

Brook et al. *Circulation*. 2004. 109:2655-2671.

RO1 ES013432

"Atherogenic effects of Ambient PM in Susceptible Animals"

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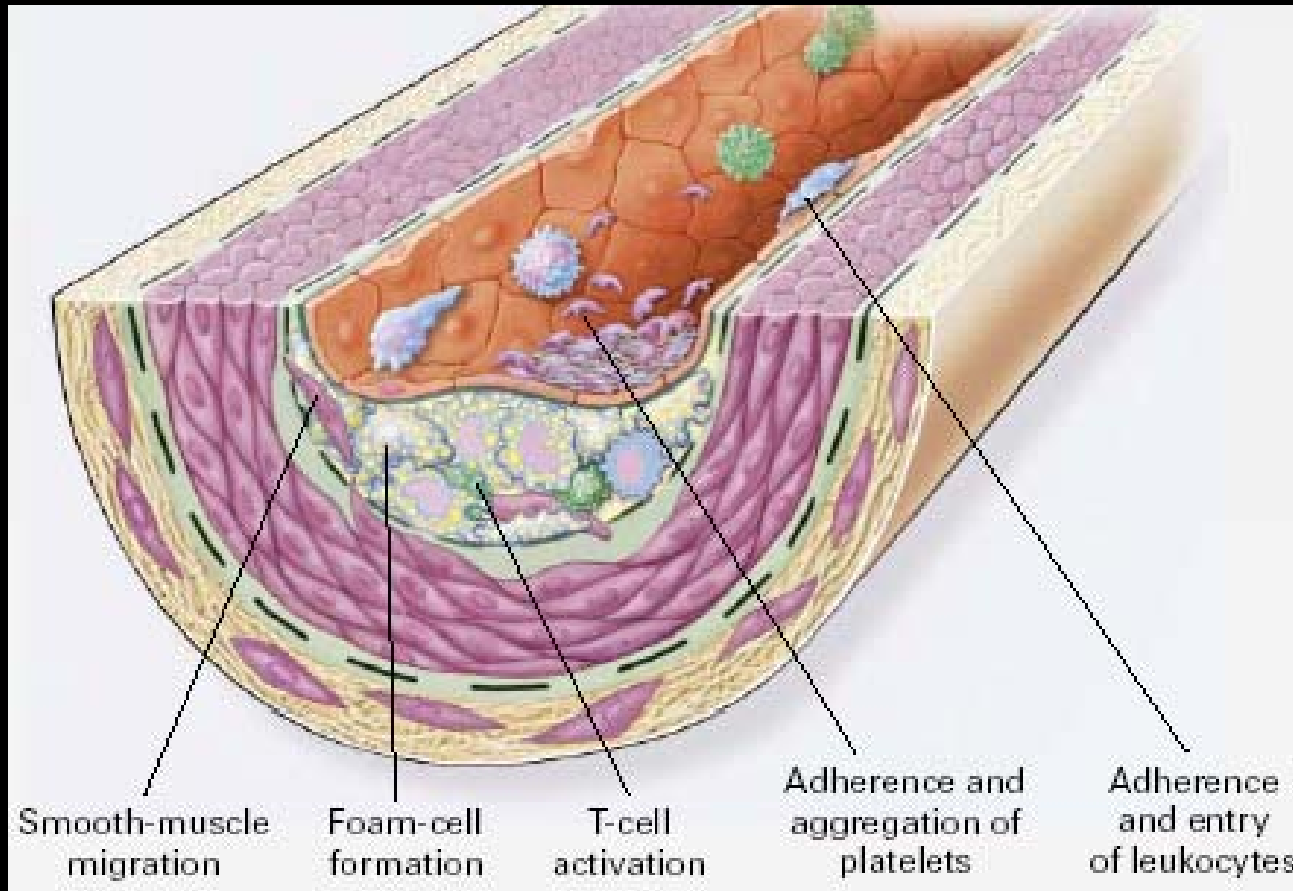
Joseph Loo, PhD*

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+ USC

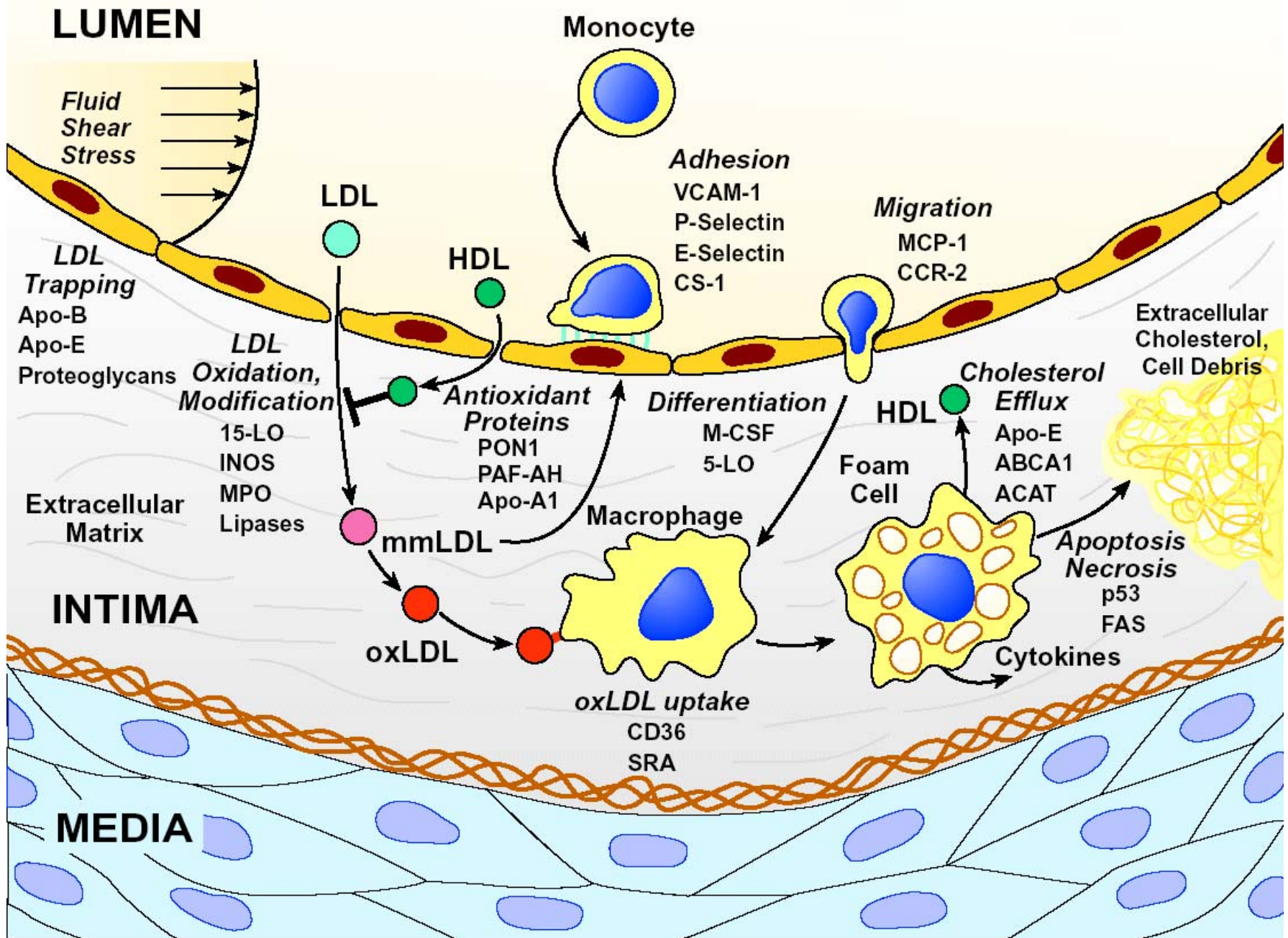
Major Hypothesis: PM-induced oxidative stress synergizes with oxidized lipid components to enhance inflammation and apoptosis in atherosclerotic lesions

PM is now a recognized risk factor for Atherosclerosis



A disease of oxidative stress, chronic inflammation and apoptosis.....

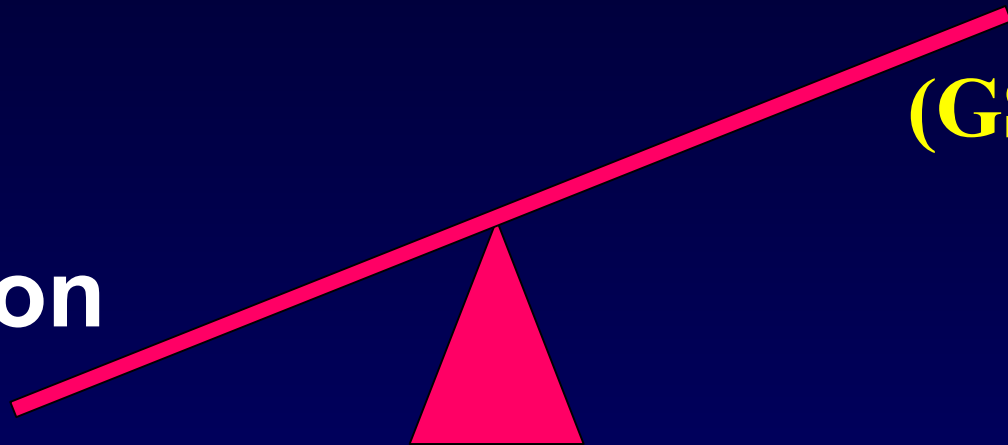
Synergy between oxidized LDL and ambient PM ?



Normal

**ROS
Inactivation**
(GSSG lo)

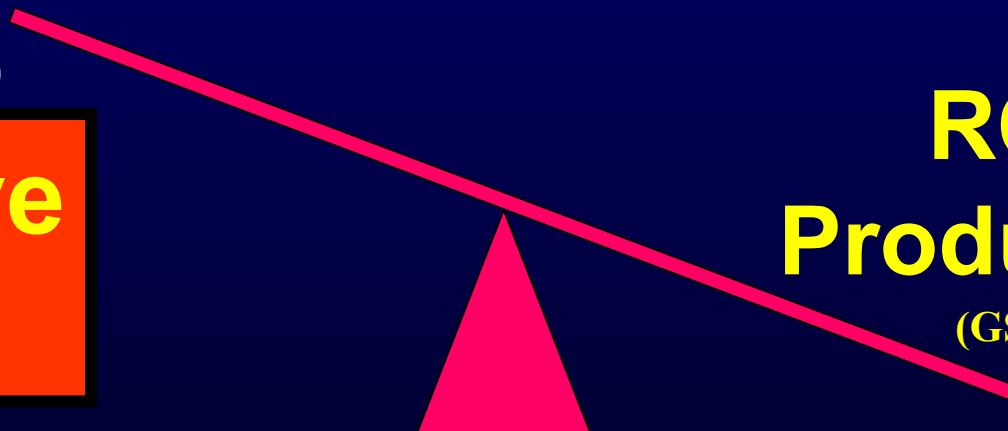
**ROS
Production**
(GSH hi)



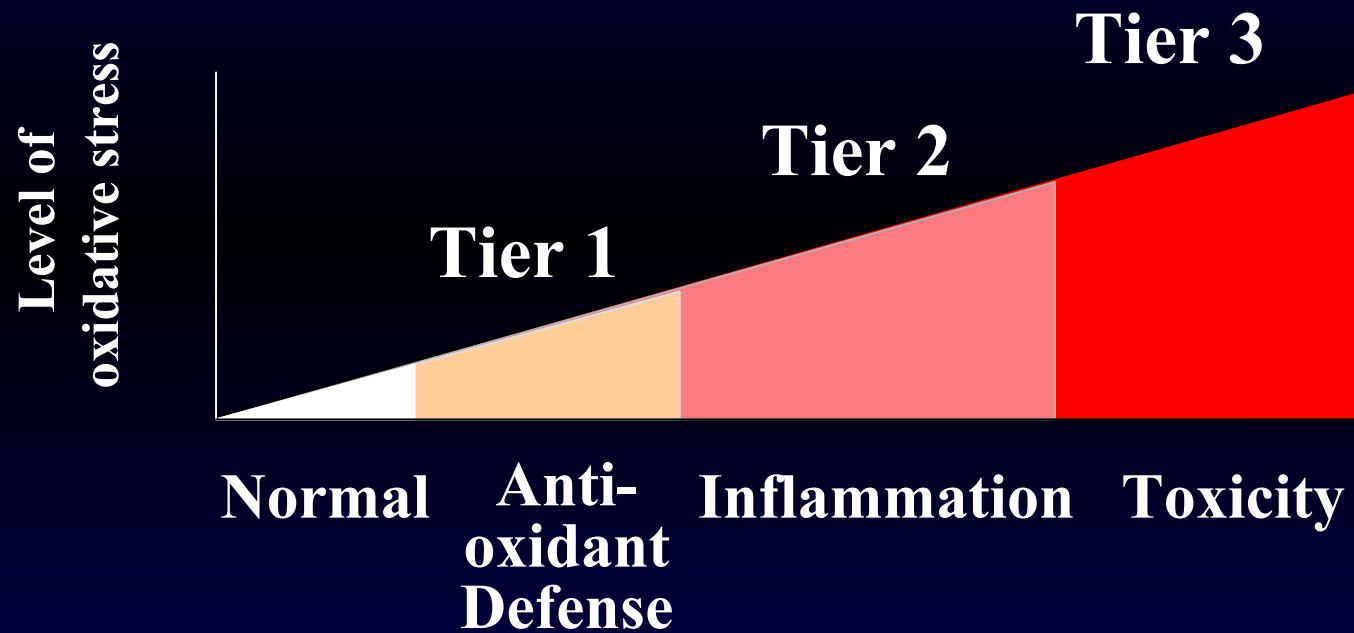
**ROS
Inactivation**
(GSSG hi)

**Oxidative
Stress**

**ROS
Production**
(GSH lo)



Stratified Oxidative Stress Hypothesis



Specific Aims:

Aim 1: To determine whether exposure to ambient PM exacerbate the rate and magnitude of atherosclerosis in apoE and LDL-R knockout mice.

Aim 2: To determine the role of PM-induced oxidative stress in the development of inflammation and apoptosis in atherosclerotic lesions in apoE and LDL-R deficient mice.

Aim 3: To determine whether modified antioxidant defense mechanisms affect the induction of atherosclerotic lesions by ambient PM in apoE deficient mice.

Aim 1

Particle concentrators (VACES): 1. Fine; 2. Ultrafine; 3. Filtered Air

Inhalation chambers

In vivo exposures

Animal strains: ApoE^{-/-} & LDL-R^{-/-}

Exp 1 : Early lesion development

Exp 2 : Late lesion development

Endpoints

Lesion scoring

Histology

Immunocytochemistry (inflammation,
& apoptosis)

Impinger samples

In vitro cellular exposures

Cell types: Endothelial, macrophage,
& smooth muscle cells

Exp 3 : Stimulation by CAPs ±Ox

PAPC

Endpoints

Inflammation

Signaling : MAPK

Apoptosis assays

Proteomics

Aim 2

In vivo Endpoints: Animal sacrifice

Lesional HO-1 expression, phospholipid
oxidation
MAPK activation

Apoptosis

Serum SAA, fibrinogen, IL-6, GM-CSF

HDL antioxidant activity (oxidative PON
modification)

In vivo luciferase imaging HO-1 promoter

In vitro Endpoints for oxidative stress

ROS generation: DCF & HE

GSH/GSSG ratios

Hierarchical oxidative: HO-1,
cytokines, MAPK,
apoptosis

2D gel electrophoresis and
protein MS

Aim 3

Particle concentrators (VACES): 1. Fine; 2. Ultrafine; 3. Filtered Air



In vivo exposures

Exp. 1 Tg HO-1/apoE ^{-/-}

Exp. 2 Nrf2 x apoE double KO

Exp. 3 apoE /PON1 double KO



Endpoints:

Atherosclerotic scores & markers inflammation & apoptosis (Aim 1)

Lesional markers for oxidative stress, including ARE-dependant genes (Aim 2)

HDL antioxidant activity

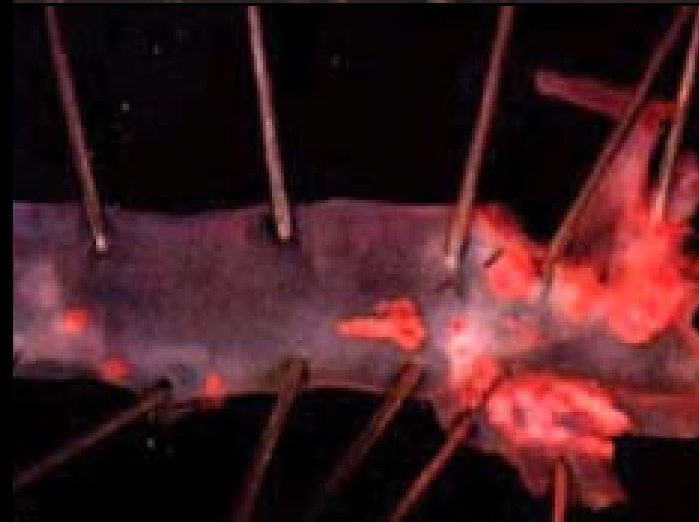
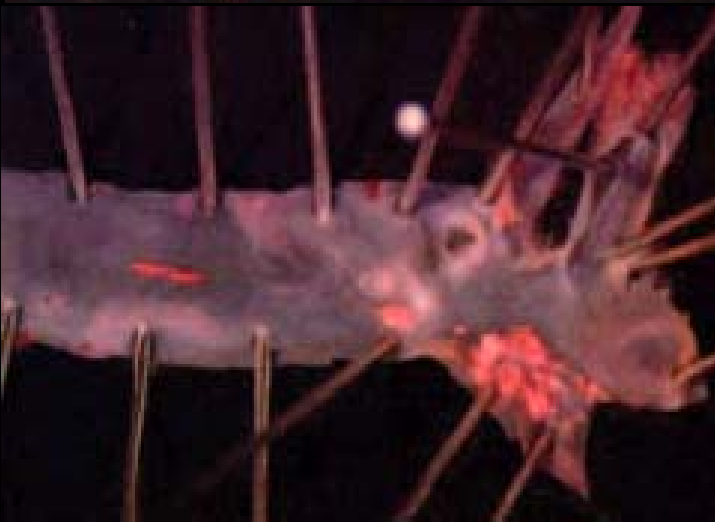
High Chol diet/ No Arsenite

High Chol diet/Arsenite

C57Bl/6 wildtype

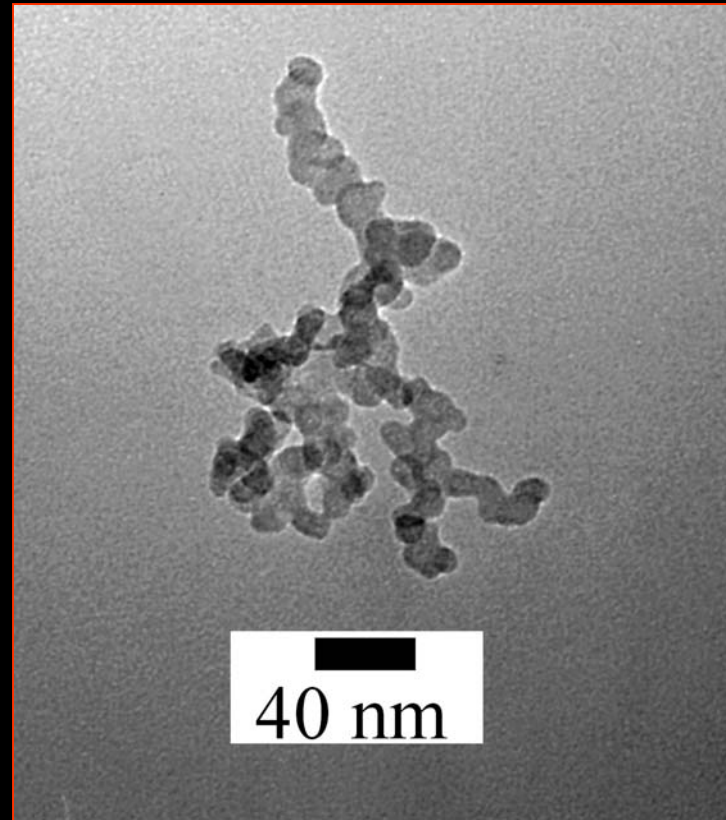


ApoE knockout

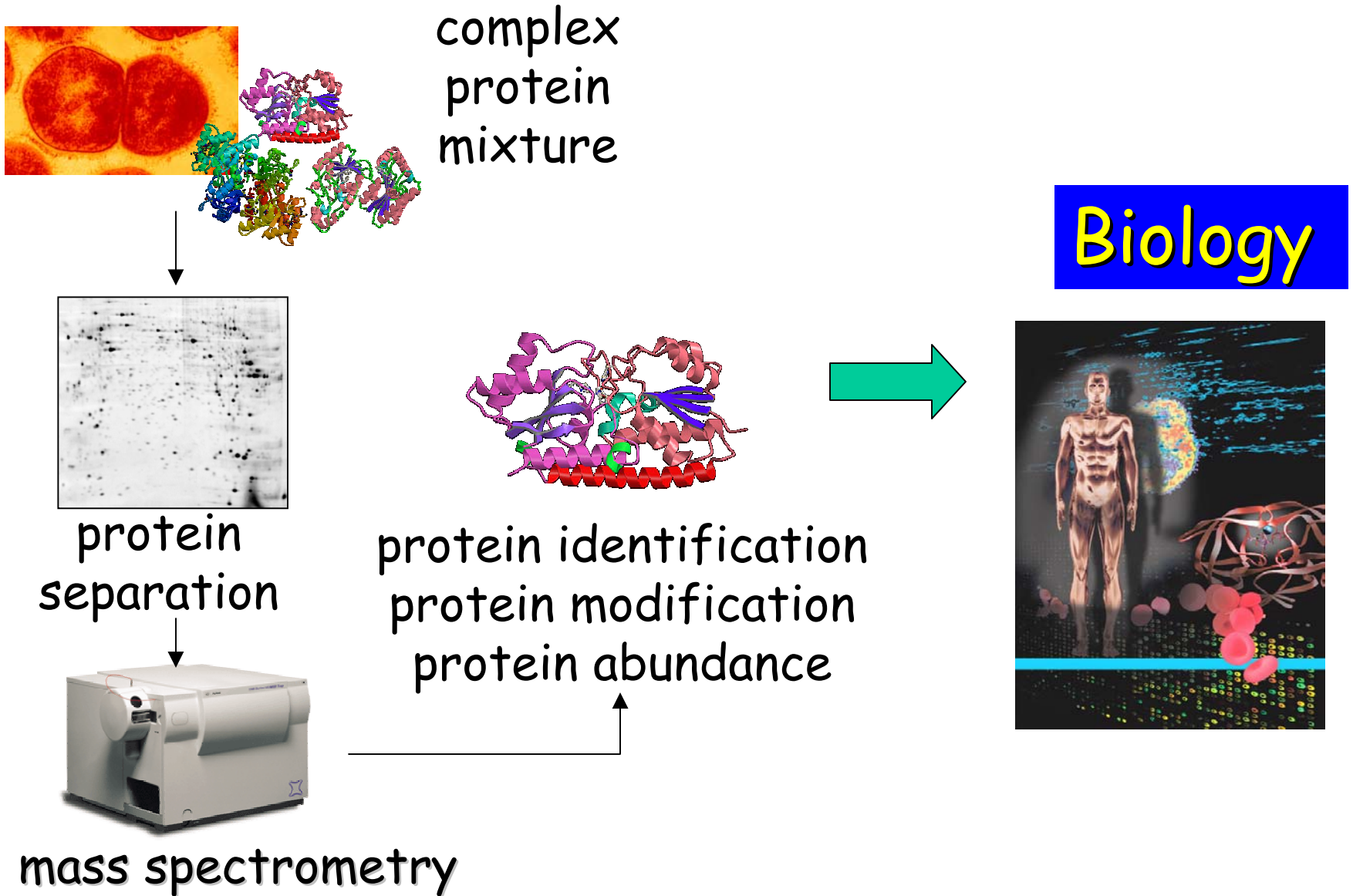


Petia P. Simeonova et al. Environ Health Perspect 111:1744–1748 (2003).

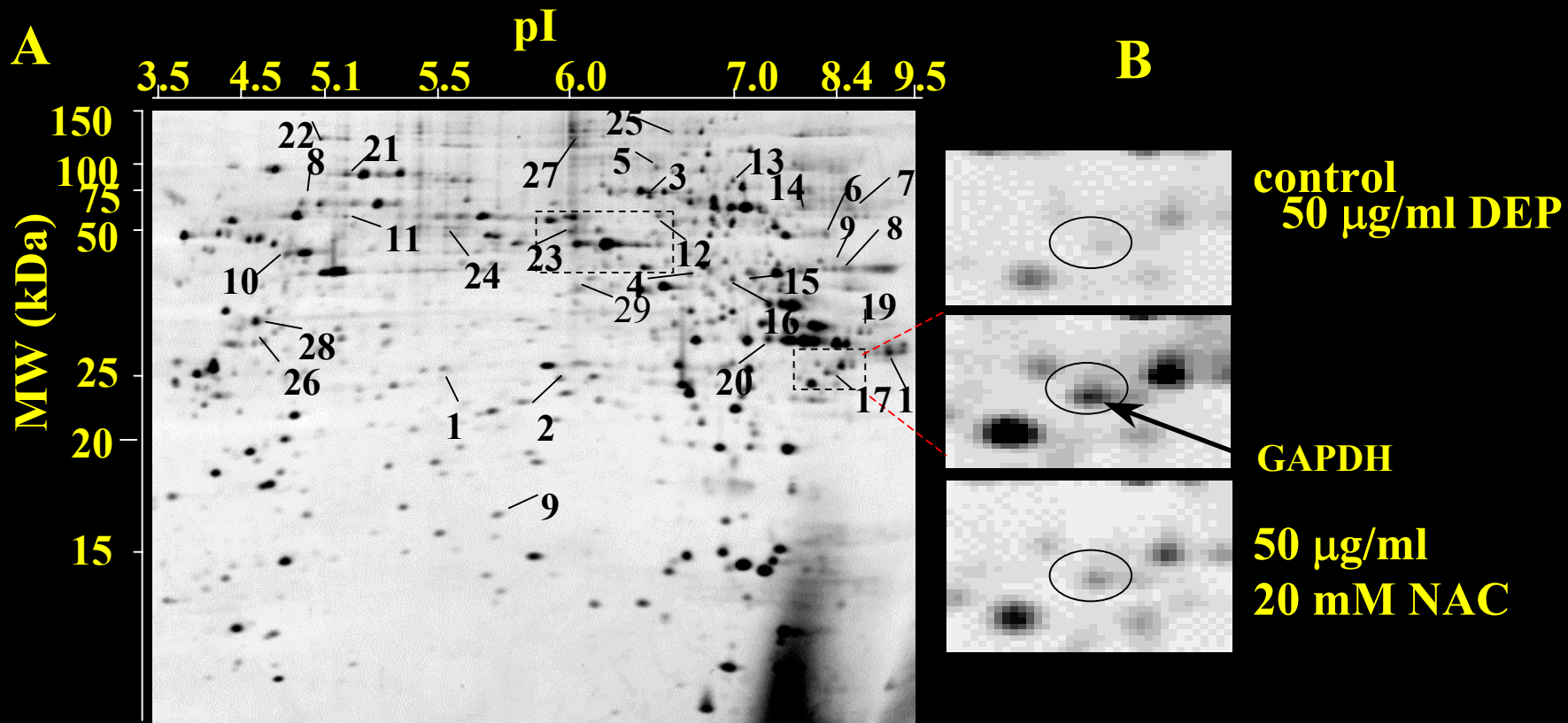
Passage of Inhaled Particles Into the Blood Circulation in Humans



Proteomics Aids Biological Research



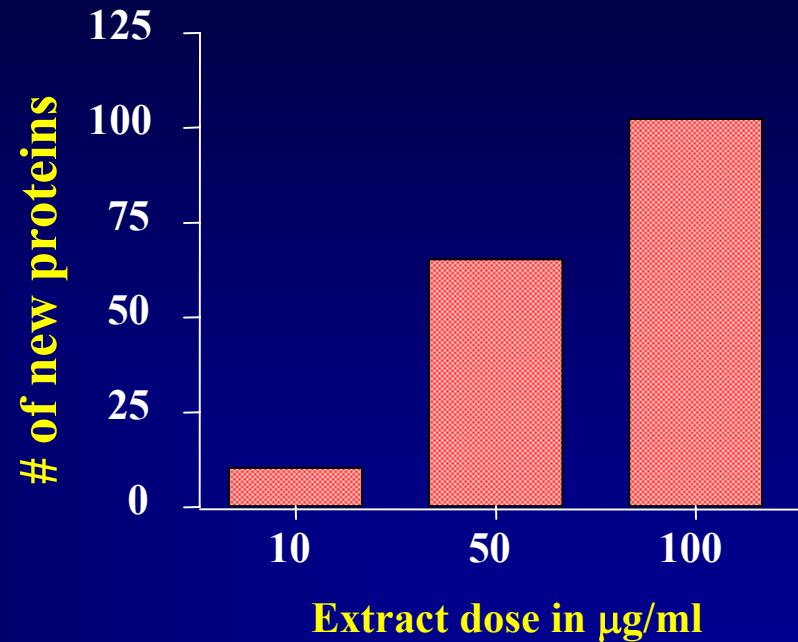
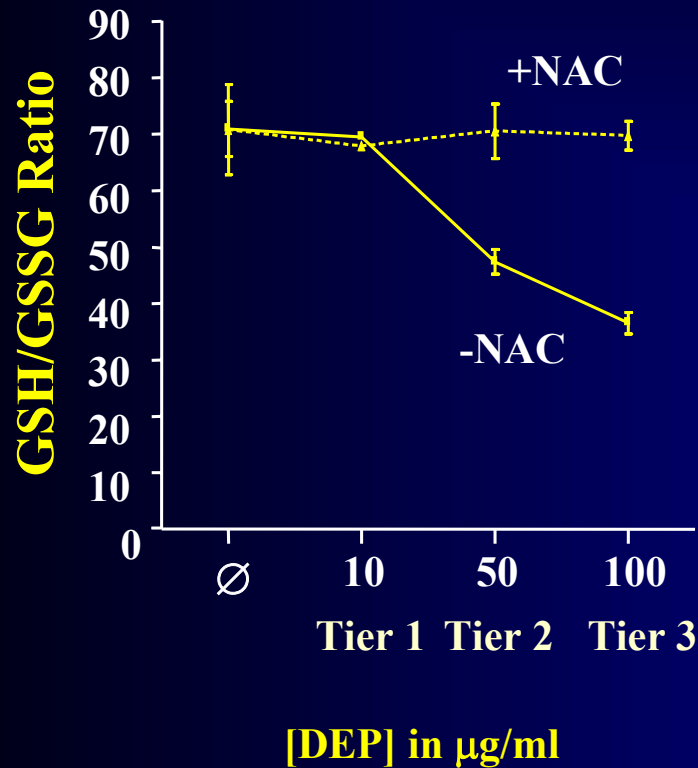
Exploration of the role of Oxidative Stress through the use of Proteomics



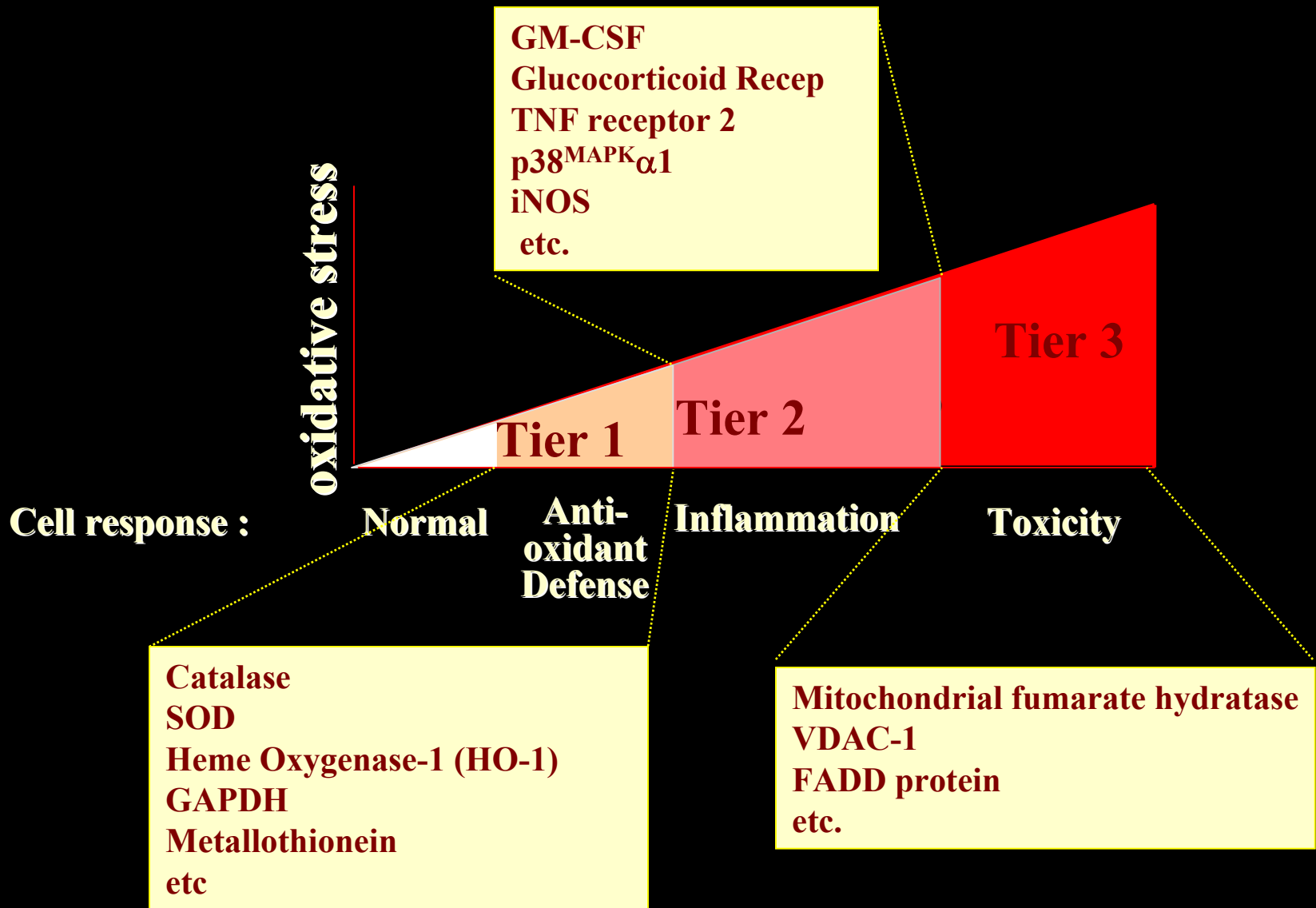
Oxidative stress response ?

Inflammatory markers ?

Dose-dependant induction of new Oxidative Stress Proteins by DEP



Macrophage/epithelial oxidative stress analysis (in vitro)



Adaptive Genetic Programming

Signal
Sensing

Signal
Transduction

Trans. Factor
Activation

Target Gene
Regulation

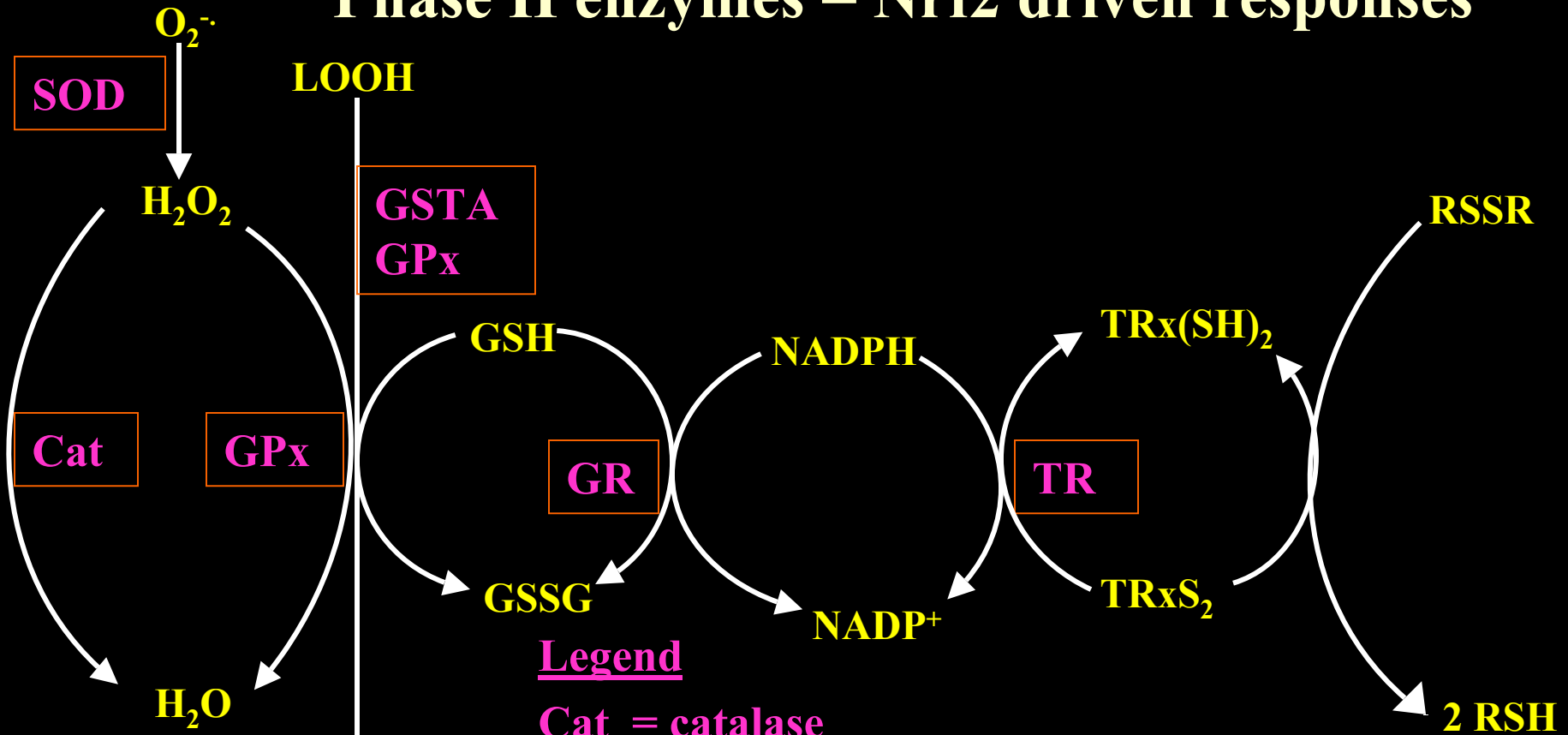
Ox. Stress
Sensors ?

Keap 1

Nrf2

Antioxidant
enzymes
Phase II met
enzymes

Phase II enzymes = Nrf2 driven responses



Legend

Cat = catalase

GPx = glutathione peroxidase

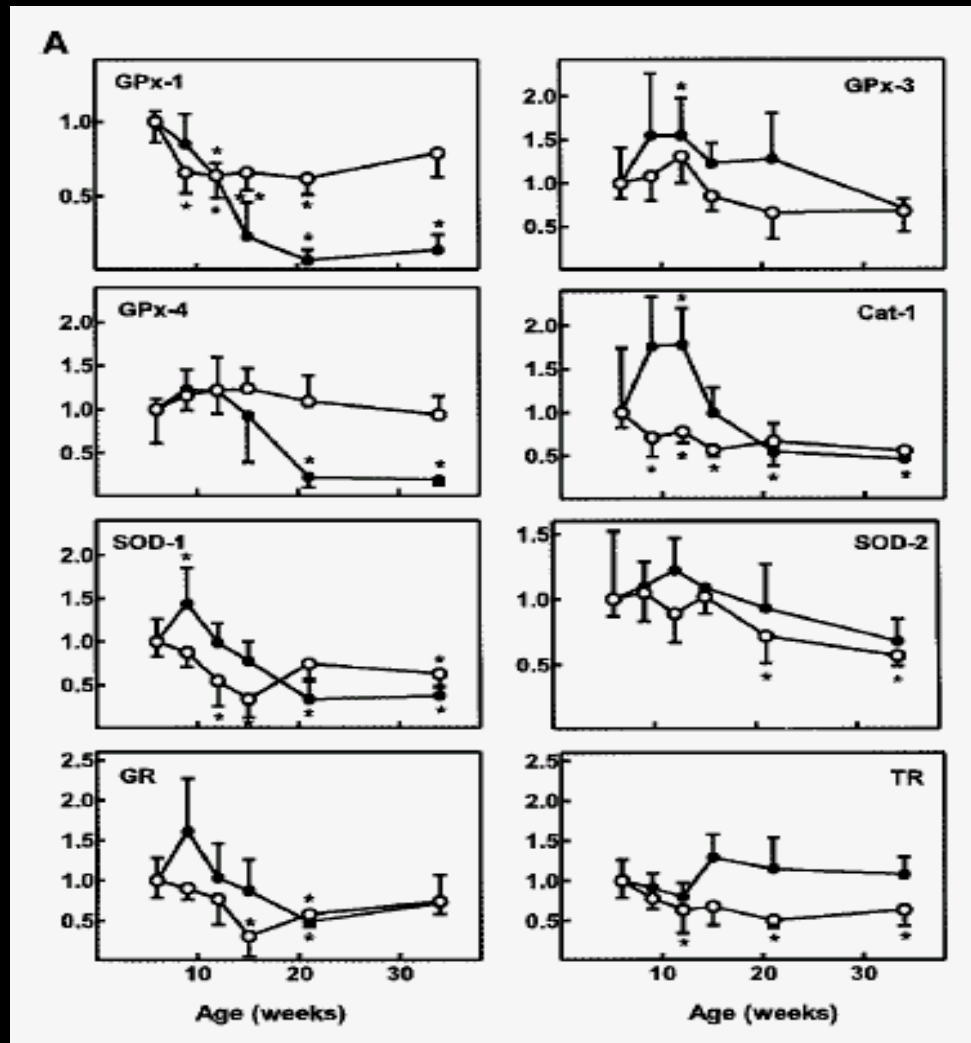
GR = glutathione reductase

SOD = superoxide dismutase

GST = glutathione S transferase

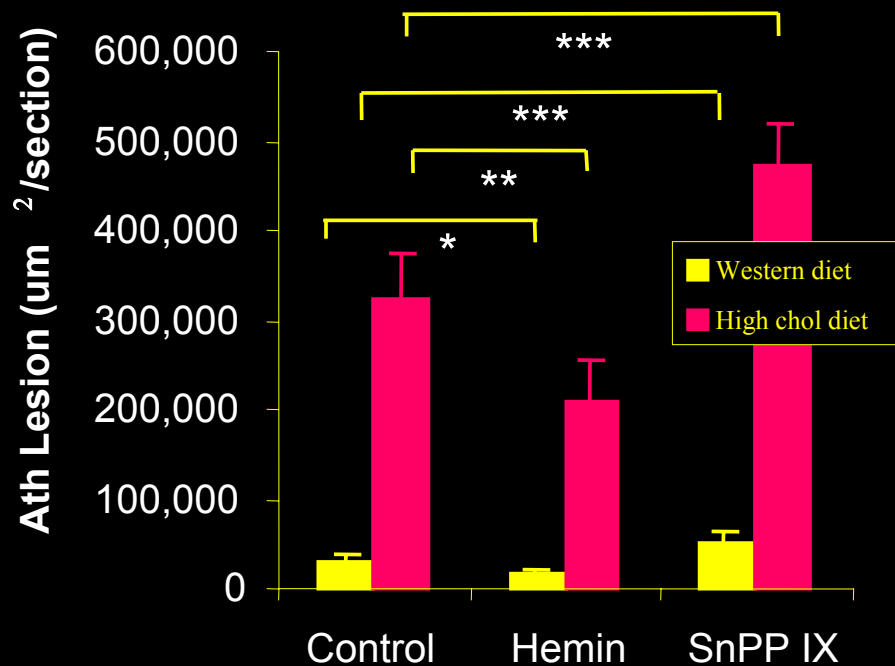
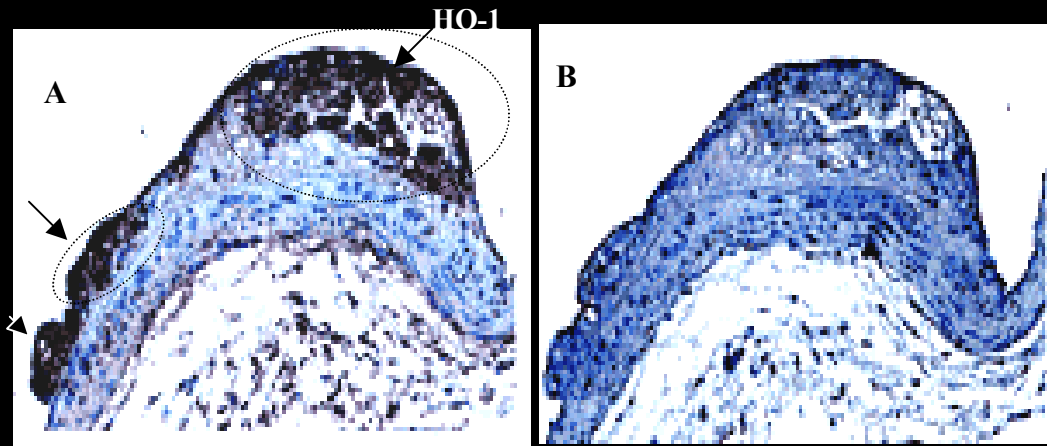
TR = thioredoxin reductase

Aorta of ApoE-Deficient Mice Responds to Atherogenic Stimuli by a Prelesional Increase and Subsequent Decrease in the Expression of Phase II Antioxidant Enzymes



Peter t' Hoen et al
Circ Res. 2003;93:262-269

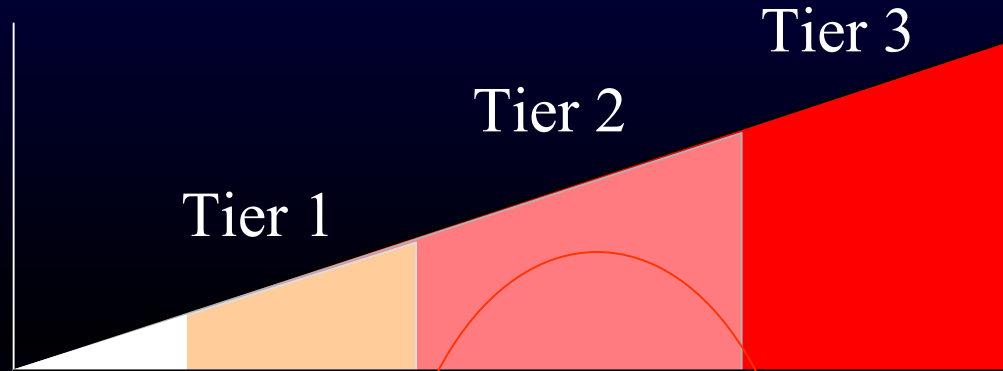
HO-1 expression in atherosclerotic lesions in LDL-R null mice.



Hemin-induced HO-1 expression inhibits while SnPP Rx accelerates atherosclerosis in LDL-R^{-/-} mice

Xiao, et al.

Level of
oxidative stress



Cell response pathway: Normal Anti-oxidant Defense Inflammation Toxicity

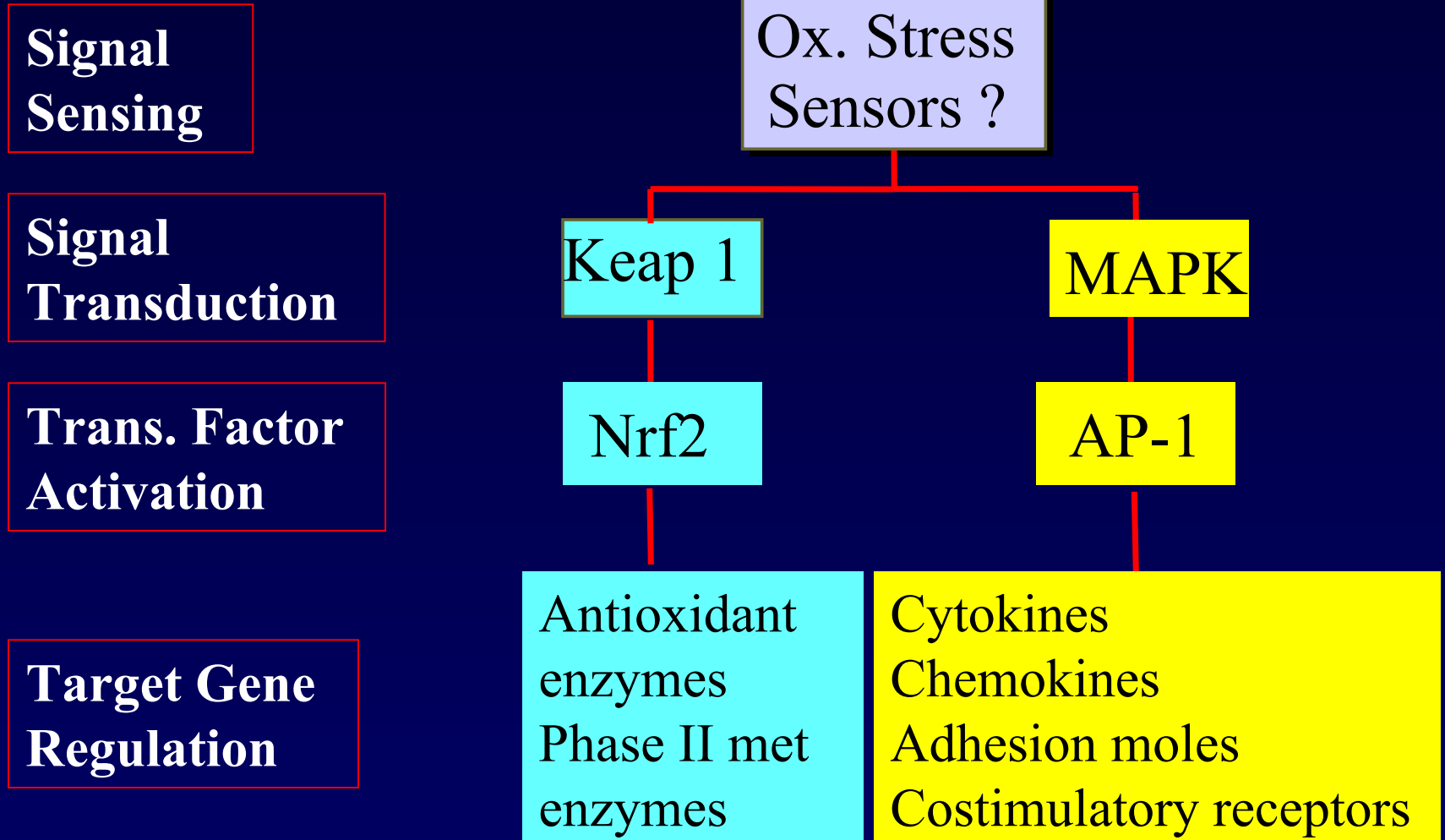
Signaling pathway:

MAP
Kinases

Genetic response:

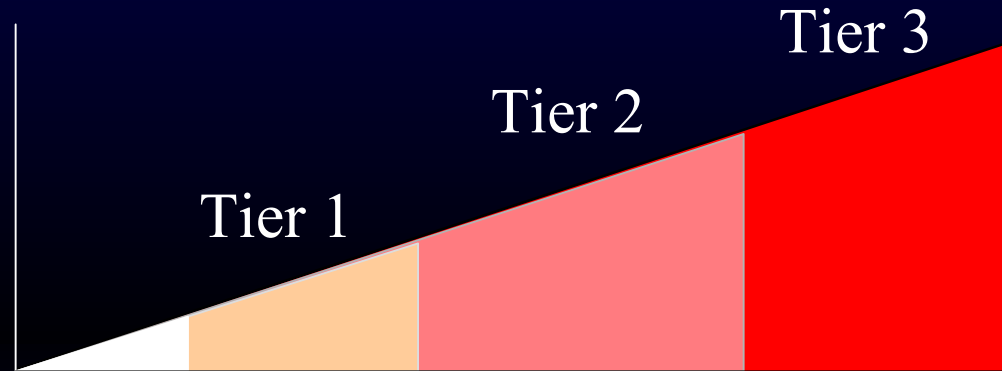
AP-1

Adaptive Genetic Programming



Xiao, et al.

Level of
oxidative stress



Cell response pathway: Normal

Anti-oxidant
Defense

Inflammation

Toxicity

Signaling pathway: —

Nrf-2

MAPK

Mitochondrial
perturbation

Genetic response: —

ARE

AP-1

PT pore

Ultrafines lodge in and destroy mitochondria

Mag. x 6000

Mag. x 21000

UFP

