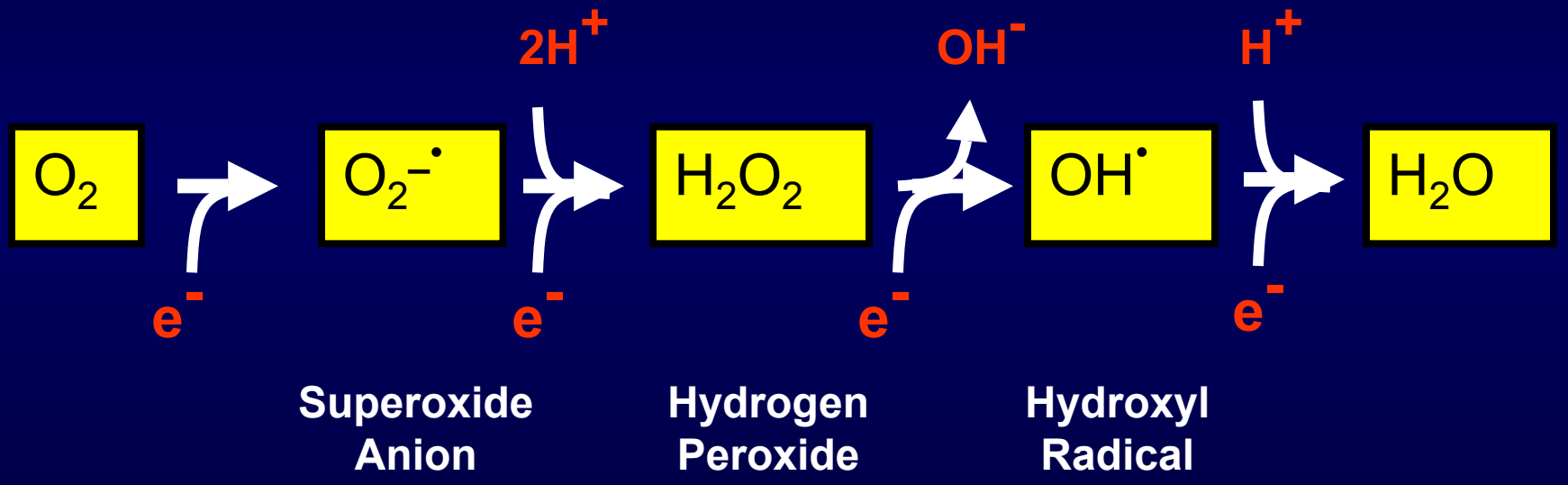
Three Tiers of Oxidative Stress in Response to Particulate Air Pollutants

Andre Nel MD/ PhD

Professor of Medicine at UCLA

Reactive Oxygen Species (ROS)



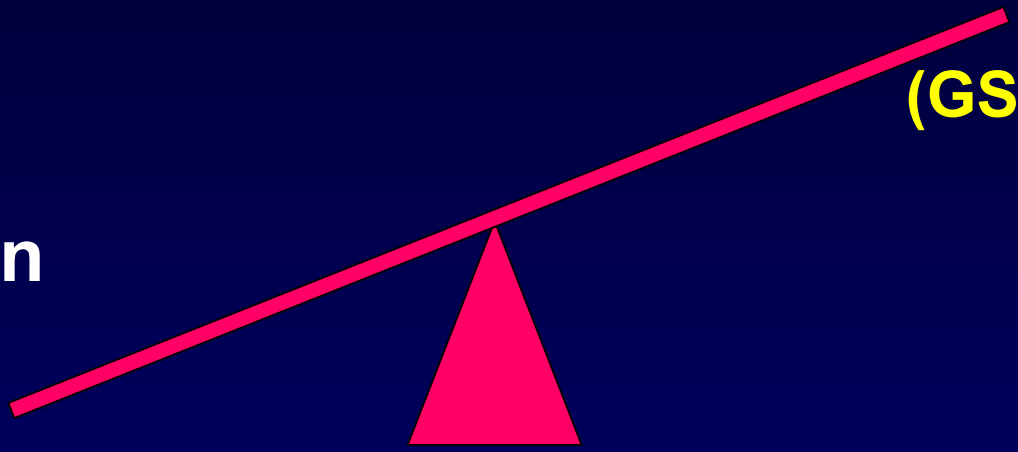
Normal

ROS Production

(GSH hi)

ROS Inactivation

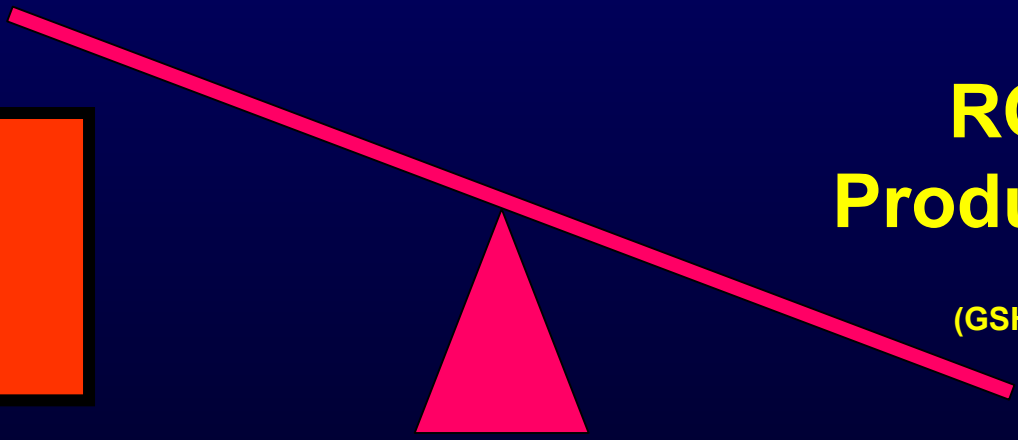
(GSSG lo)



ROS Inactivation

(GSSG hi)

Oxidative Stress



ROS Production

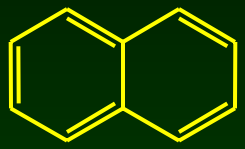
(GSH lo)

Oxidative Stress Hypothesis

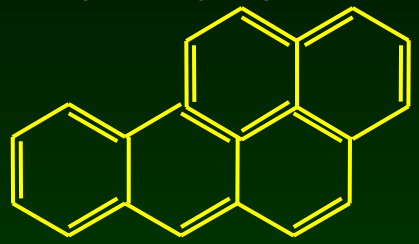
1. PM contains pro-oxidative chemicals
2. PM chemicals generate ROS $\rightarrow$ Oxidative stress
3. Oxidative stress $\rightarrow$ inflammation

Important Organic Chemicals

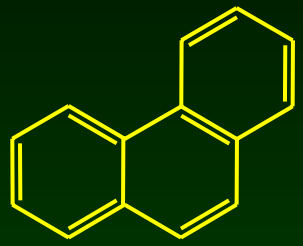
PAHs = Aromatic fraction



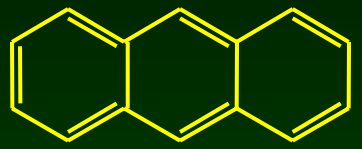
Naphthalene



Benzo(a)pyrene

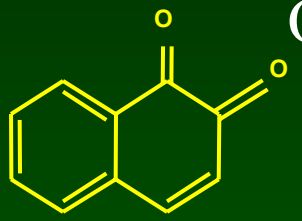


Phenanthrene

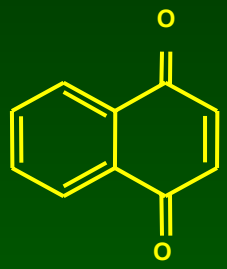


Anthracene

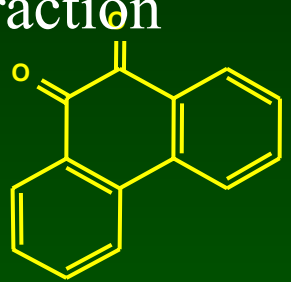
Quinones = Polar fraction



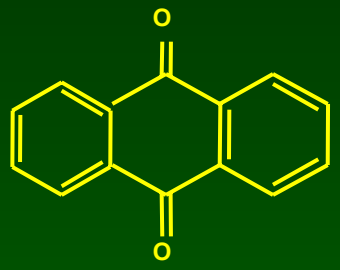
1,2-Napthaquinone
(1,2 NQ)



1,4-Naphthaquinone
(1,4-NQ)

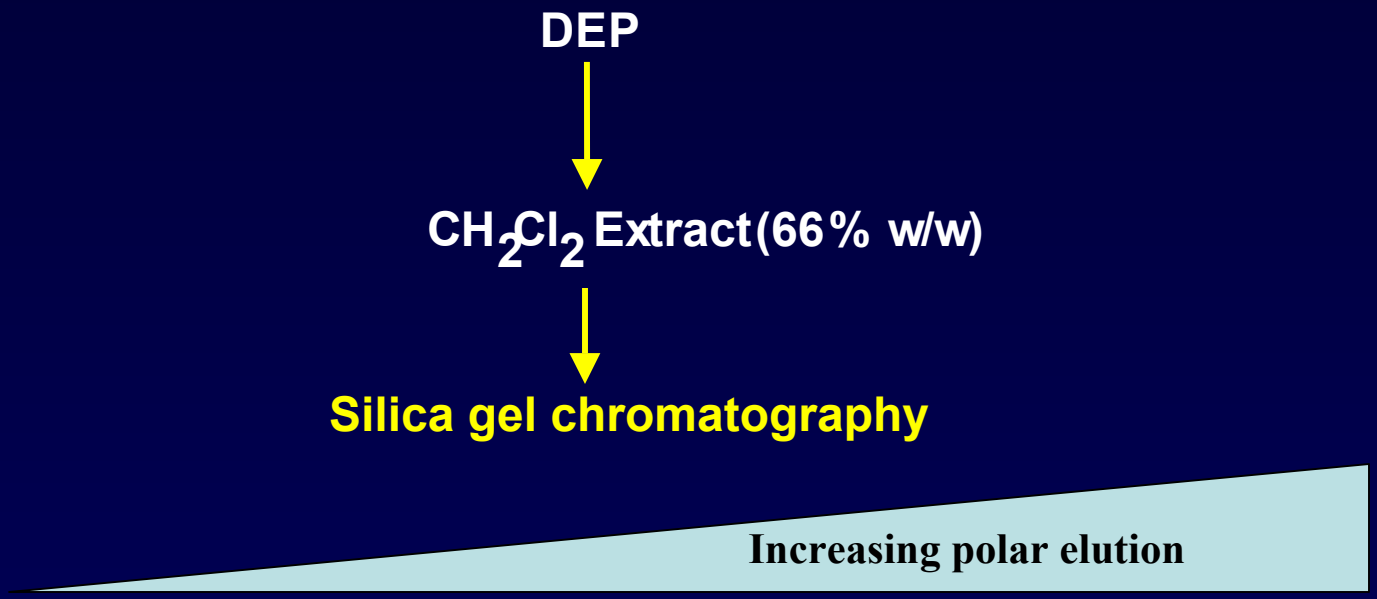


9,10-Phenanthraquinone
(9,10-PQ)



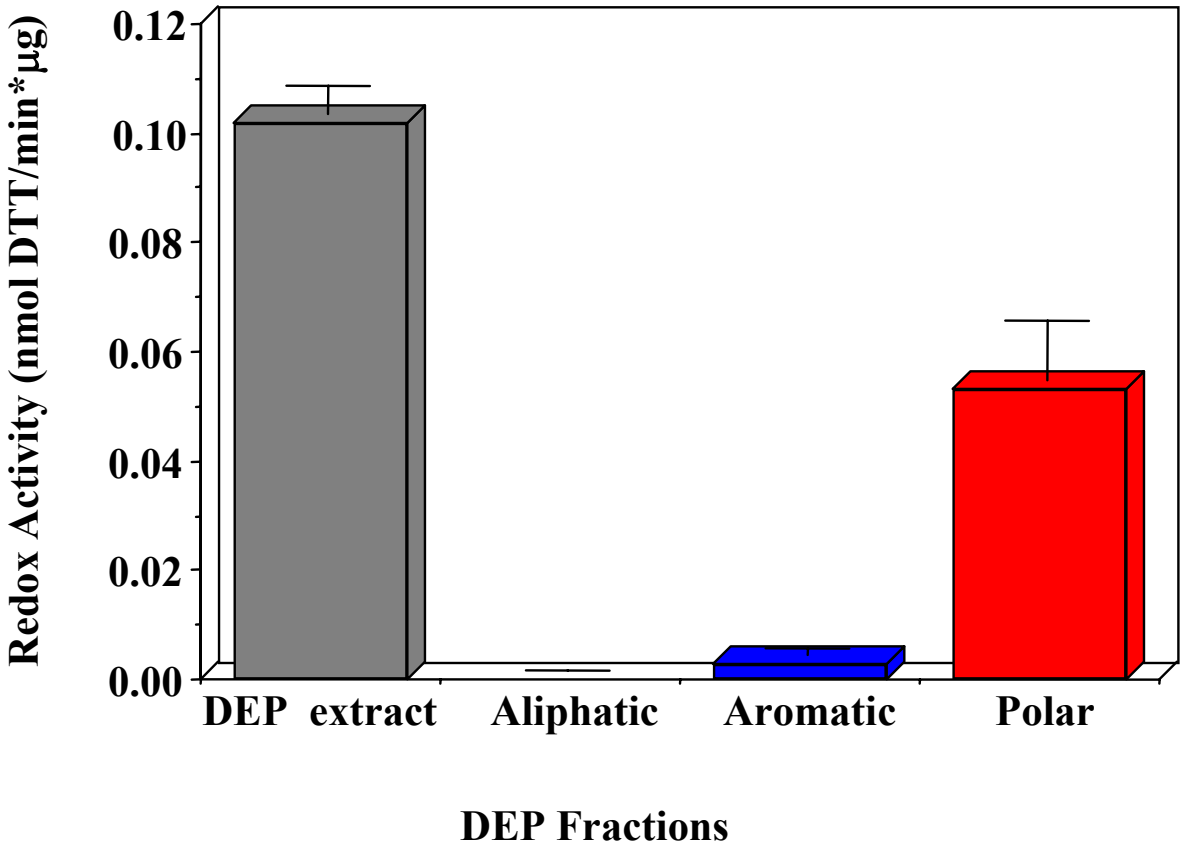
9,10-Anthraquinone
(9,10-AQ)

Organic chemical fractionation of DEP

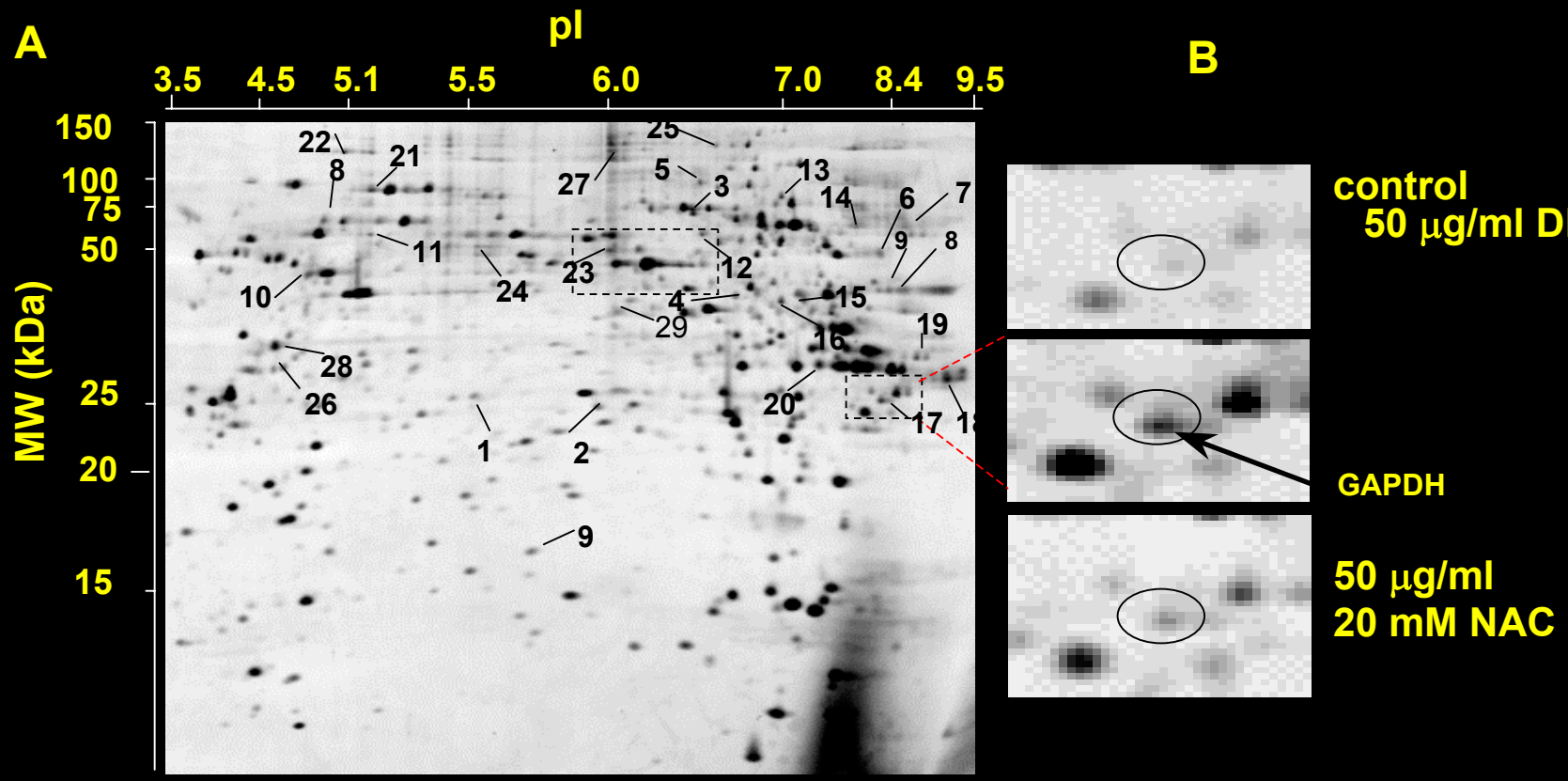


Li et al. J Immunol. 165: 3393-3401; 2000.

Differential Redox Cycling ability of individual DEP chemical fractions



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

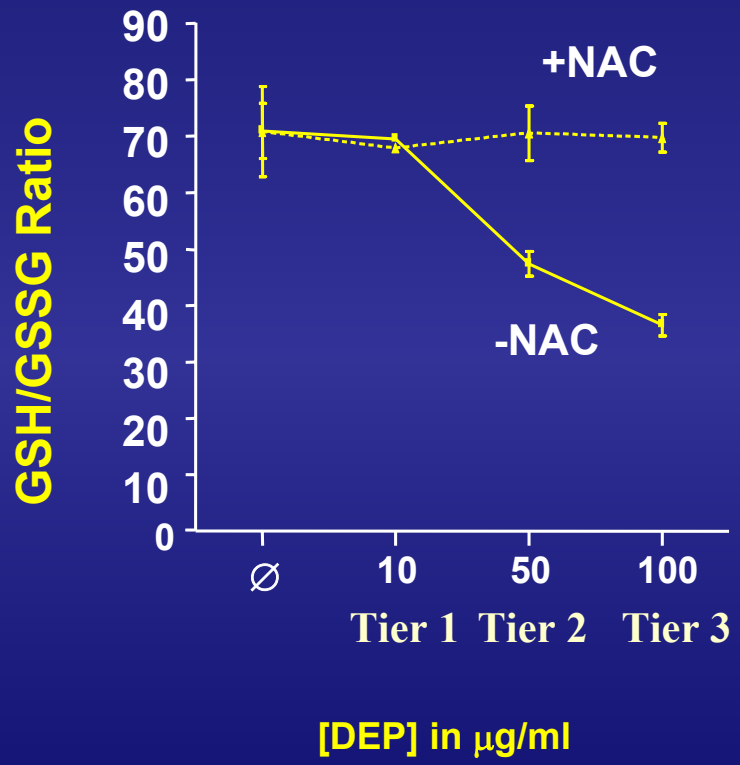


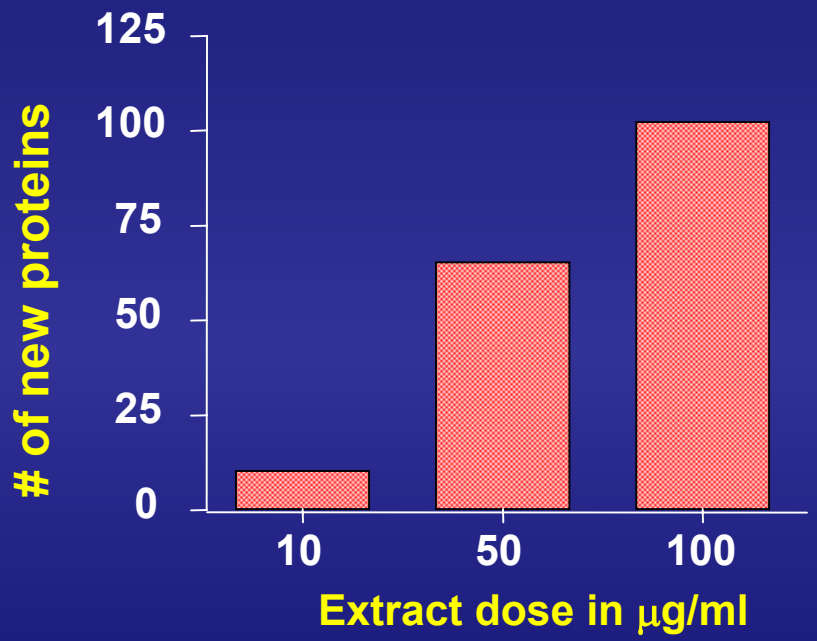
Oxidative stress response ?

Inflammatory markers ?

Nel, Xiao, Loo, et al. JBC. 2003; 278:50781

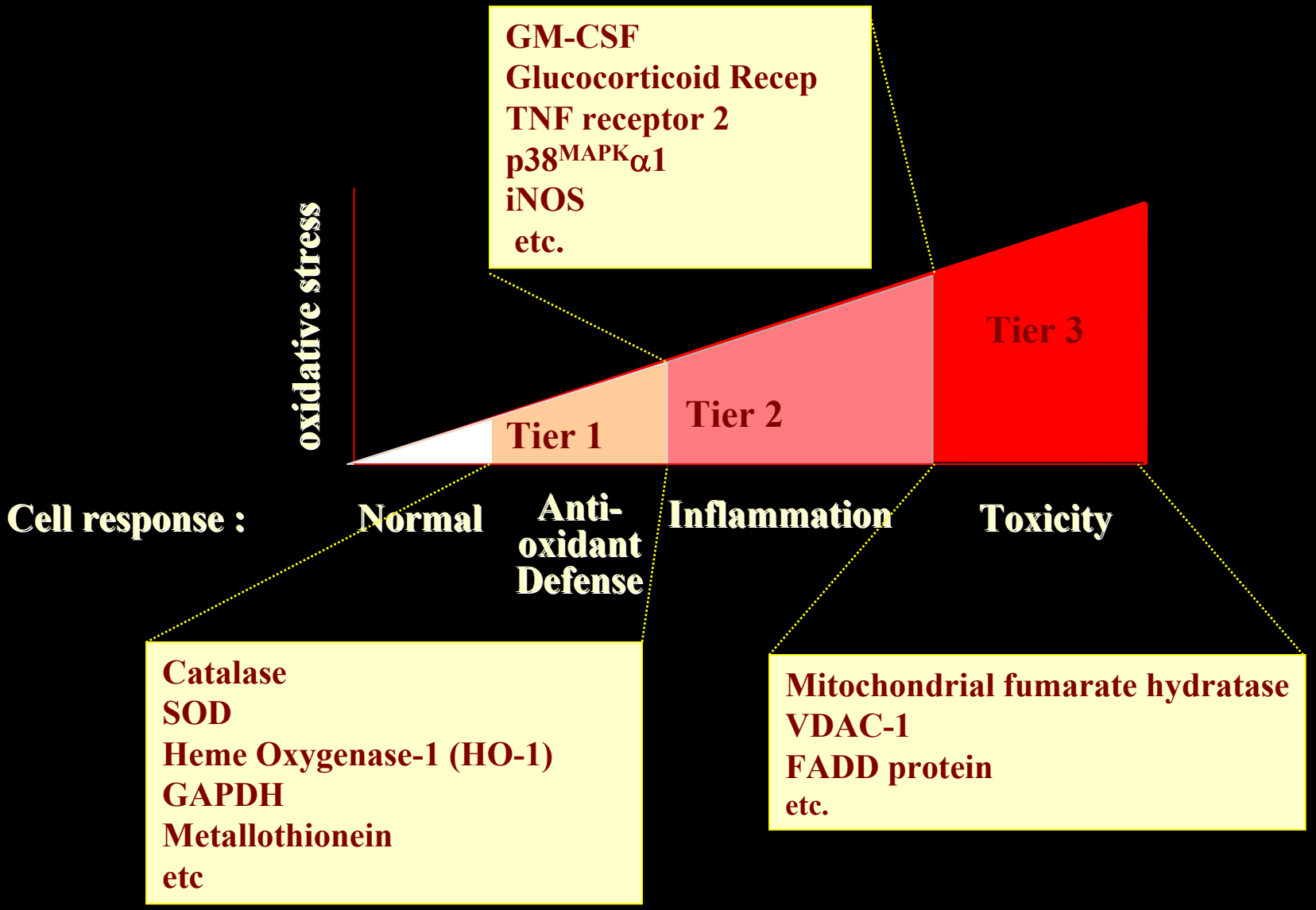
Dose-dependant induction of new Oxidative Stress Proteins by DEP



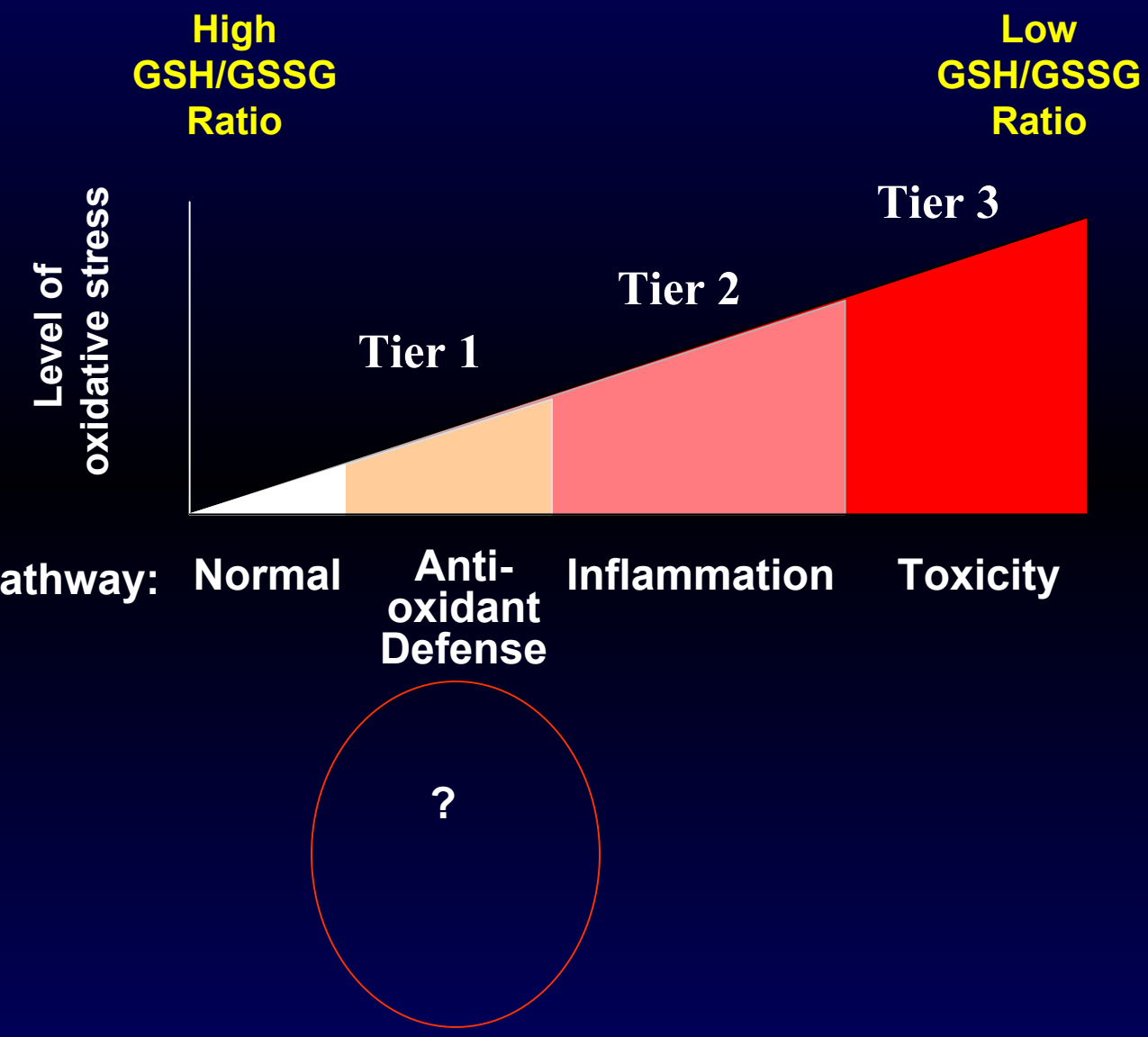


Xiao, Loo, Nel et al. JBC. 2003; 278:50781

Macrophage/epithelial oxidative stress analysis (in vitro)



Xiao, et al.


High GSH/GSSG Ratio
Low GSH/GSSG Ratio
Level of oxidative stress
Tier 1
Tier 2
Tier 3
Cell response pathway:
Normal
Anti-oxidant Defense
Inflammation
Toxicity
?

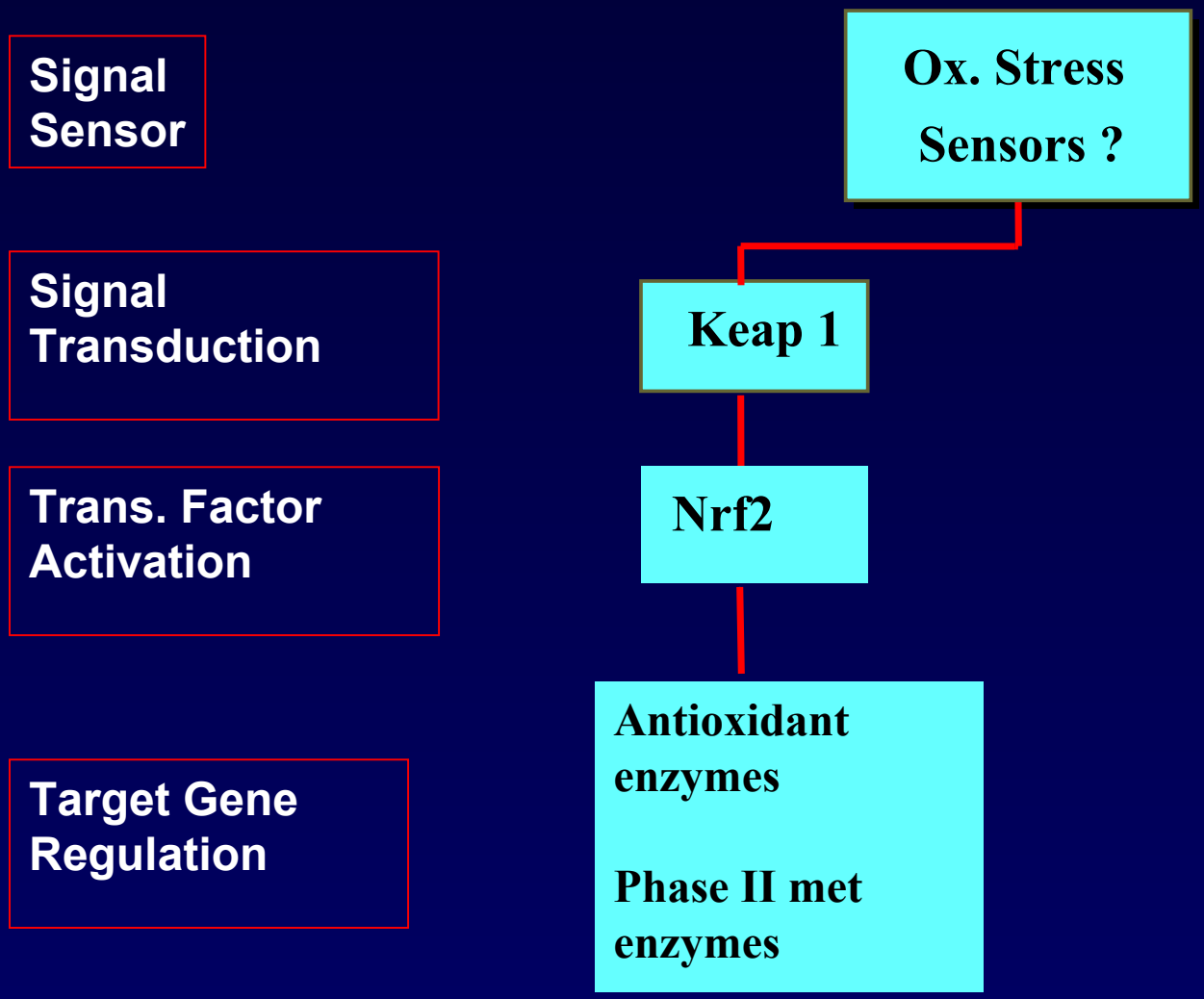
Extrapolation of the Stratified Oxidative Stress hypothesis to Asthma & Atherosclerosis

Prediction: people with defective anti-oxidant defense will more readily develop airway inflammation in response to exposure to particulate pollutants

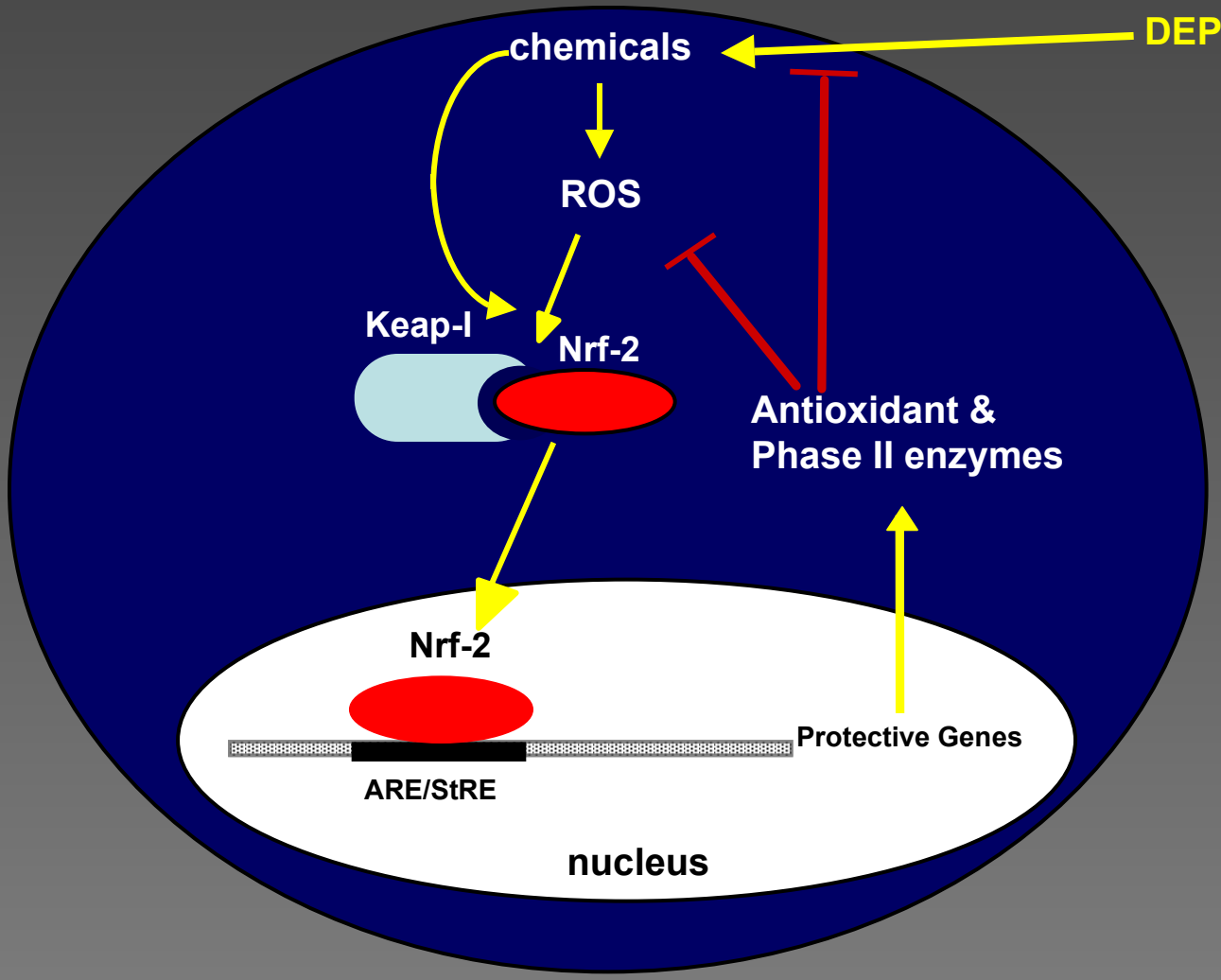
…. and may help to answer

Who is susceptible to PM?

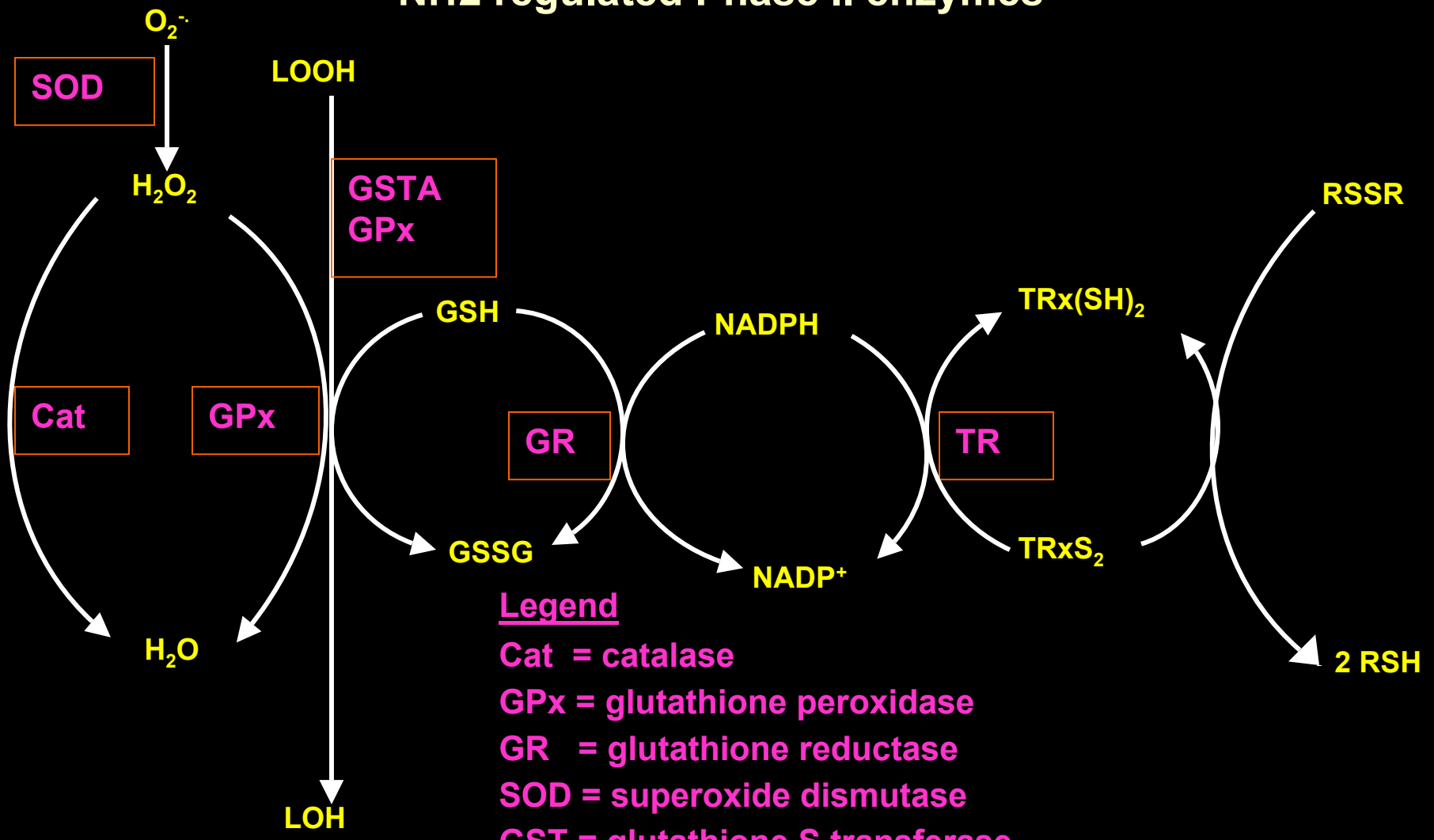
Adaptive Genetic Programming



Role of the Antioxidant Response Element (ARE) and the transcription factor, Nrf-2



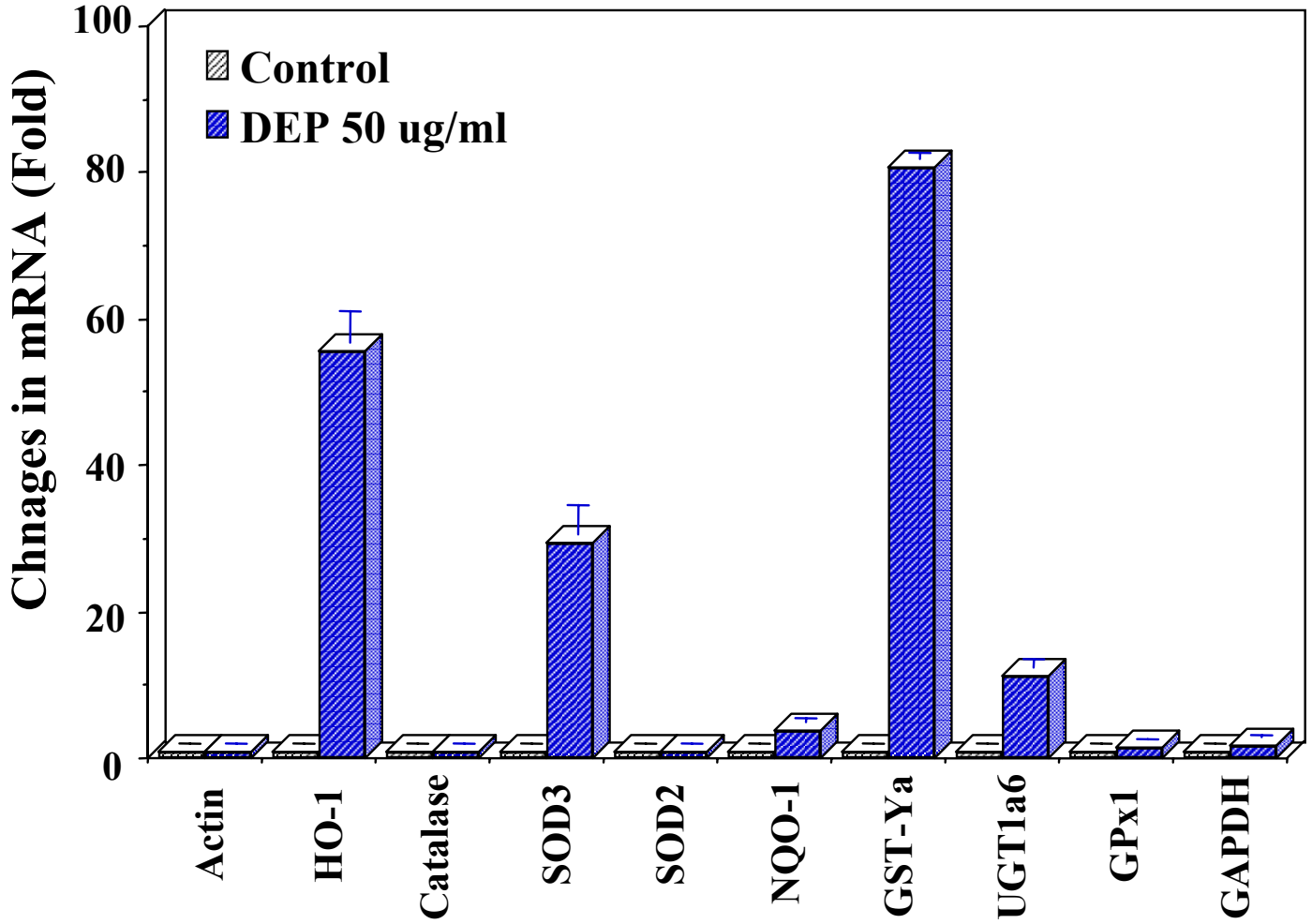
Nrf2 regulated Phase II enzymes



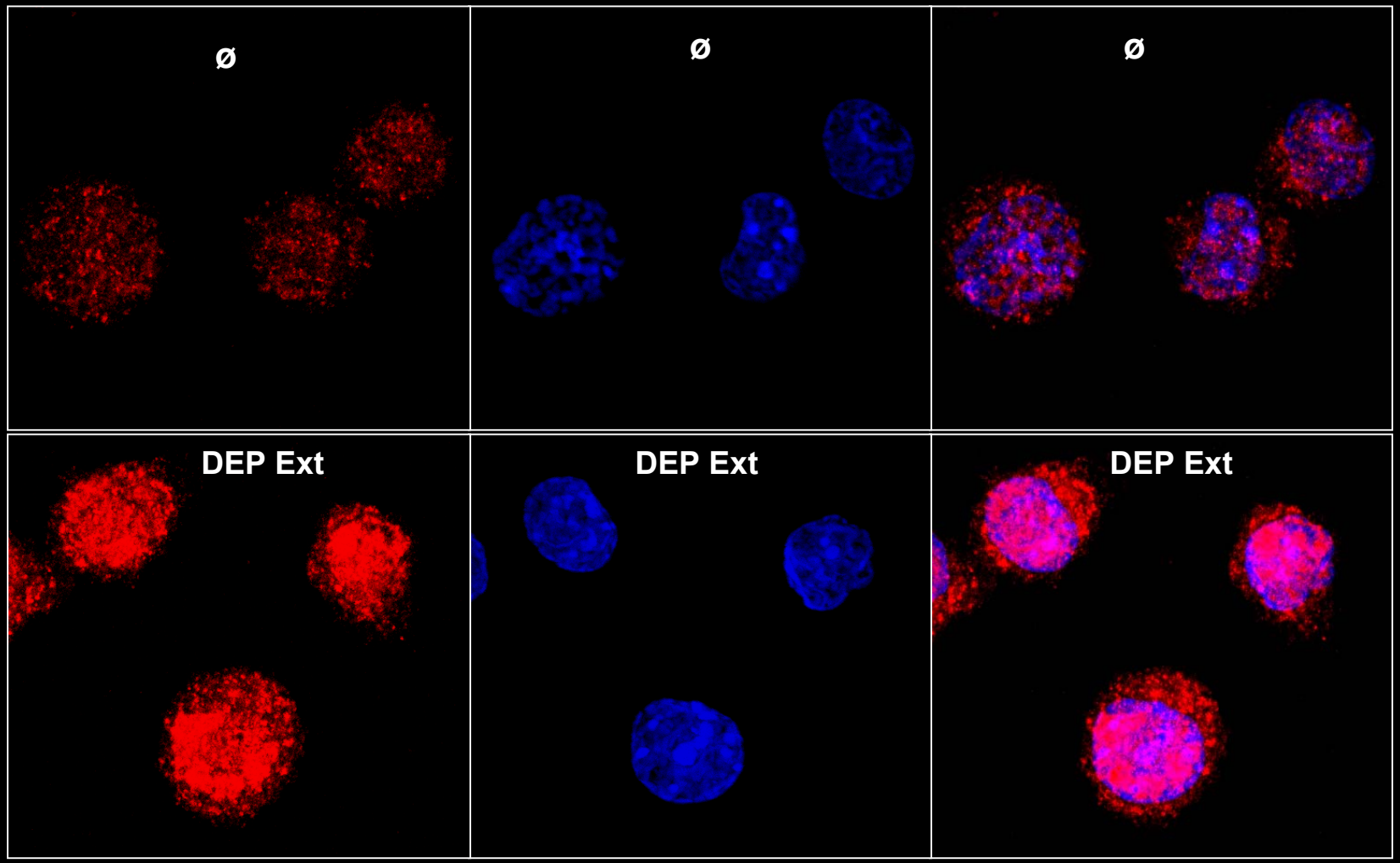
Legend

Cat = catalase
GPx = glutathione peroxidase
GR = glutathione reductase
SOD = superoxide dismutase
GST = glutathione S transferase
TR = thioredoxin reductase

Phase II Enzyme expression by real-time PCR in macrophages

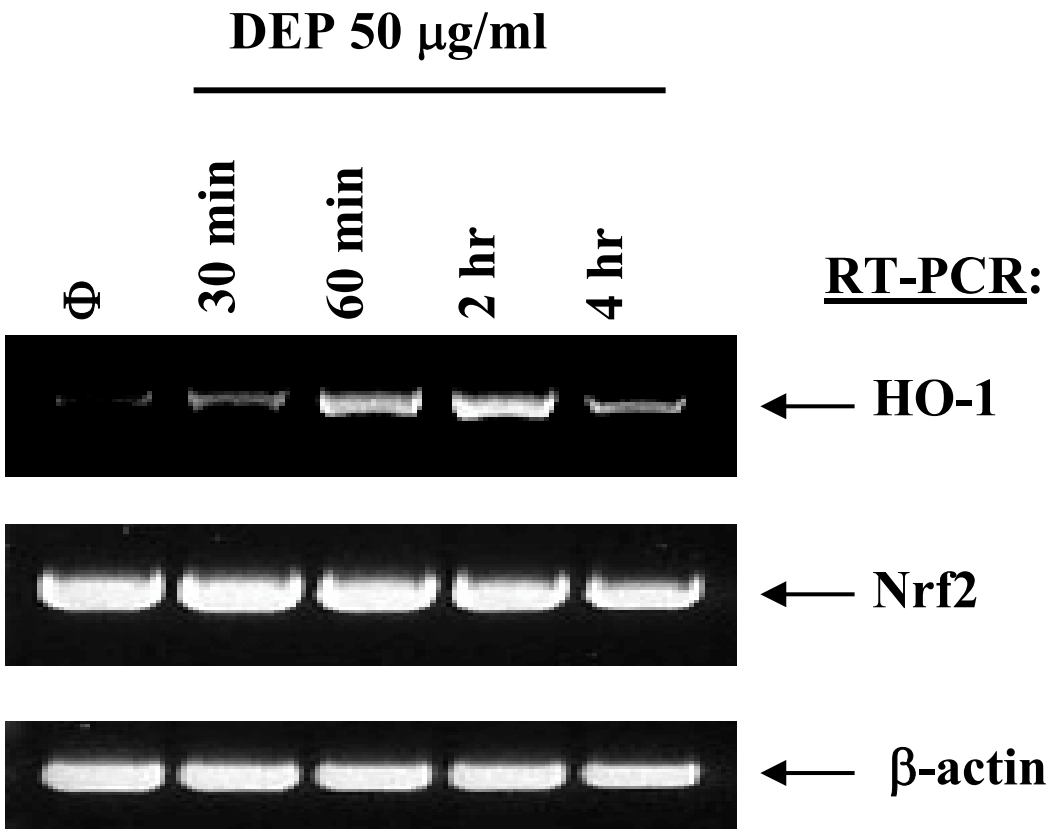


Ning Li et al. J Immunol. 2004. 173: 3467-3481.



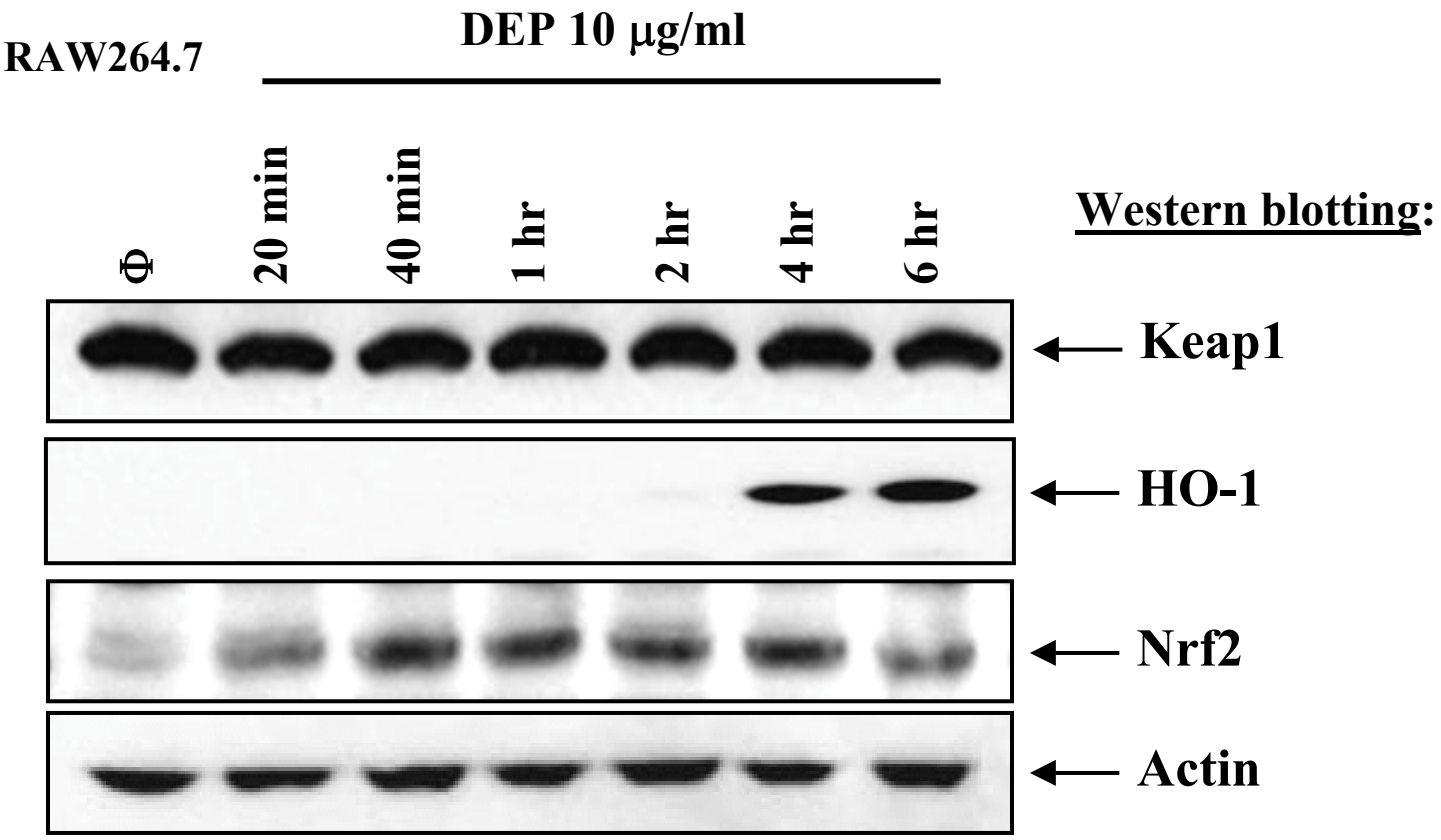
Ning Li et al. J Immunol. 2004. 173: 3467-3481.

Nrf2 message is not increased during DEP treatment



Ning Li et al. J Immunol. 2004. 173: 3467-3481.

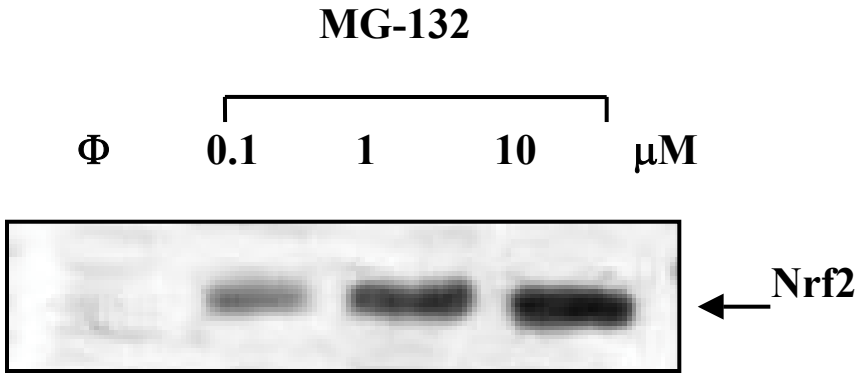
… but Nrf2 protein levels are increased by DEP treatment



…suggesting post-transcriptional regulation

Ning Li et al. J Immunol. 2004. 173: 3467-3481.

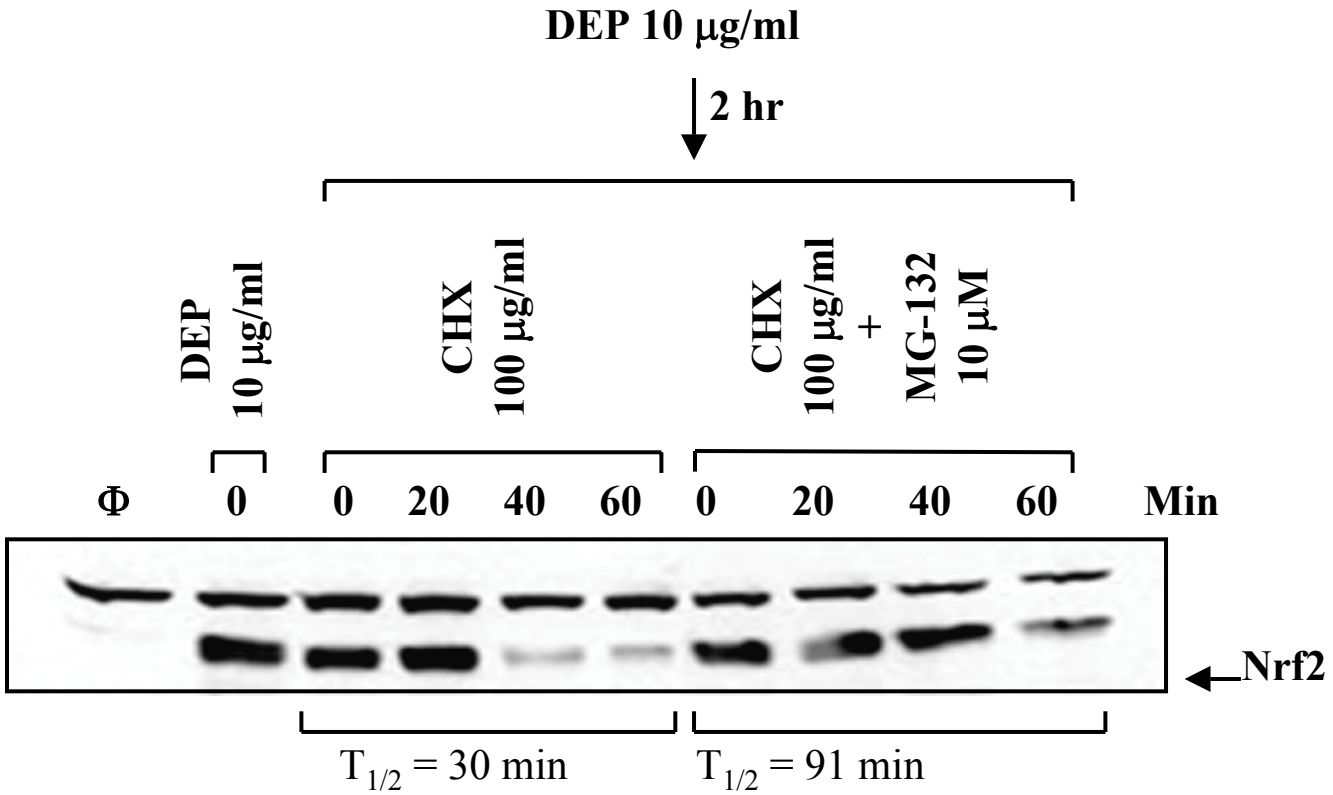
Nrf2 protein levels are increased by MG-132 - an inhibitor of the 26S proteosome



…demonstrating that Nrf2 levels are regulated by proteosomal degradation

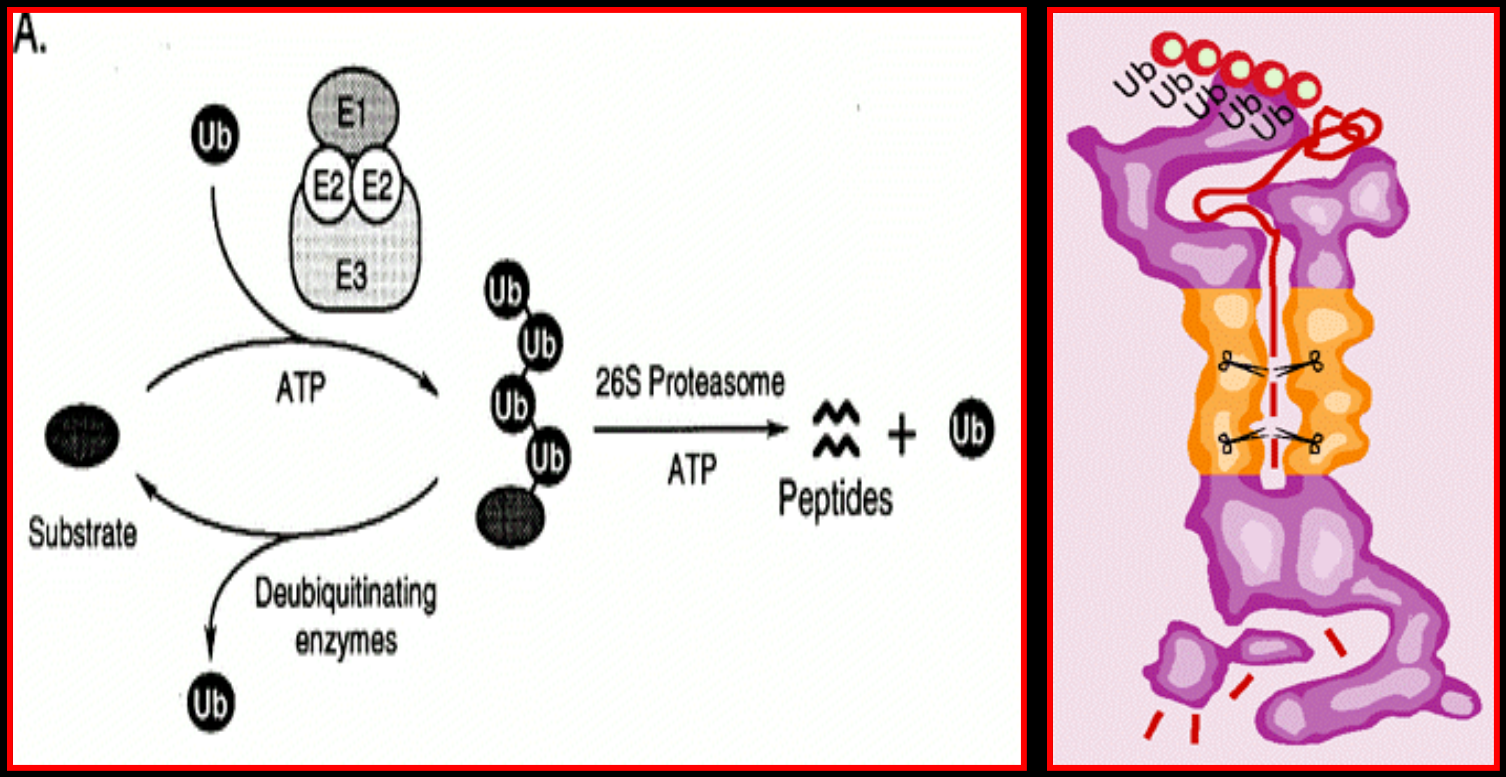
Ning Li et al. J Immunol. 2004. 173: 3467-3481.

Nrf2 protein accumulation is regulated through an effect on protein stability (half life)



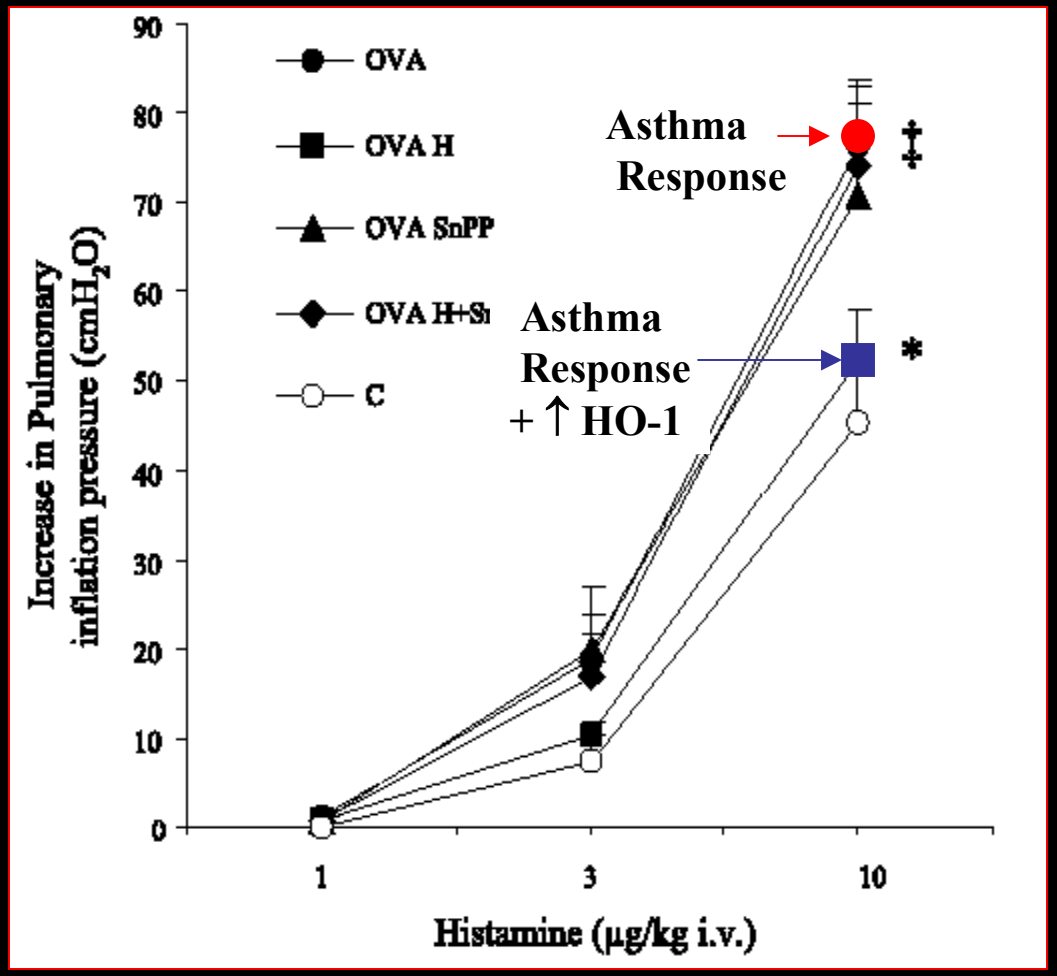
Ning Li et al. J Immunol. 2004. 173: 3467-3481.

Proteosomal degradation plays an important role in determining protein half-life



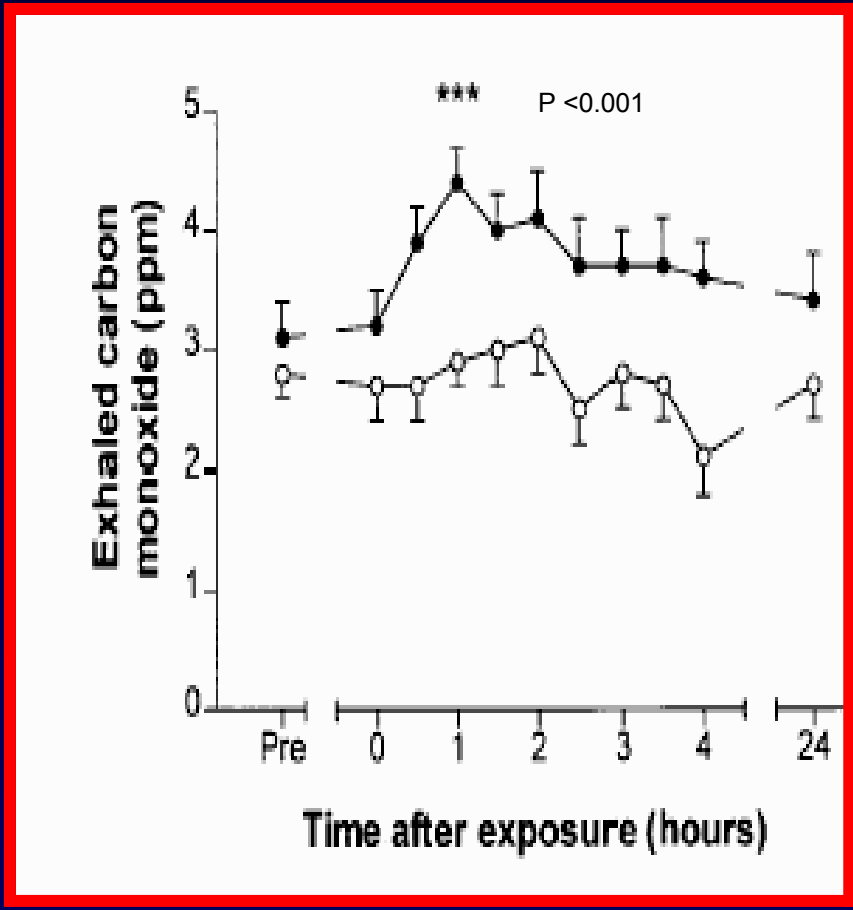
…and may be an important target for oxidative stress events including Nrf2, catalase, SOD...

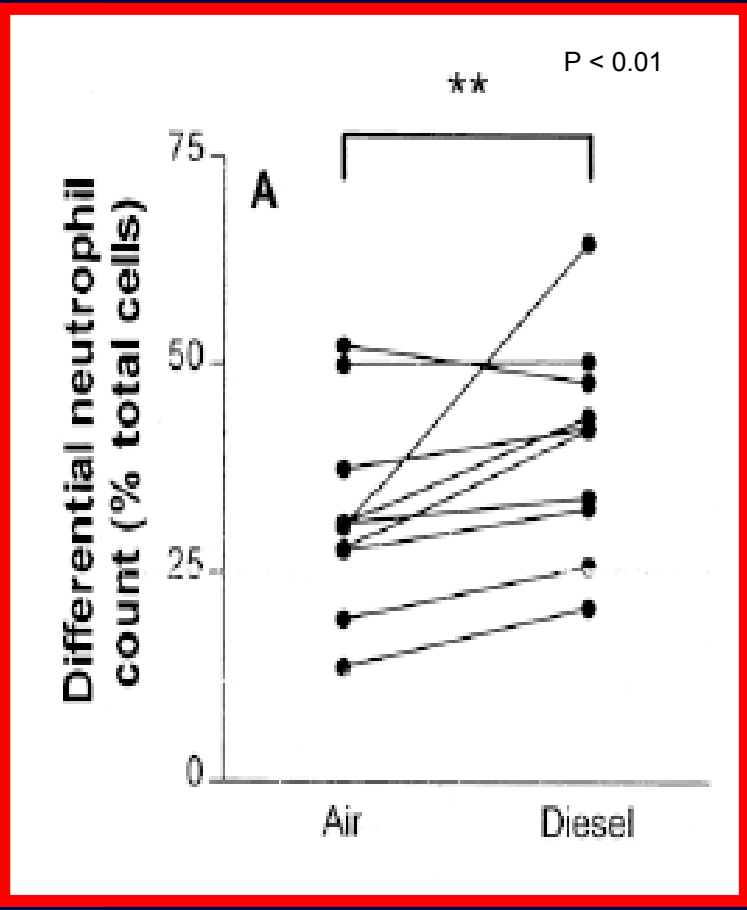
Heme Oxygenase attenuates Allergen-induced Airway Inflammation and Hyperreactivity in Guinea Pigs



Almolki et al. Am J Physiol Lung Cell Mol Physiol. 2004; 287(1):L26-34.

CO exhalation is a sensitive clinical marker for the effects of DEP on the HO-1 system in vivo





NIGHTINGALE et al .Am. J. Respir. Crit. Care Med., Volume 162, Number 1, July 2000, 161-166

Altered Phase II Antioxidant Defense in Asthma

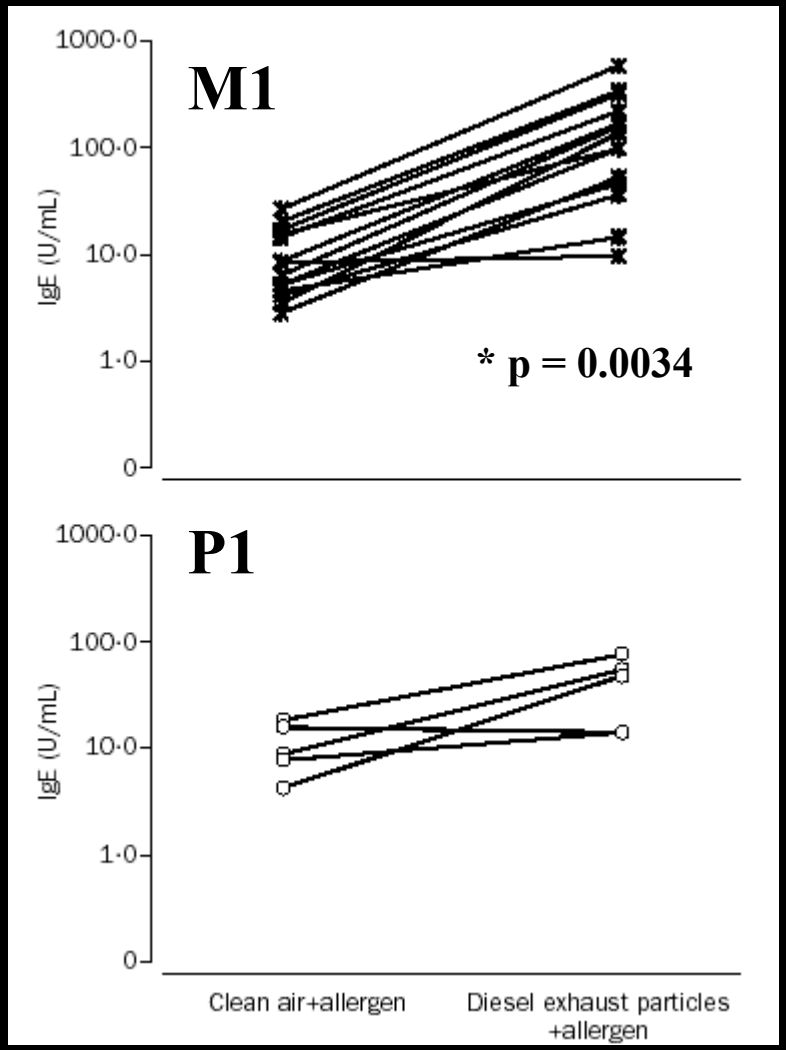
Association of GST genotypes/polymorphisms with asthma, including occupational asthma

a. GST-M1 null genotype: Asthma risk ↑ 3.5 fold

b. GST-P1 (Val^{105}/Val^{105}) 6-fold lower risk of asthma vs Ile^{105}/Ile^{105} genotype

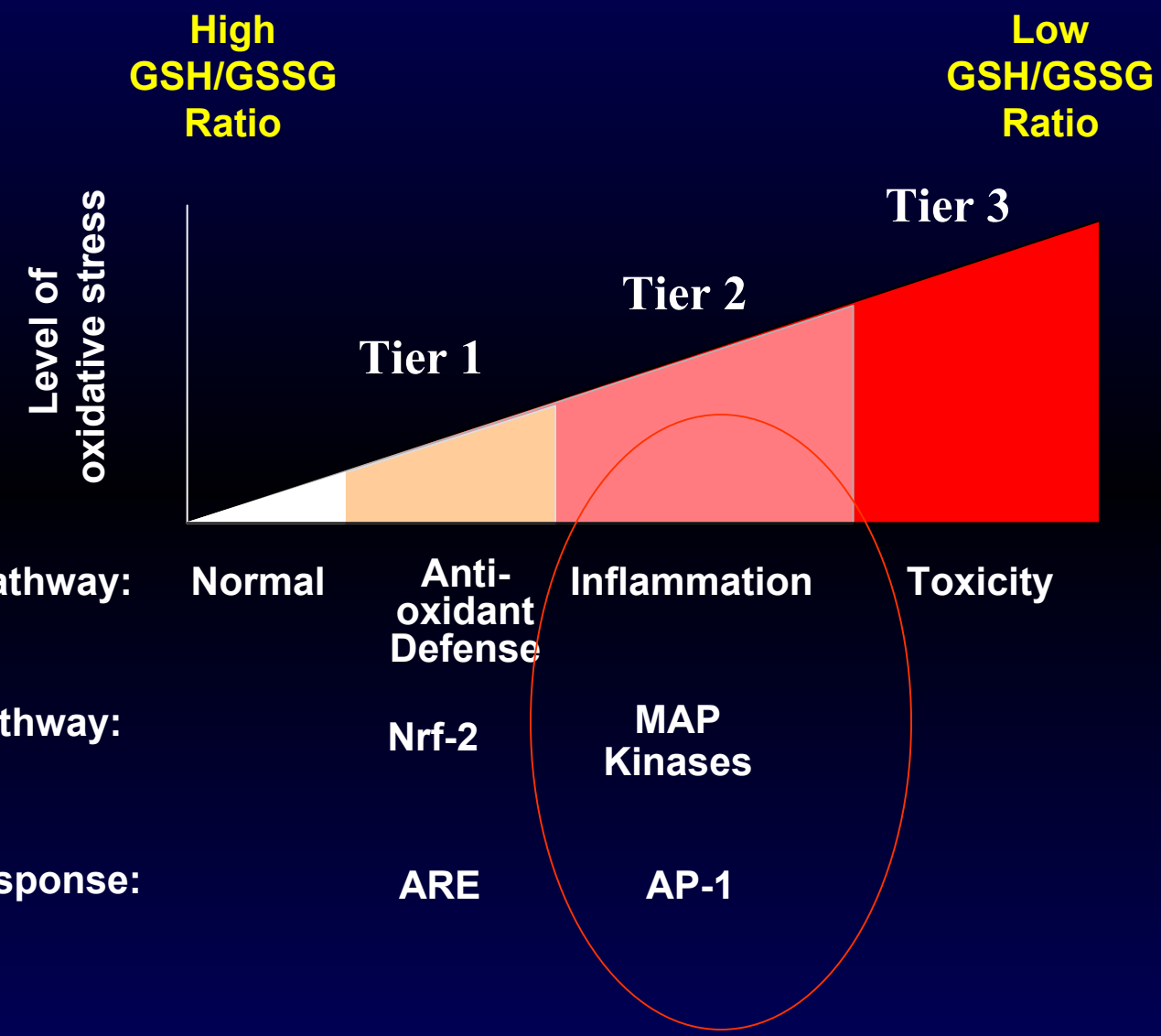
c. GST-P1 (Val^{105}/Val^{105}) protects vs TDI-induced asthma

Effect of GST M1 and P1 genotypes on xenobiotic enhancement of allergic responses

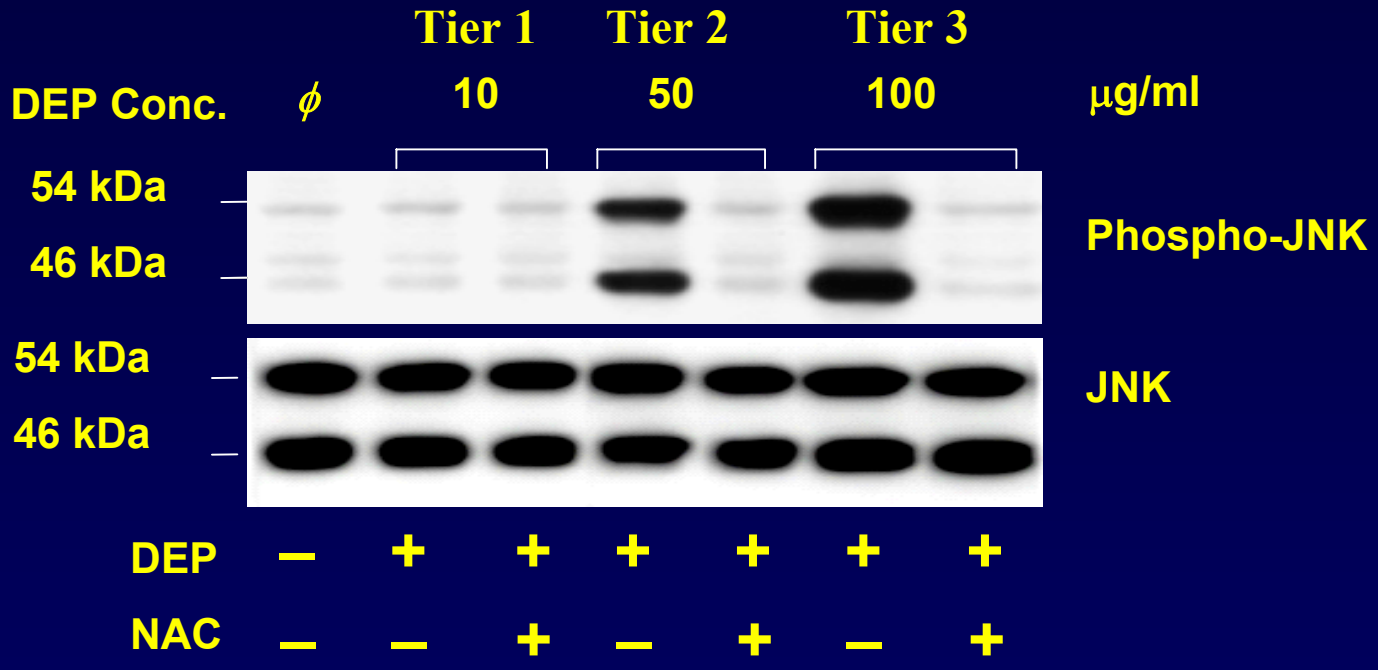


Gilliland et al. Lancet, 363:119; 2004

Xiao, et al.


High GSH/GSSG Ratio
Low GSH/GSSG Ratio
Level of oxidative stress
Tier 1
Tier 2
Tier 3
Cell response pathway:
Normal
Anti-oxidant Defense
Inflammation
Toxicity
Signaling pathway:
Nrf-2
MAP Kinases
Genetic response:
ARE
AP-1

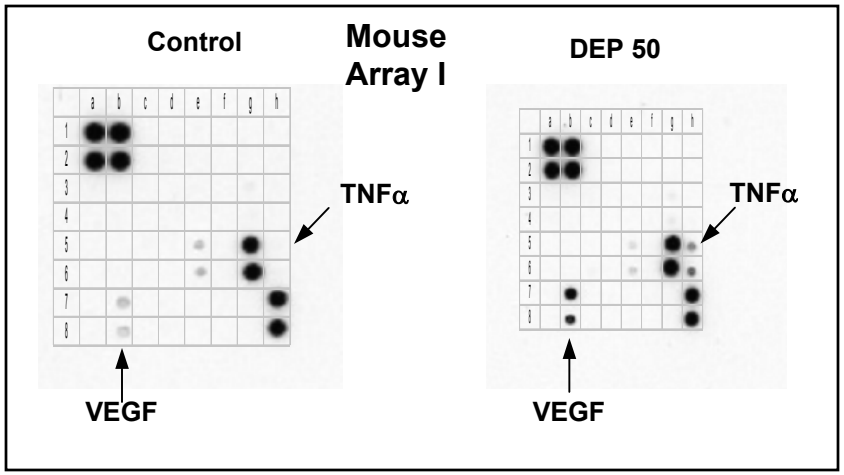
MAP kinase activation at intermediary dose range

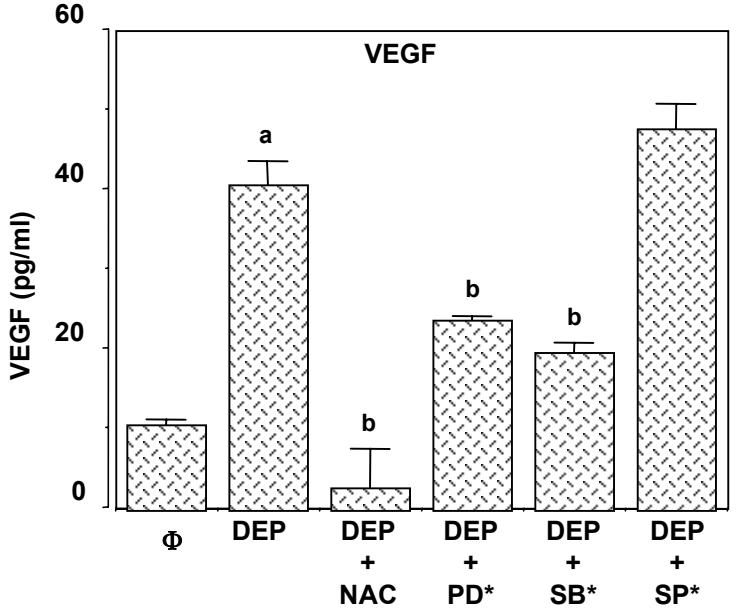


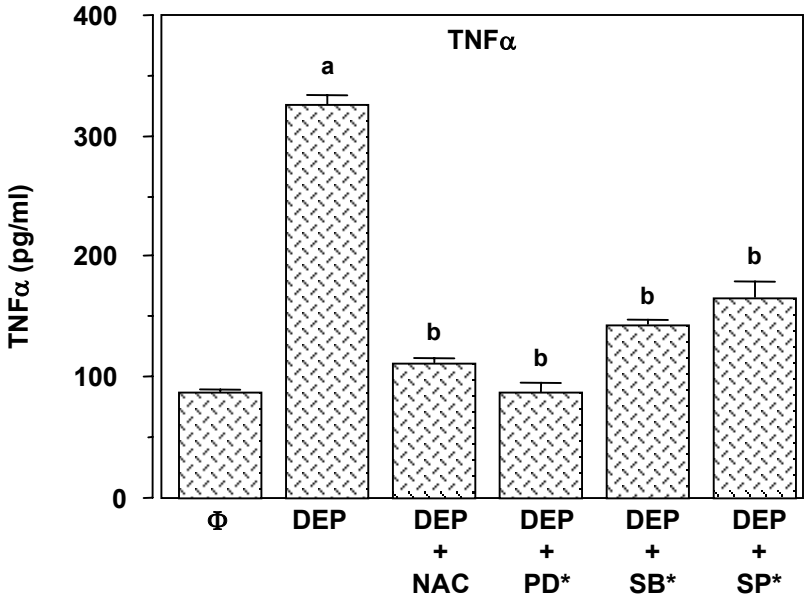
M Wang et al. Submitted

Cytokine Array Analysis and ELISA to demonstrate Pro-inflammatory Effects of DEP

RAW 264.7



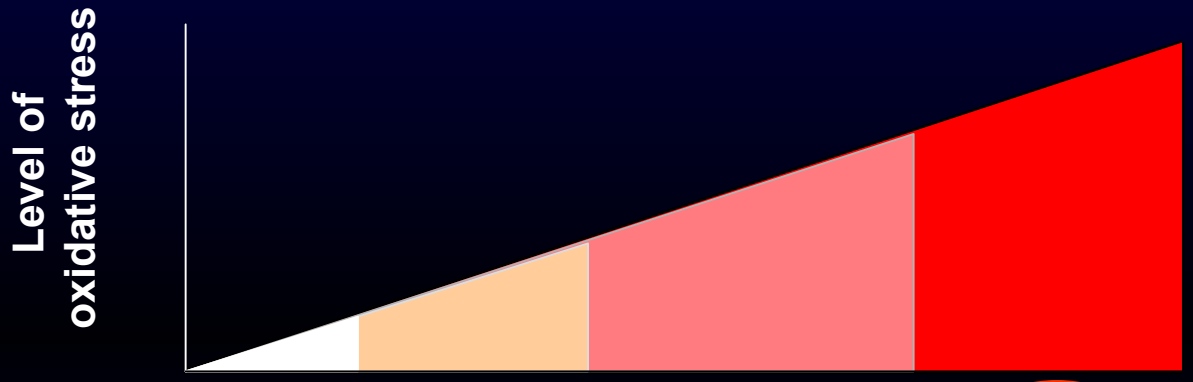




Wang et al

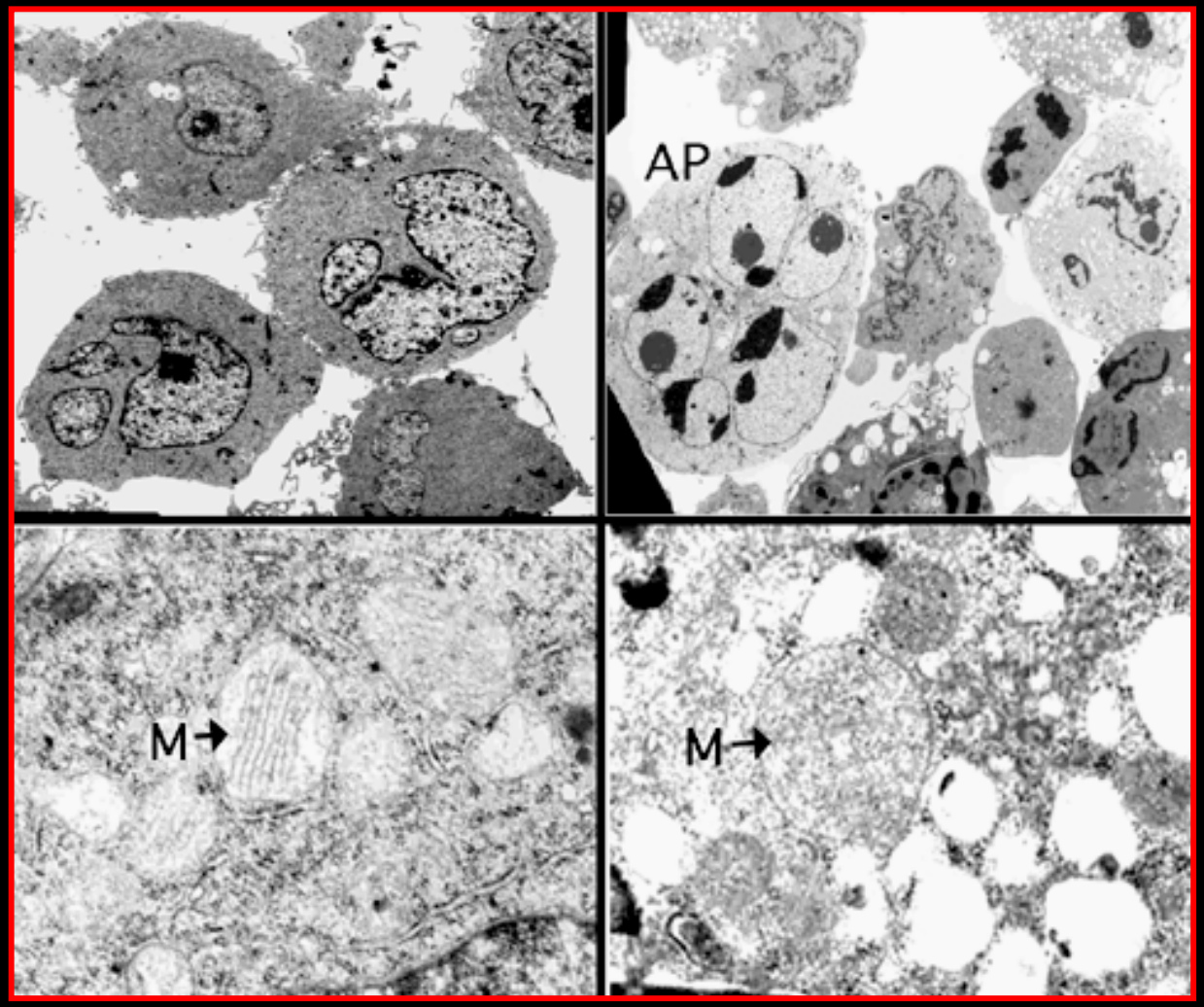
M Wang et al. Submitted

Xiao, et al.



Cell response pathway:	Normal	Anti-oxidant Defense	Inflammation	Toxicity
Signaling pathway:	___	Nrf-2	MAPK	Mitochondrial perturbation
Genetic response:	___	ARE	AP-1	PT pore

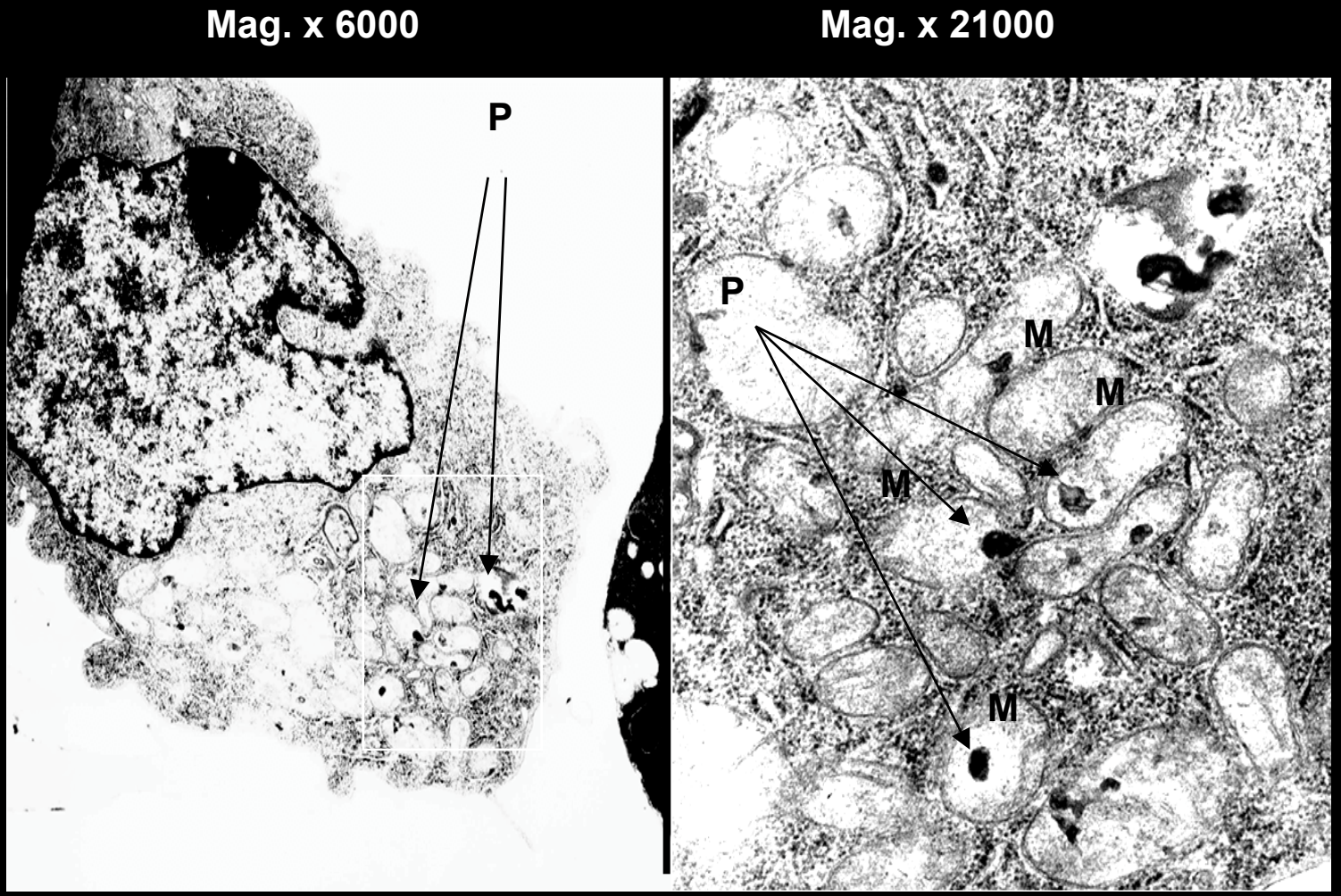
Electron micrograph showing that organic DEP chemicals induce apoptosis via mitochondrial effects



Untreated DEP extract

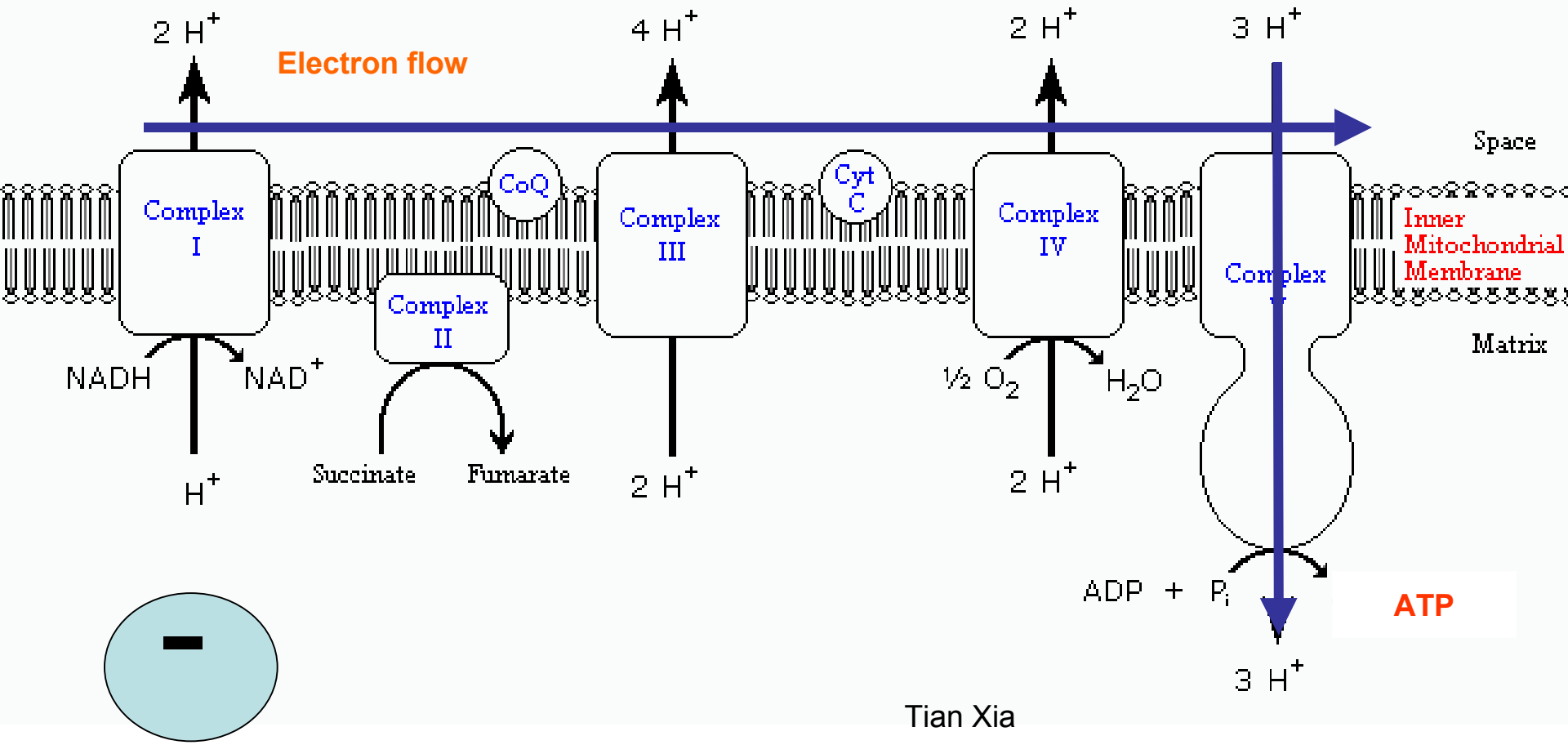
Tian Xia et al. Environ Health Pers. 2004. In Press.

Ultrafines lodge in and destroy mitochondria



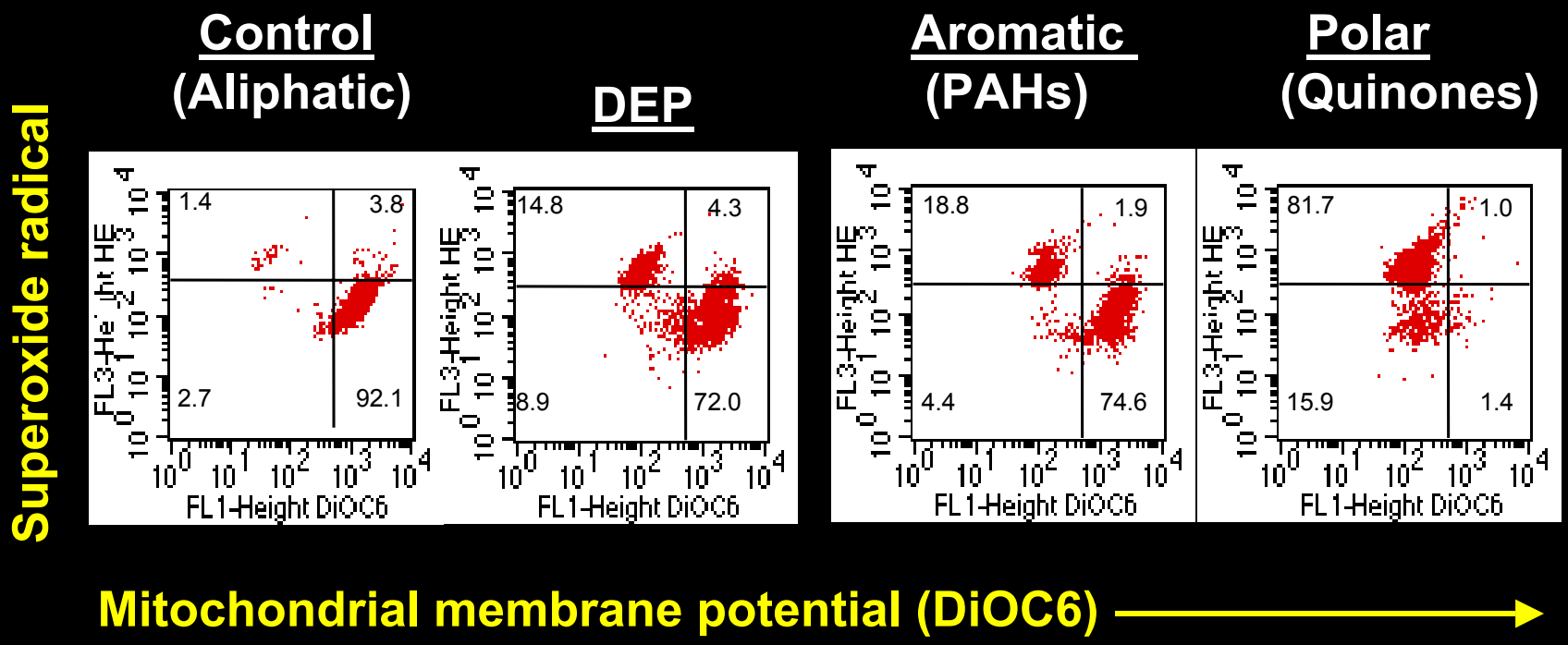
Ning Li et al. Environmental Health Perspectives. 2003. 111:455-460

Mitochondria membrane potential



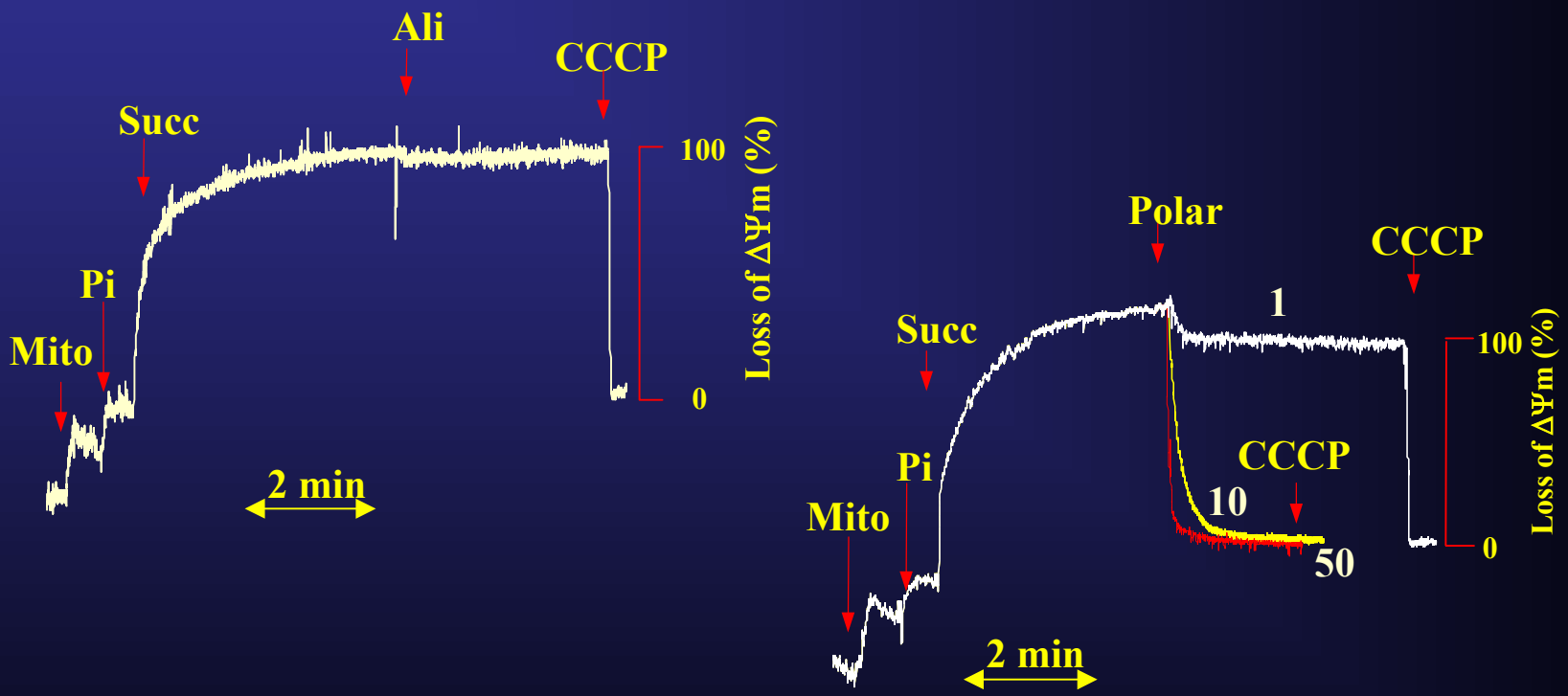
Tian Xia

Differential Toxic Effects by different DEP components

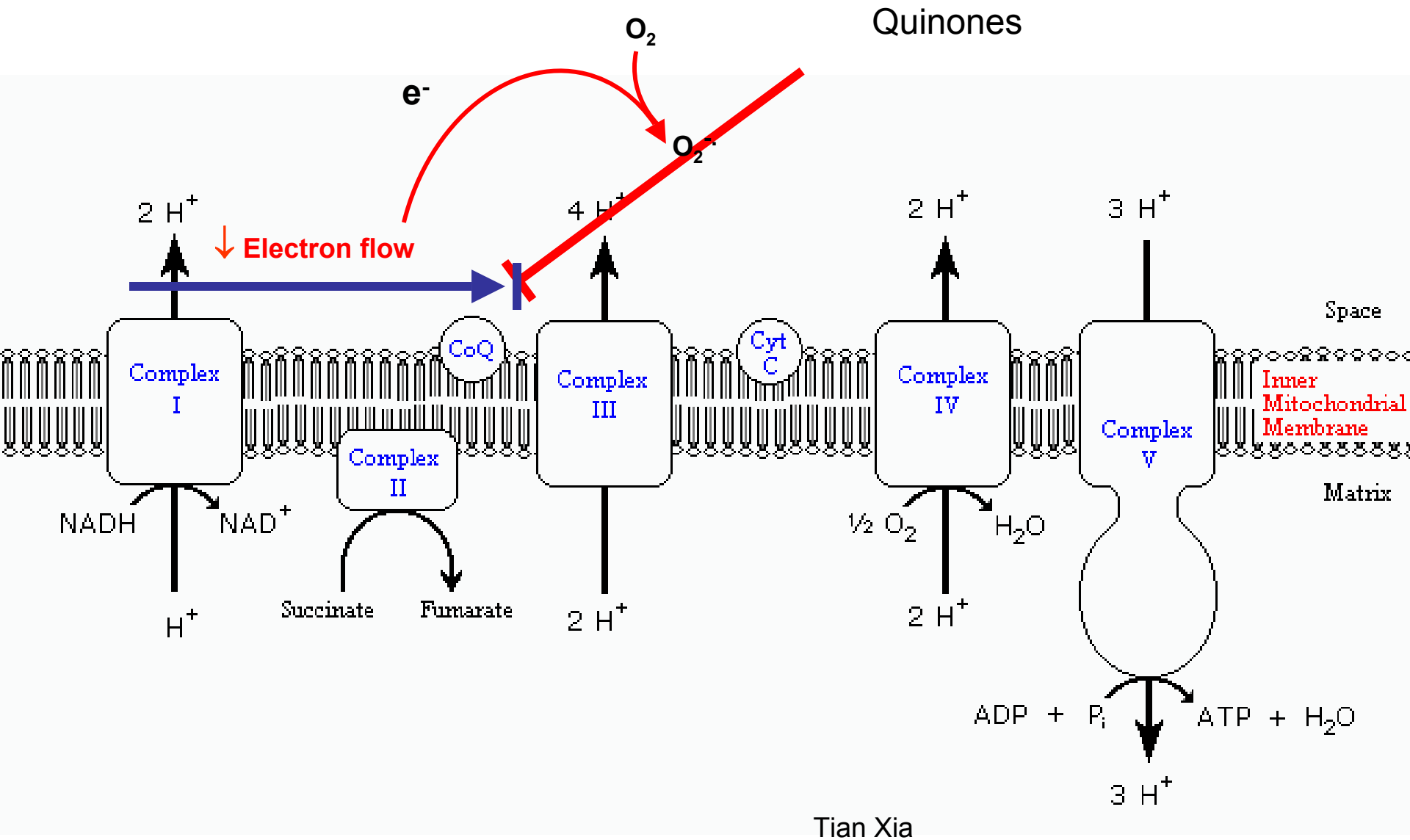


Tian Xia et al. Environ Health Pers. 2004. In Press.

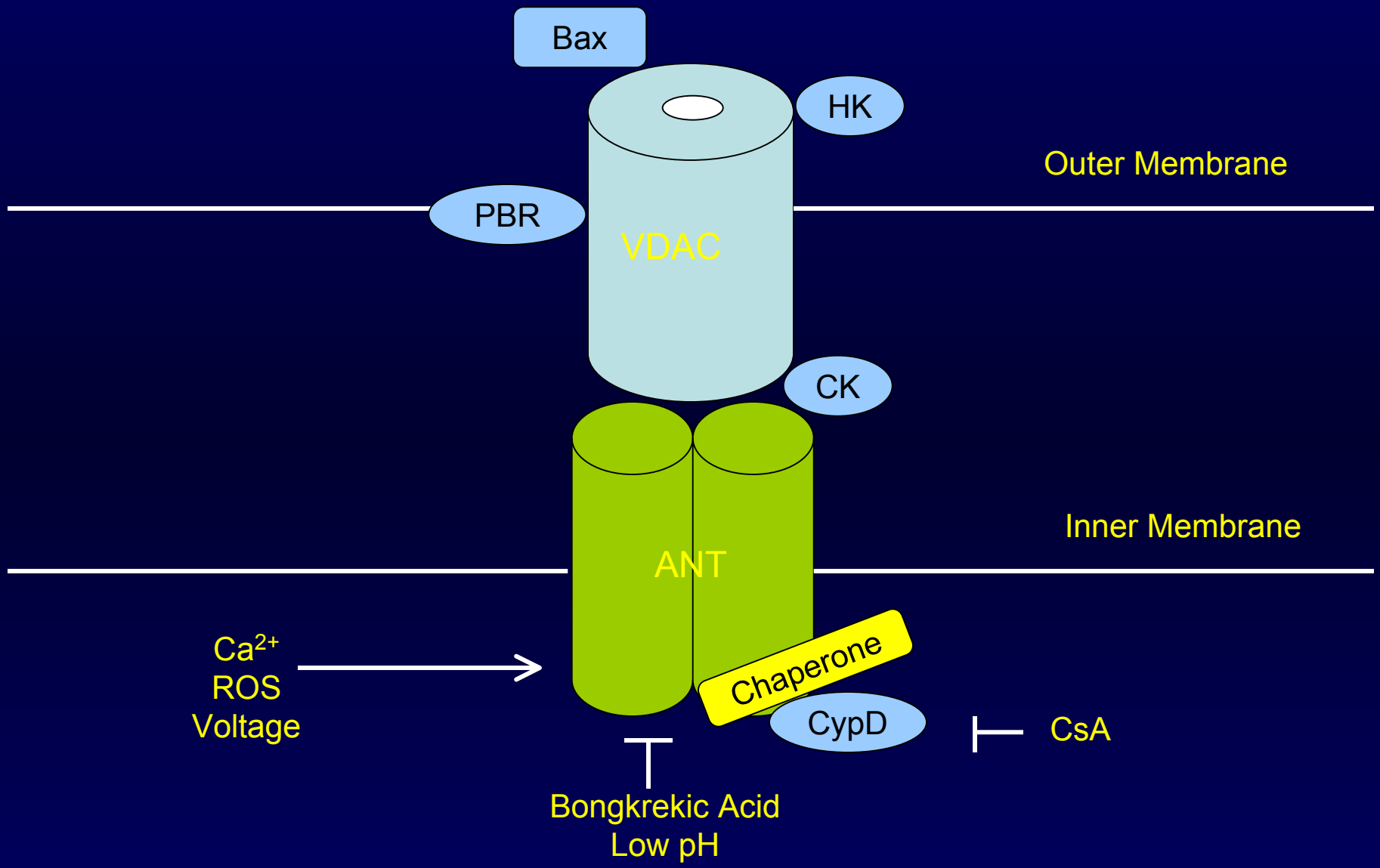
Polar material induces rapid mitochondrial depolarization



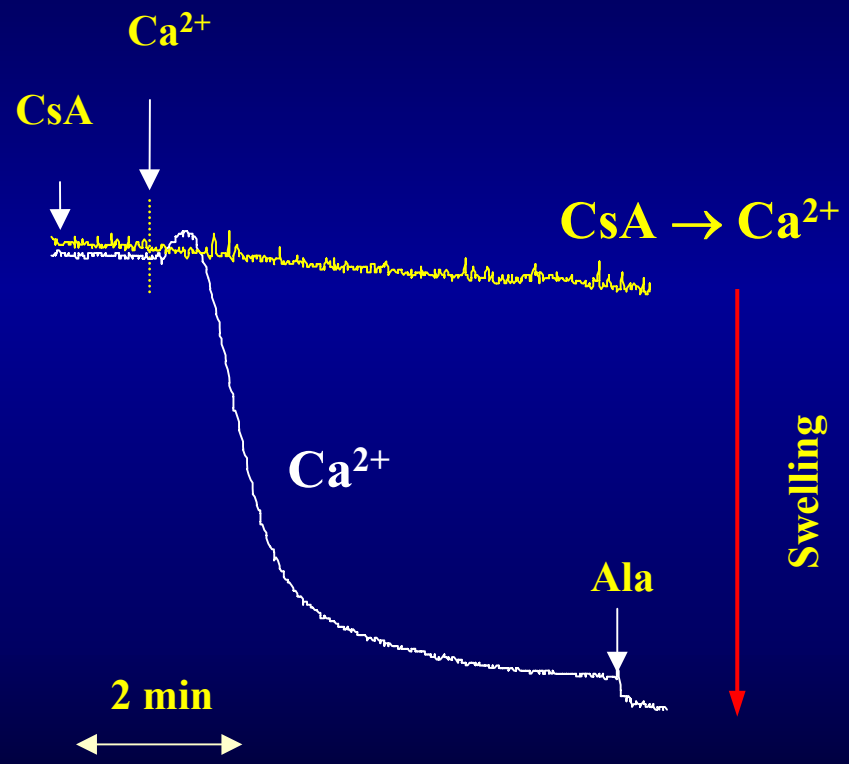
Tian Xia et al. Environ Health Pers. 2004. In Press.


Quinones
O_2
e^-
$O_2^{\cdot-}$
2 H^+
Electron flow
4 H^+
2 H^+
3 H^+
Space
Complex I
CoQ
Complex III
Cyt C
Complex IV
Complex V
Inner Mitochondrial Membrane
Complex II
Matrix
NADH
NAD^+
H^+
Succinate
Fumarate
2 H^+
½ O_2
H_2O
2 H^+
ADP + P_i
ATP + H_2O
3 H^+

Tian Xia

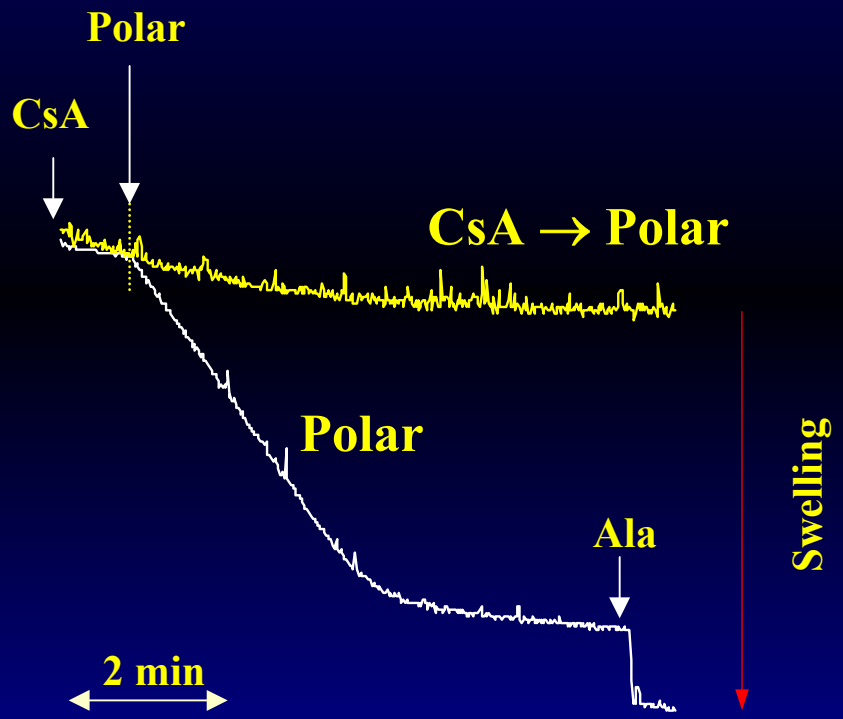

Bax
HK
Outer Membrane
PBR
VDAC
CK
Inner Membrane
ANT
Ca2+
ROS
Voltage
Chaperone
CypD
CsA
Bongkrekic Acid
Low pH

Calcium dependant PT pore opening is sensitive to the effect of Cyclosporin A



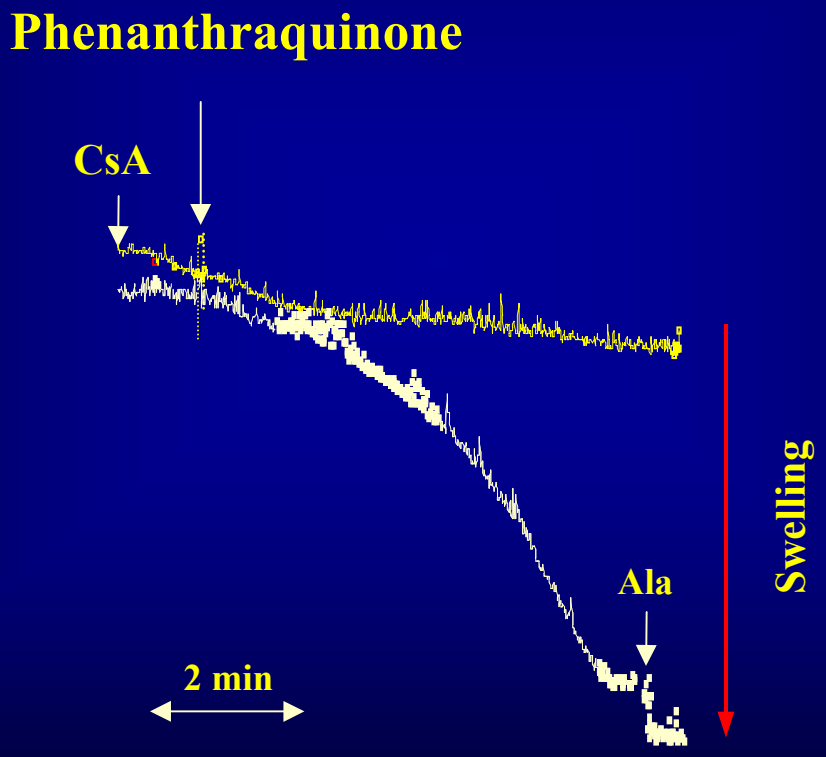
Tian Xia et al. Environ Health Pers. 2004. In Press.

Polar-induced PT transition is Ca^{2+} dependant and CsA inhibitable

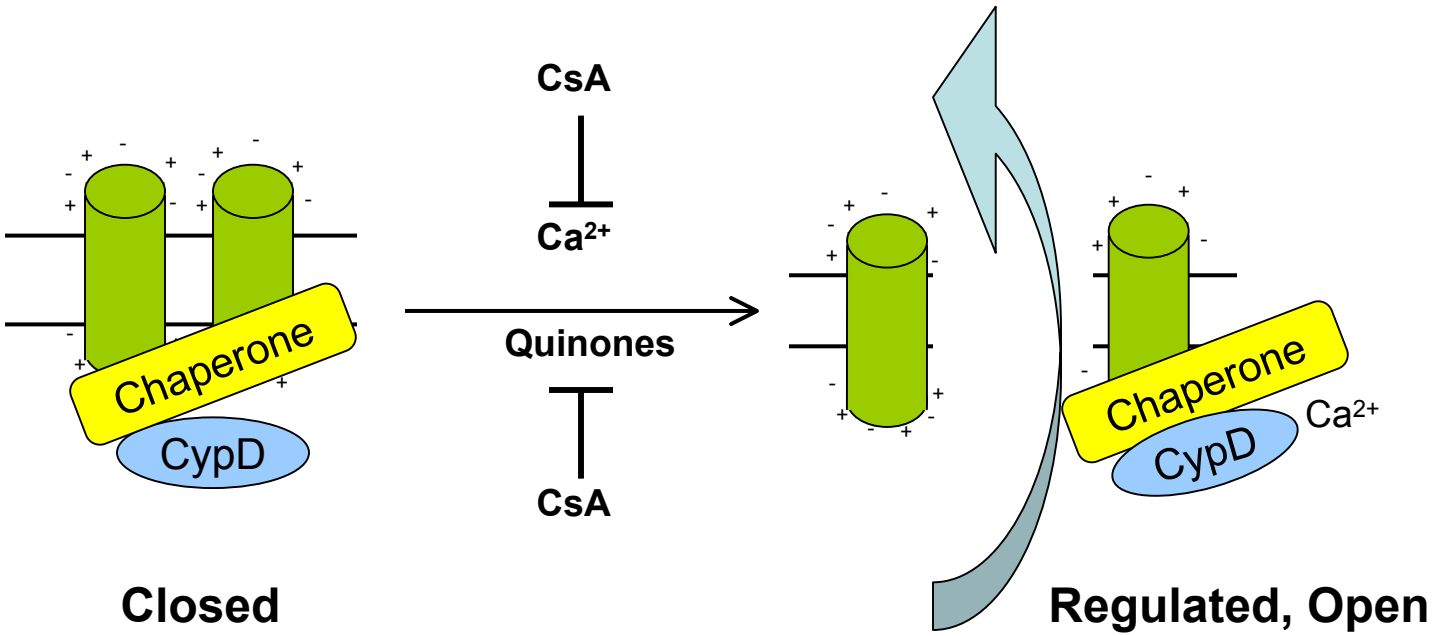


Tian Xia et al. Environ Health Pers. 2004. In Press.

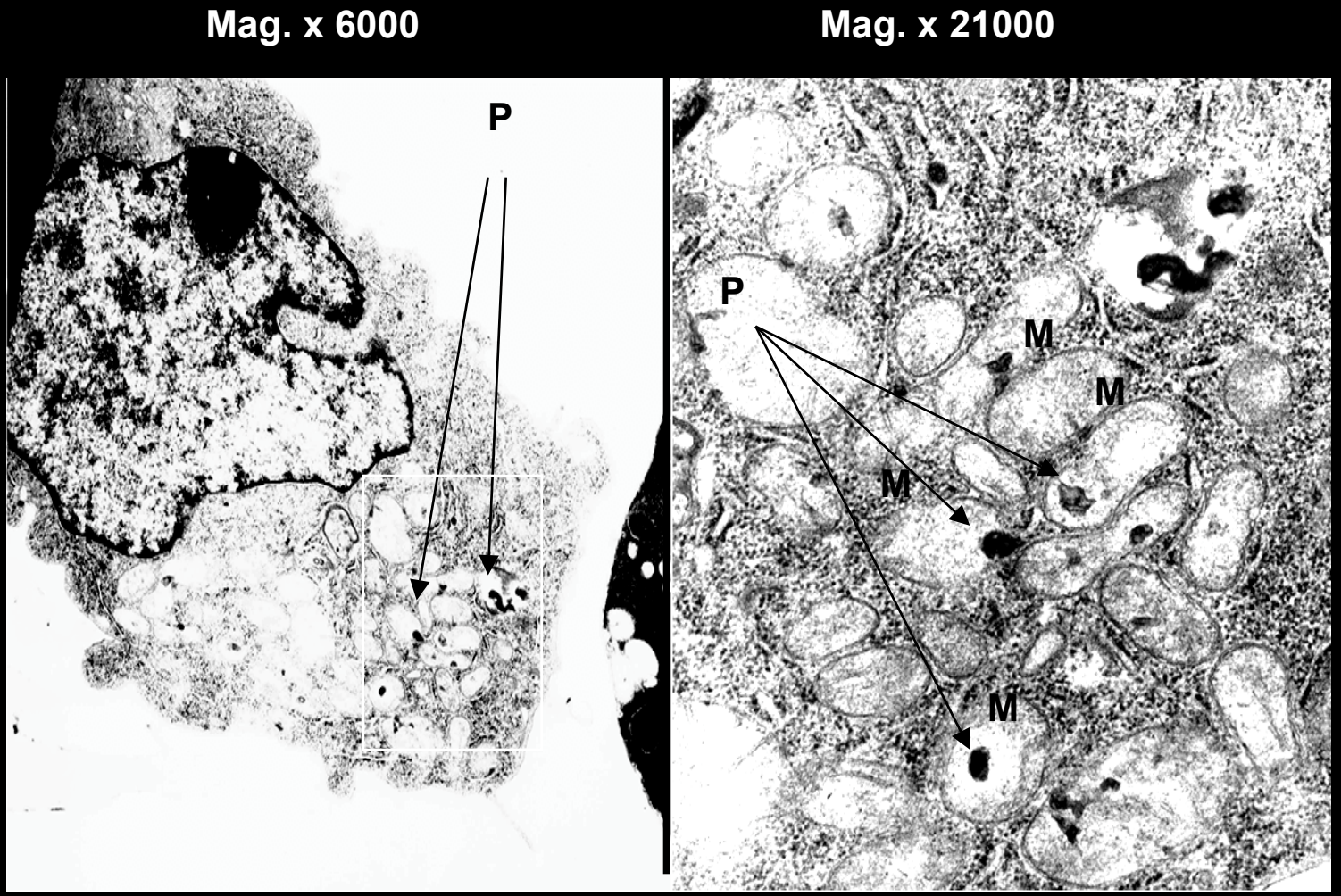
PT opening by redox cycling DEP quinones is Ca^{2+} dependant and CsA inhibitable



Tian Xia et al. Environ Health Pers. 2004. In Press.


CsA
Ca2+
Quinones
CsA
Chaperone
CypD
Chaperone
CypD
Ca2+
Closed
Regulated, Open

Ultrafines lodge in and destroy mitochondria



Ning Li et al. Environmental Health Perspectives. 2003. 111:455-460

Ambient Ultrafine Particles induce PT pore opening