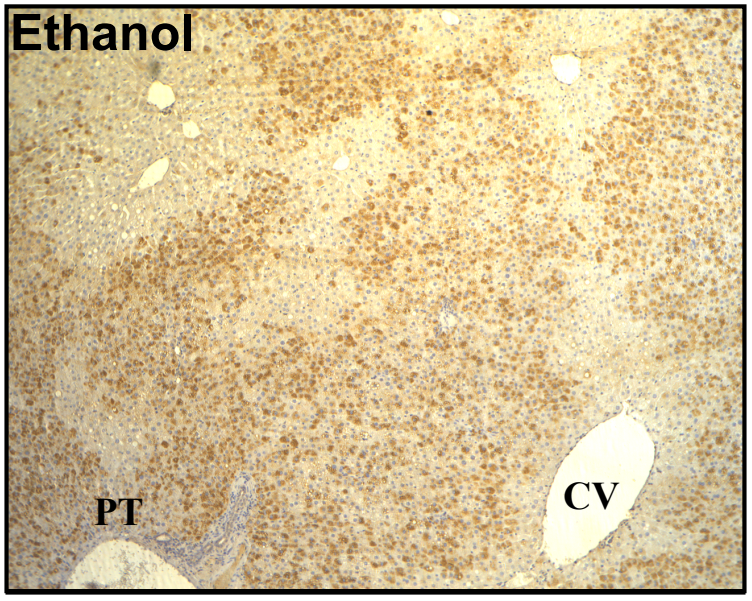

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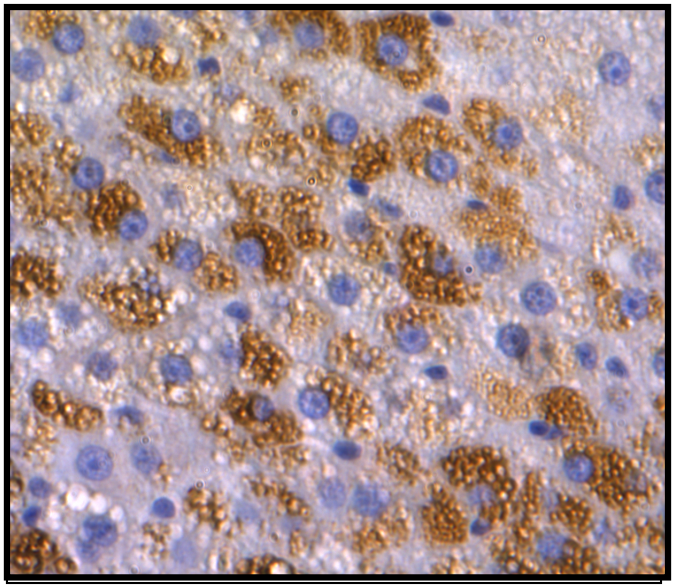
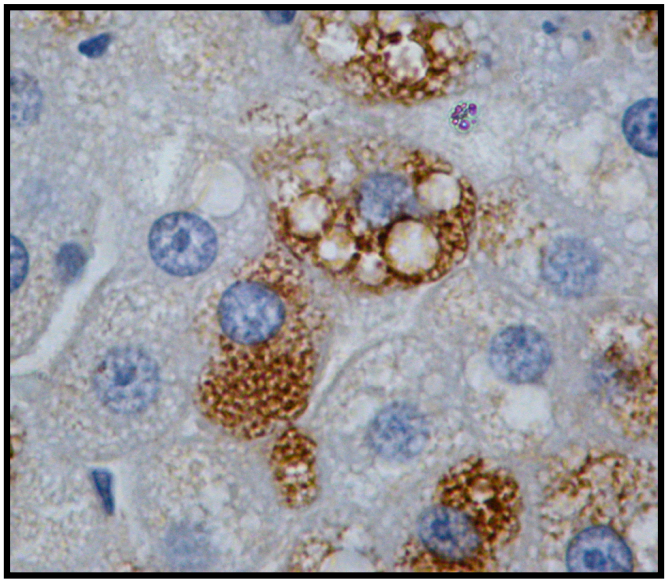
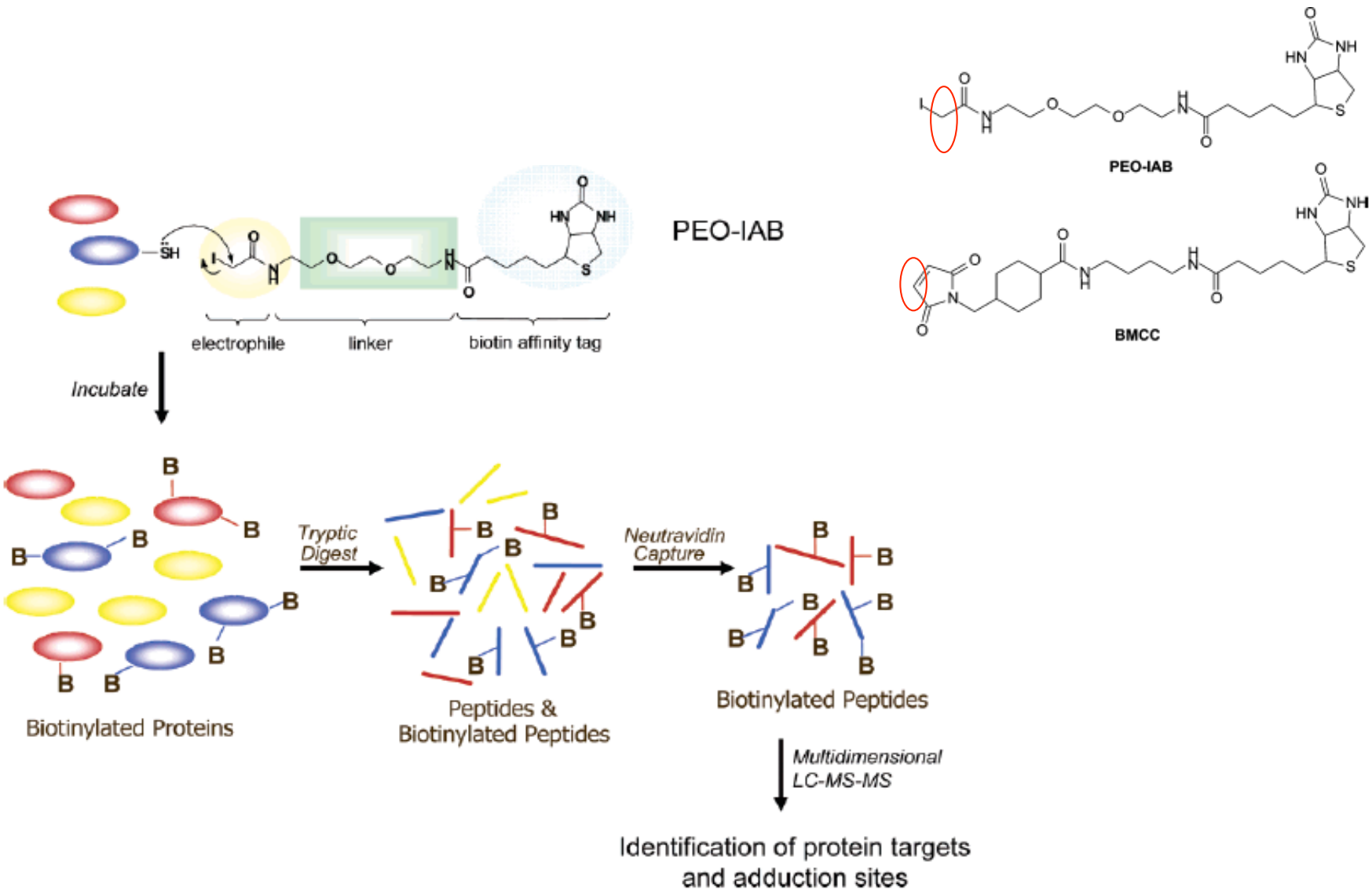


(100X)
(Magnification)



Cellular Protein Targets of Thiol-Reactive Electrophiles



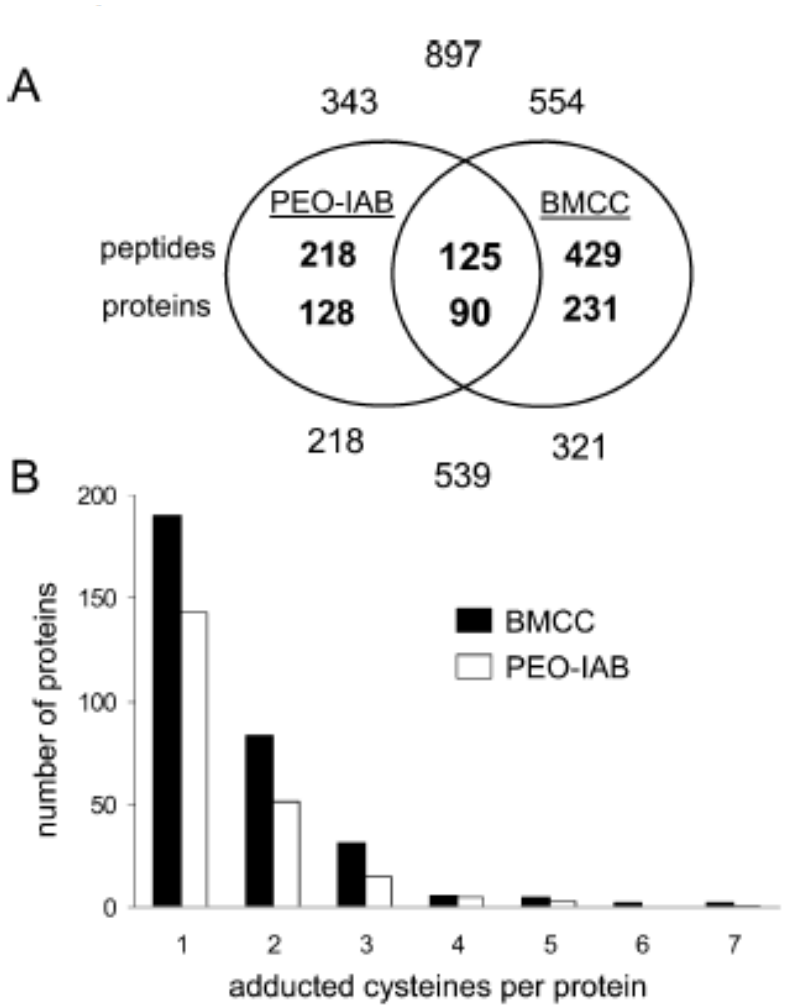
Dennehy et al. *Chem Res Tox* 19:20, 2006

Cellular Protein Targets of Thiol-Reactive Electrophiles

Only cysteine adducts were identified

Greater than 90% of adducted proteins were modified at only 1 or 2 cysteines

PEO-IAB & BMCC modified a core group of 125 cysteines



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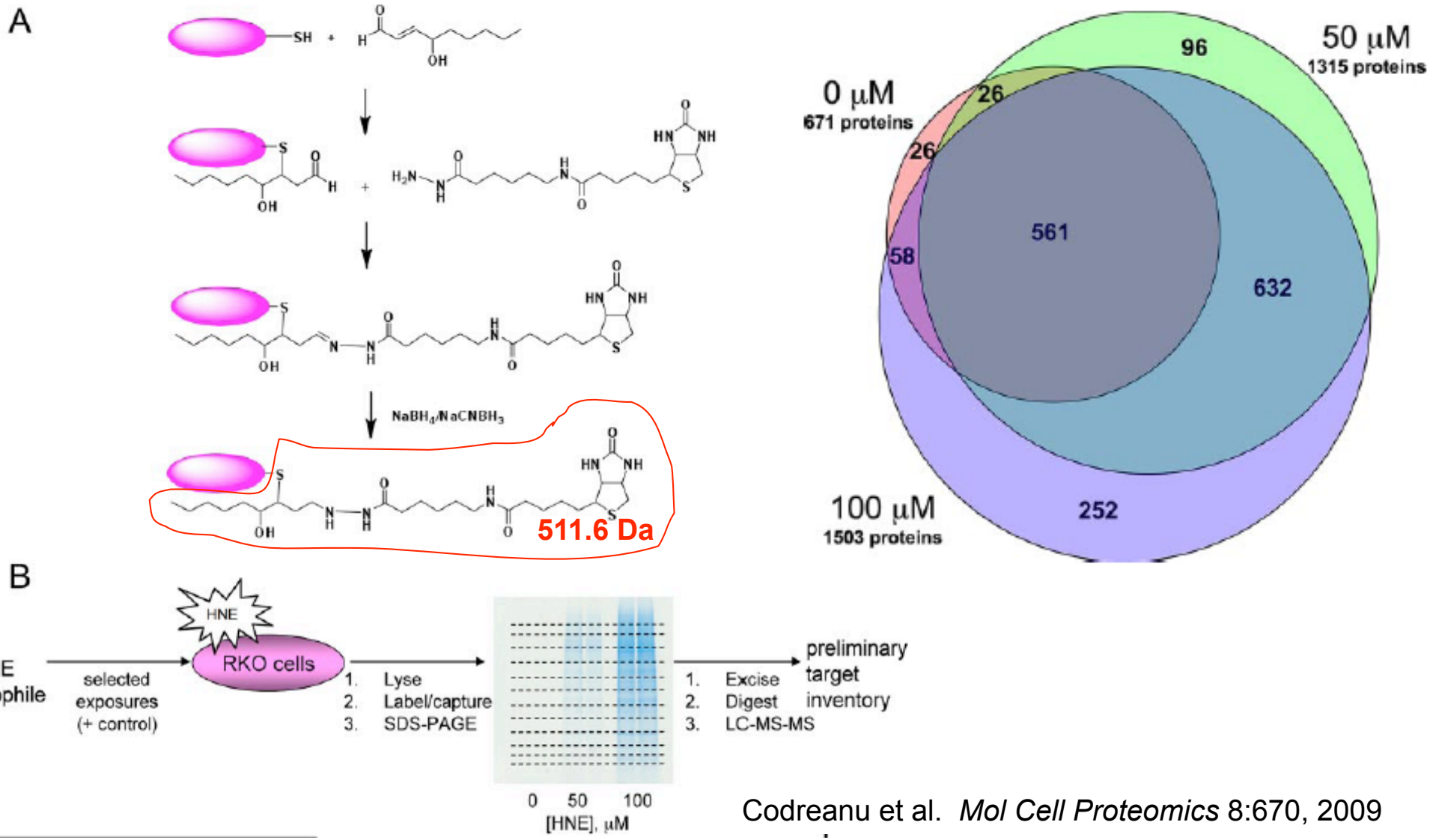
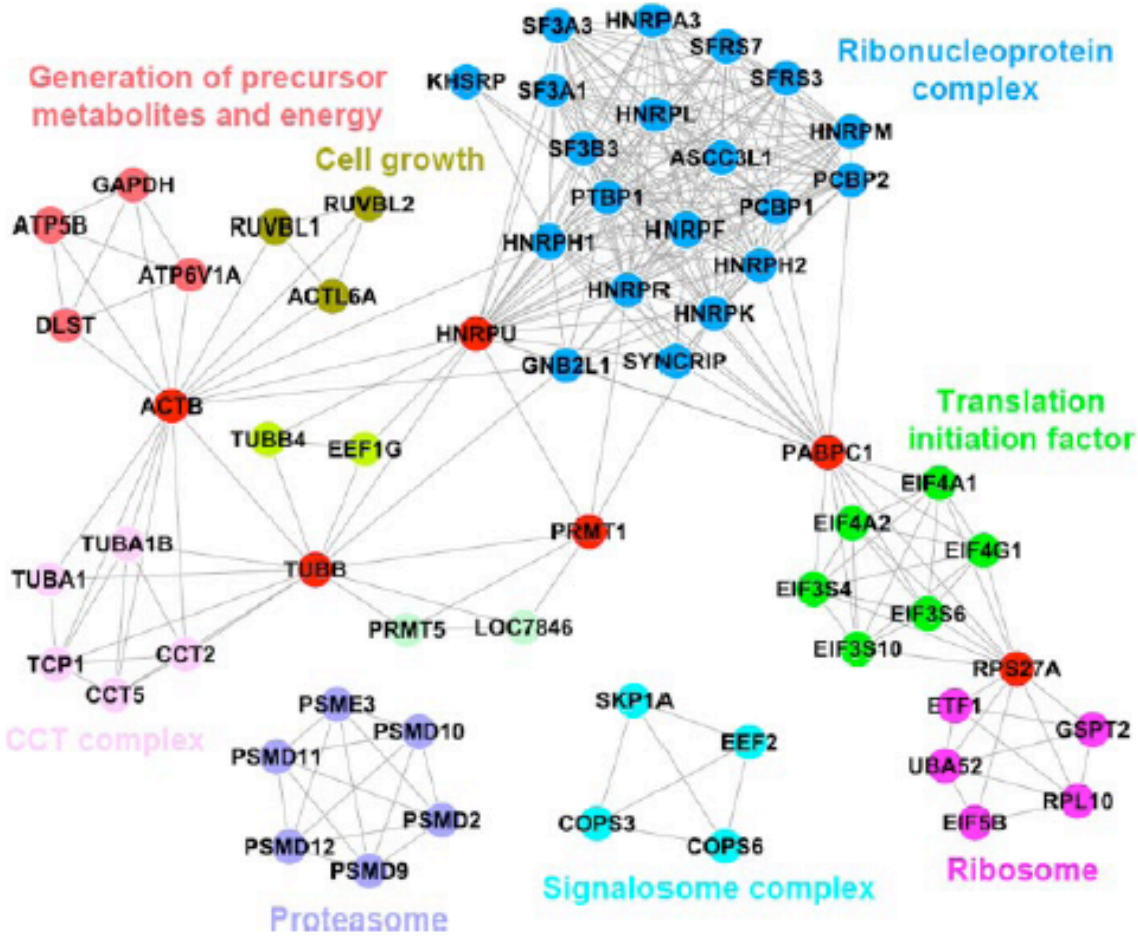
Cellular Protein Targets of Thiol-Reactive Electrophiles



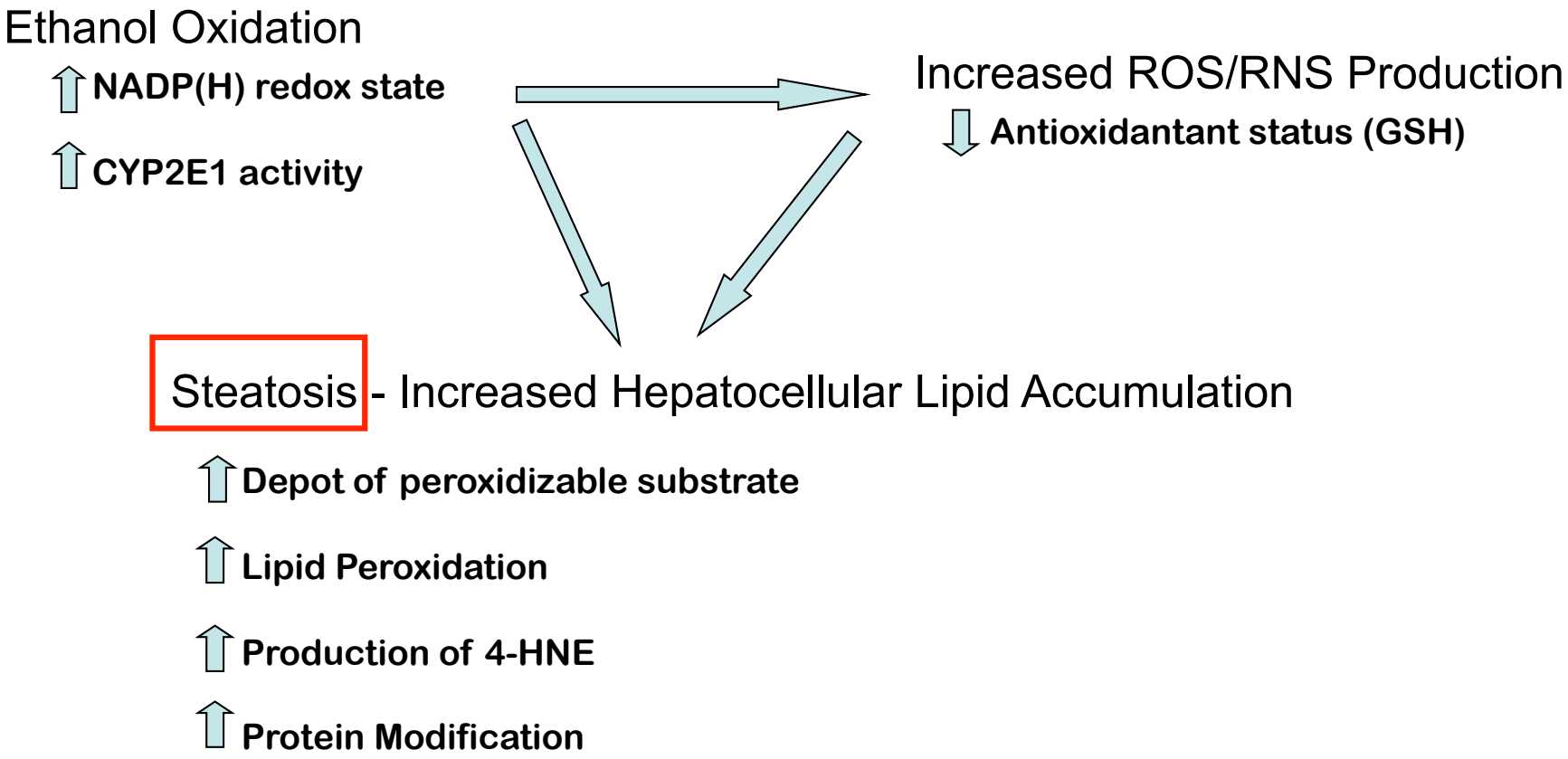
Codreanu et al. *Mol Cell Proteomics* 8:670, 2009

Protein Communities of 4-HNE-Modifed Proteins



Codreanu et al. *Mol Cell Proteomics* 8:670, 2009

Alcoholic Liver Disease: A multifactorial Disorder Involving Oxidative Stress



Experimental Procedures

Harvest hepatic tissue & subcellular fractions

↓

2-D Gel electrophoresis analysis for detection of 4-HNE modified proteins

↓

Extract 4-HNE immunopositive spots for tryptic digestion

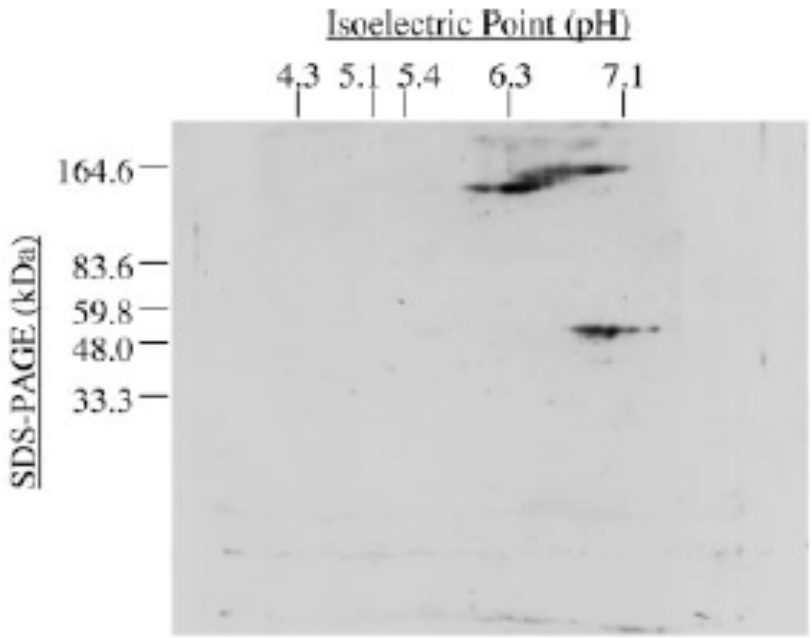
↓

Identify 4-HNE modified proteins using MALDI-TOF/TOF or LC-MS/MS

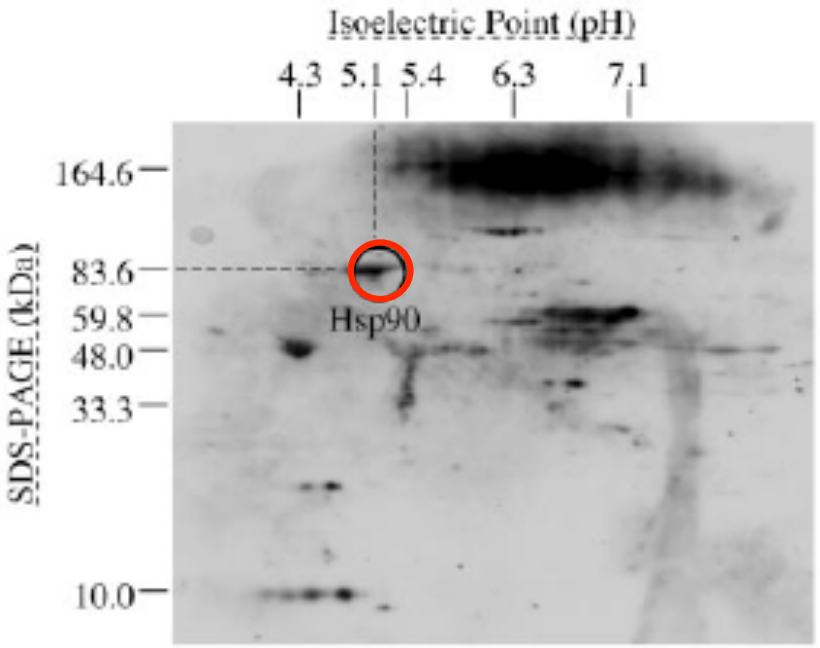
↓

Characterize functional effects of 4-HNE modification using recombinant protein

HSP90 Detection by Anti-4-HNE Immunoblots of 2-D Gels



Control



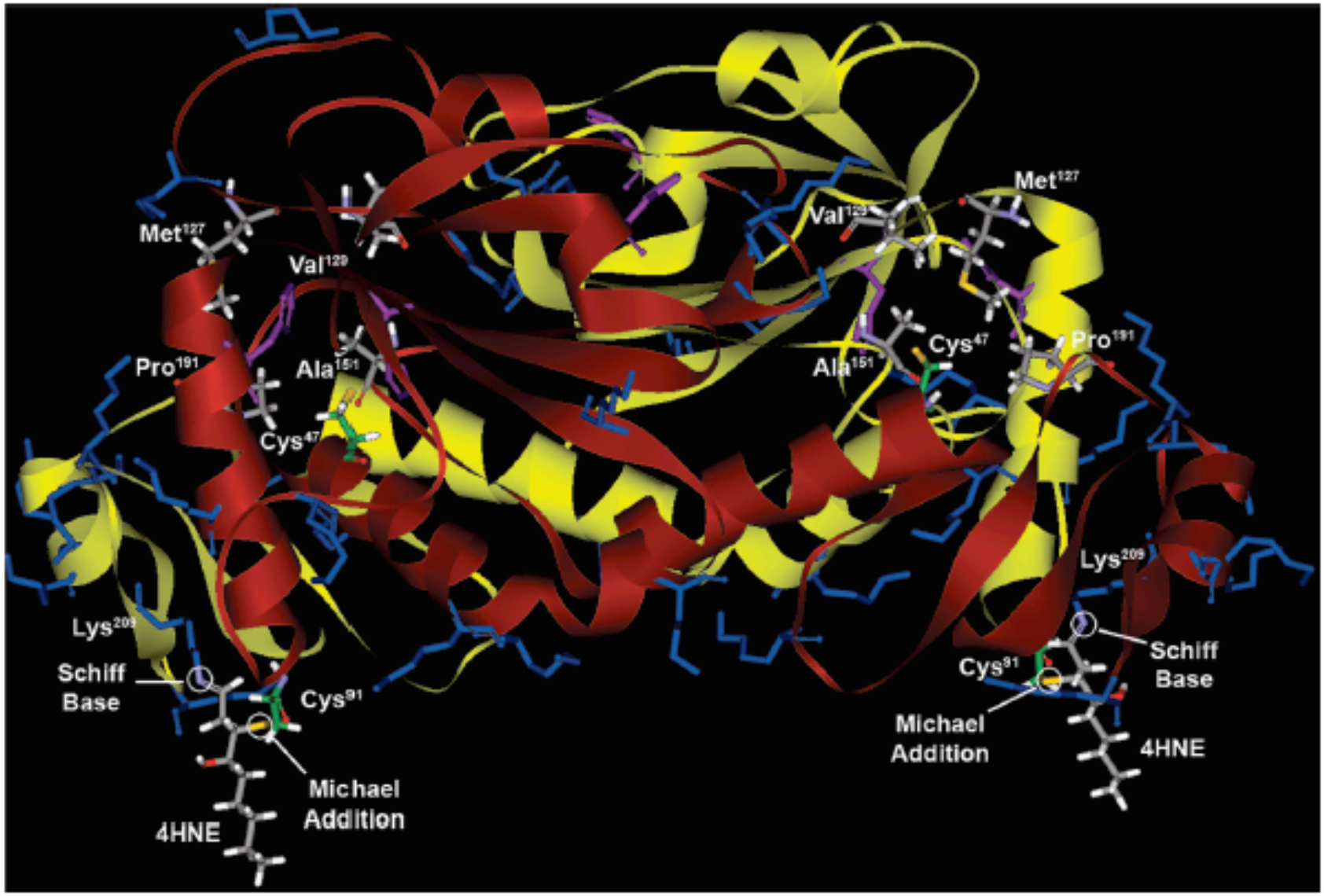
Ethanol

Carbone et al, *JPET* 2005; 315:8

Hepatic Proteins Modified by 4-HNE

Target Protein	Modified Residue	Functional Consequences
HSP70 *Chem Res Toxicol.* 2004; 17:1459	Cys 267 TACER	Impaired ATPase activity & protein folding
PDI *Chem Res Toxicol.* 2005; 18:1324	Cys 57 WCGHC	Dysregulated restoration of mismatched intraprotein disulfide bonds
ADH *Free Radic Biol Med* 2004; 37:1430	Cys 46 NFCLK	Activity not impaired; enhanced proteosomal degradation
HSP90 *JPET* 2005; 315:8	Cys 572 ENLCK	Impaired refolding of damaged proteins
ERK1/2 *JBC* 2007; 282:1925 *Molec Pharmacol* 2007; 37:1430	His 178 PDHDH	Inhibition of ERK1/2 phosphorylation and impaired liver regeneration
Prx6 *Free Rad Biol Med 2008;45:1551* *Chem Res Toxicol*. 2008; 12:2289	Cys 91 YNCEP	Modification of solvent accessible thiol; activity only marginally impaired
Tubulin *Chem Res Toxicol* 2007; 20:1111 *Biochim. Biophys Acta* 2009;1791:772	Cys 347α, CYS 376 α Cys 303ß	Inhibition of tubulin polymerization; Lipid accumulation and inhibition of lipid transport in HepG2 cells

In Silico Modeling of 4-HNE: Peroxiredoxin 6

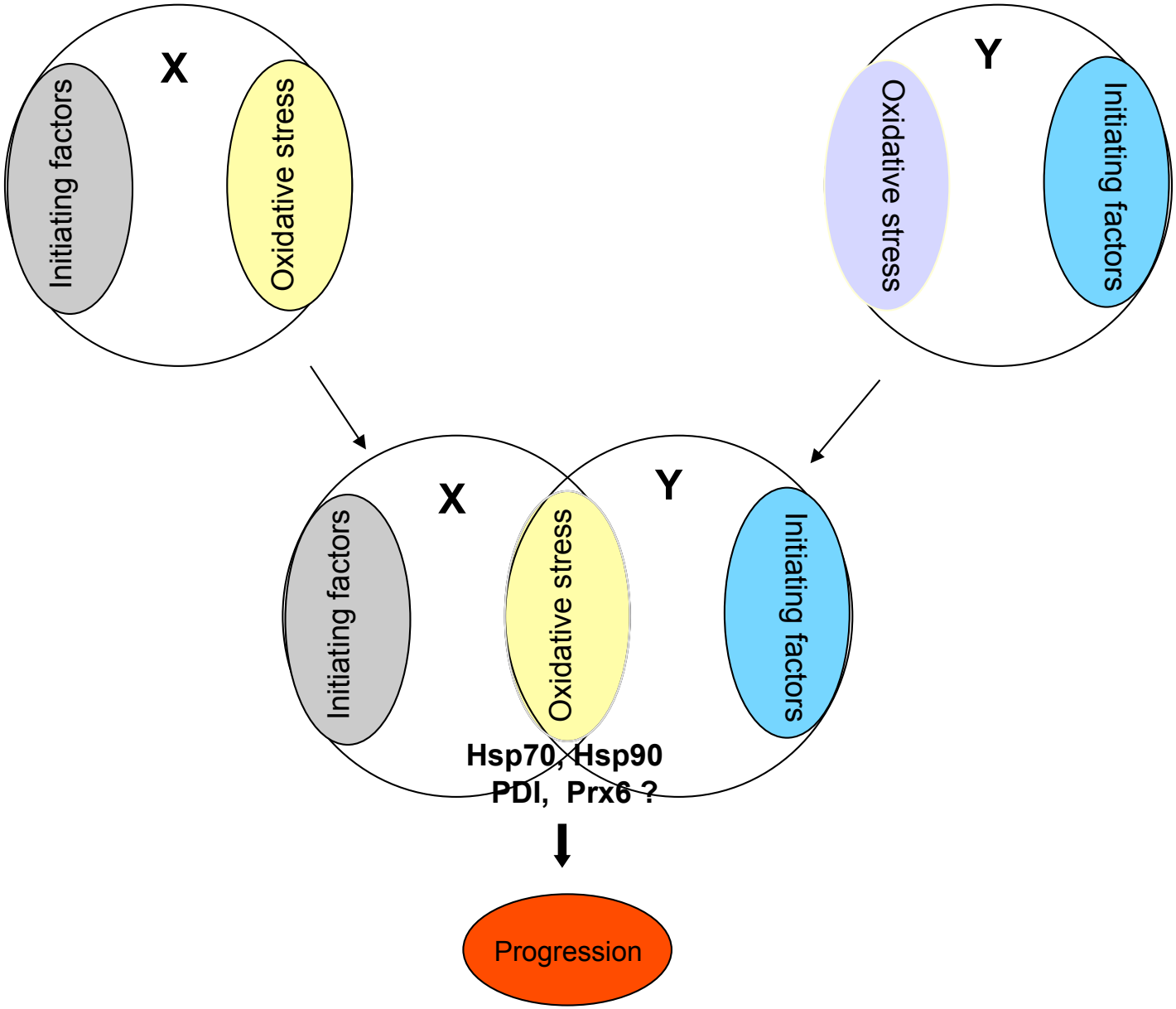


Roede et al. *Chem Res Tox* 12:2289, 2008

4-HNE Modification Does Not Impair the Function of All Proteins

Protein	Functional Consequence
Alcohol Dehydrogenase	Increased/decreased UPS degradation
Catalase	None
Glutamate Dehydrogenase	None

Proposed Model for Identification of Common Target Proteins and Roles in Disease Processes



Limitations and Challenges

- Large numbers of proteins are modified by electrophiles making it difficult to identify specific proteins involved in cellular dysregultation.

- There is a low stoichiometry of protein modification. This magnifies the analytical challenges suggesting the necessity of specific affinity enrichment procedures.

Acknowledgements

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Dr. David Carbone, University of Arizona
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Dr. Ben Stewart (LLNL)