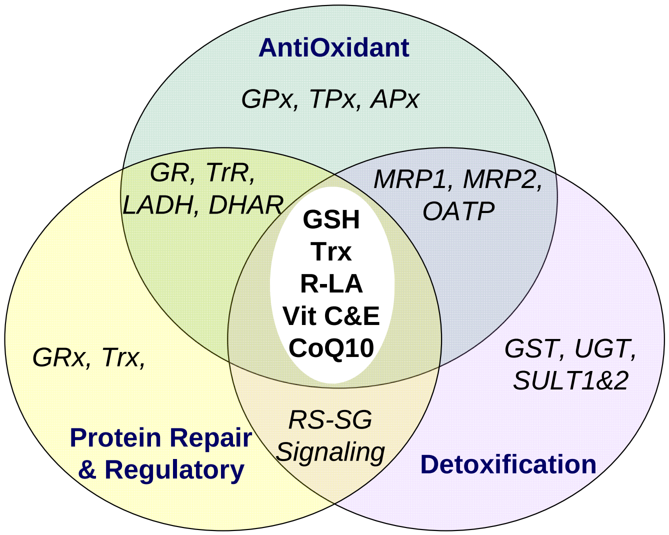
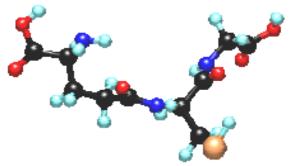
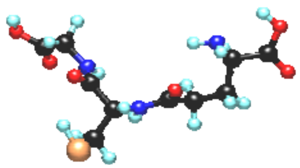
Understanding the Unified Biological System for Chemoprotection/ Detoxification and Oxidative Stress Protection

Christopher W. Shade, Ph.D.

Quicksilver Scientific, LLC

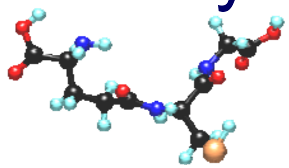




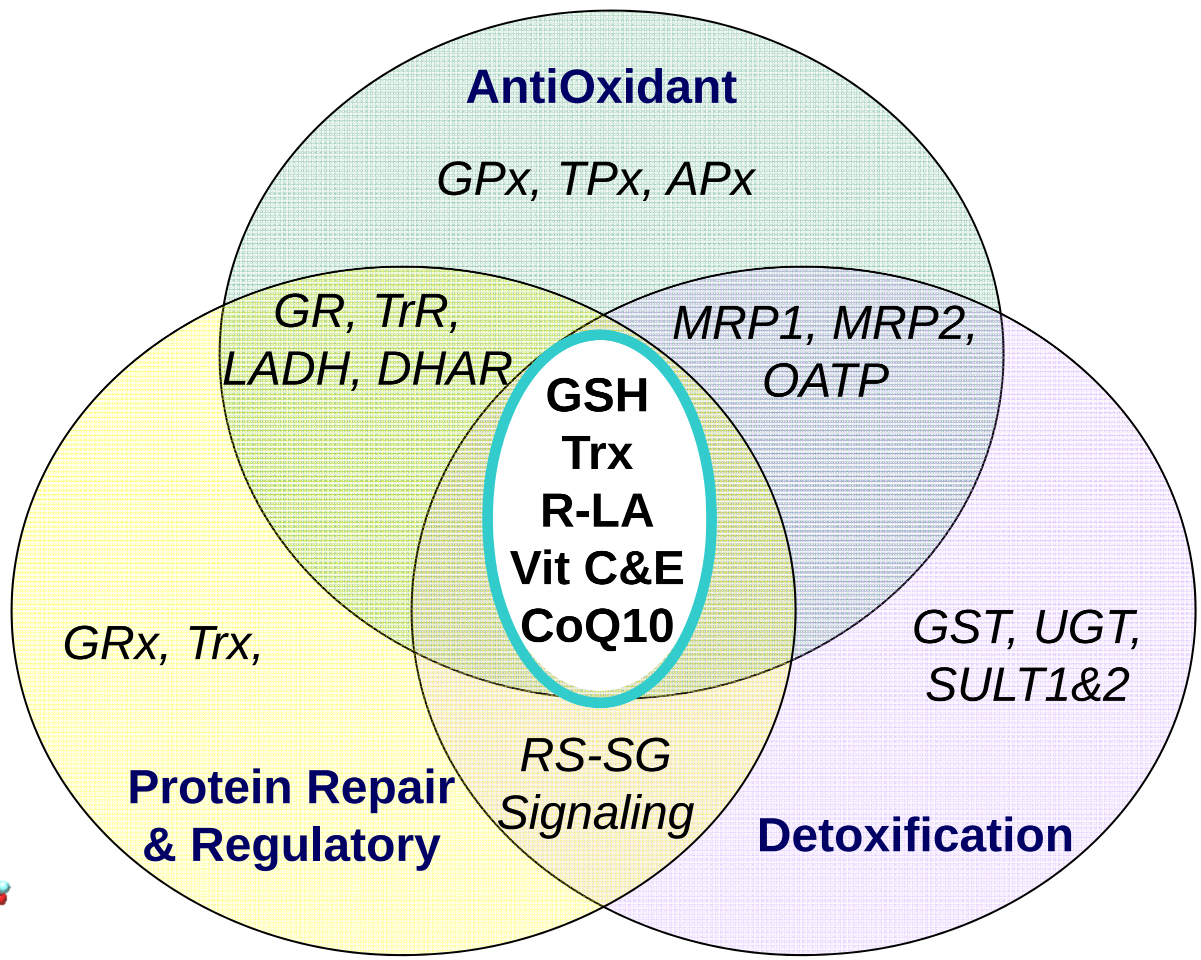


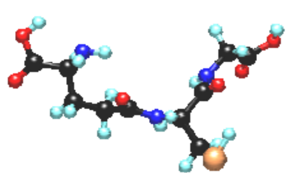
Overview

1. The enzymes as the most important players in the Super-System, distributing reducing equivalents through the system as needed and fullfilling detoxification and protein repair functions as well
2. The substrates/antioxidants as the linkers to all the compartments of the cellular (and extra-cellular space)
3. Having balance of all the players with a enzyme sufficient system enables full protection without toxicity of trying to push the system with one player (e.g. Vit E, Vit A)



AntiOxidant–Detoxification–Protein Repair SuperSystem

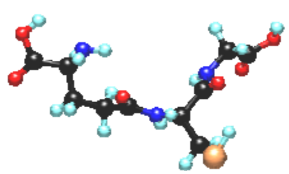


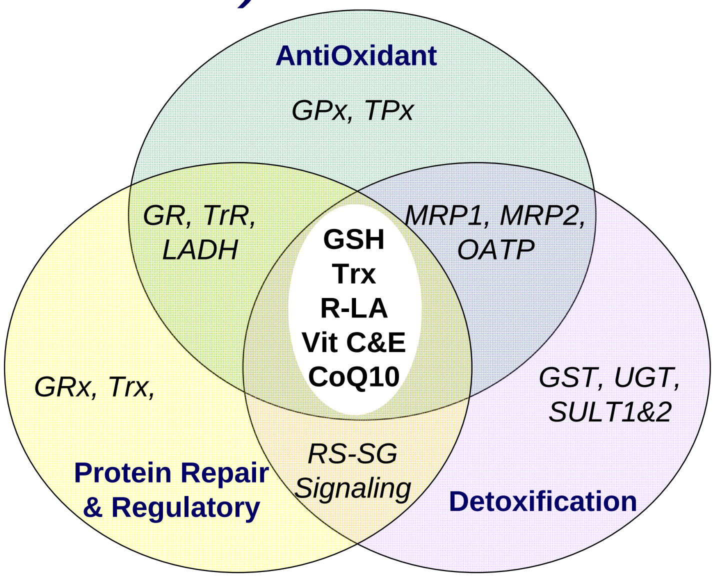


The Players

- Antioxidants – Substrates (*the ones we all know*)
  - GSH, Trx, LA, Vit C, Vit C, CoQ10, Vit A
- Antioxidant Enzymes (*quench radicals and regenerate antioxidants)*
  - GR, TrR, GPx, Trx, APx LADH, DHAR
- Protein Repair Enzymes *(thioltransferases)*
  - GRx, Trx
- Detoxification Proteins

  (Phase II and III detox)
  - GST, OATP, MRP1-3





Overlapping AntiOxidant Systems

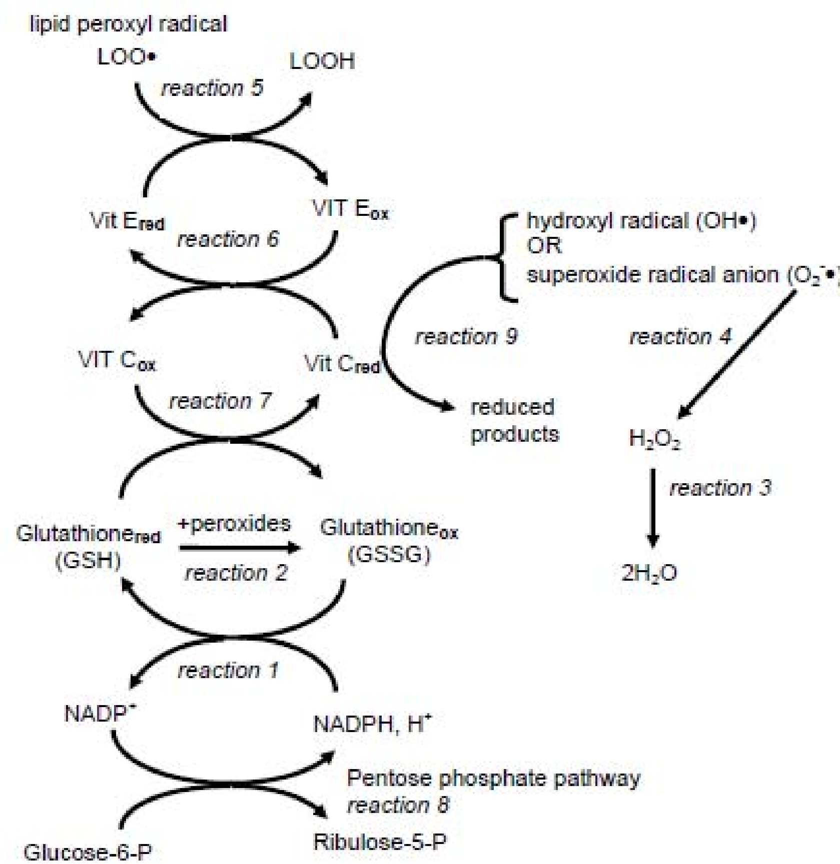
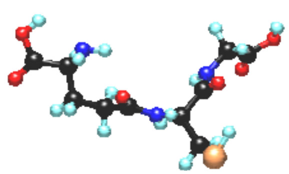


Figure 7. Antioxidant cascade depicting the interrelated roles of the pentose phosphate pathway, NADPH, glutathione and vitamins C and E. Besides being reduced by vitamin C, superoxide radical anion is also detoxified by superoxide dismutase (reaction 4) to produce peroxide removed by catalase (reaction 3) or glutathione peroxidase (reaction 2). GSH for the latter reaction is regenerated by glutathione reductase (reaction 1).



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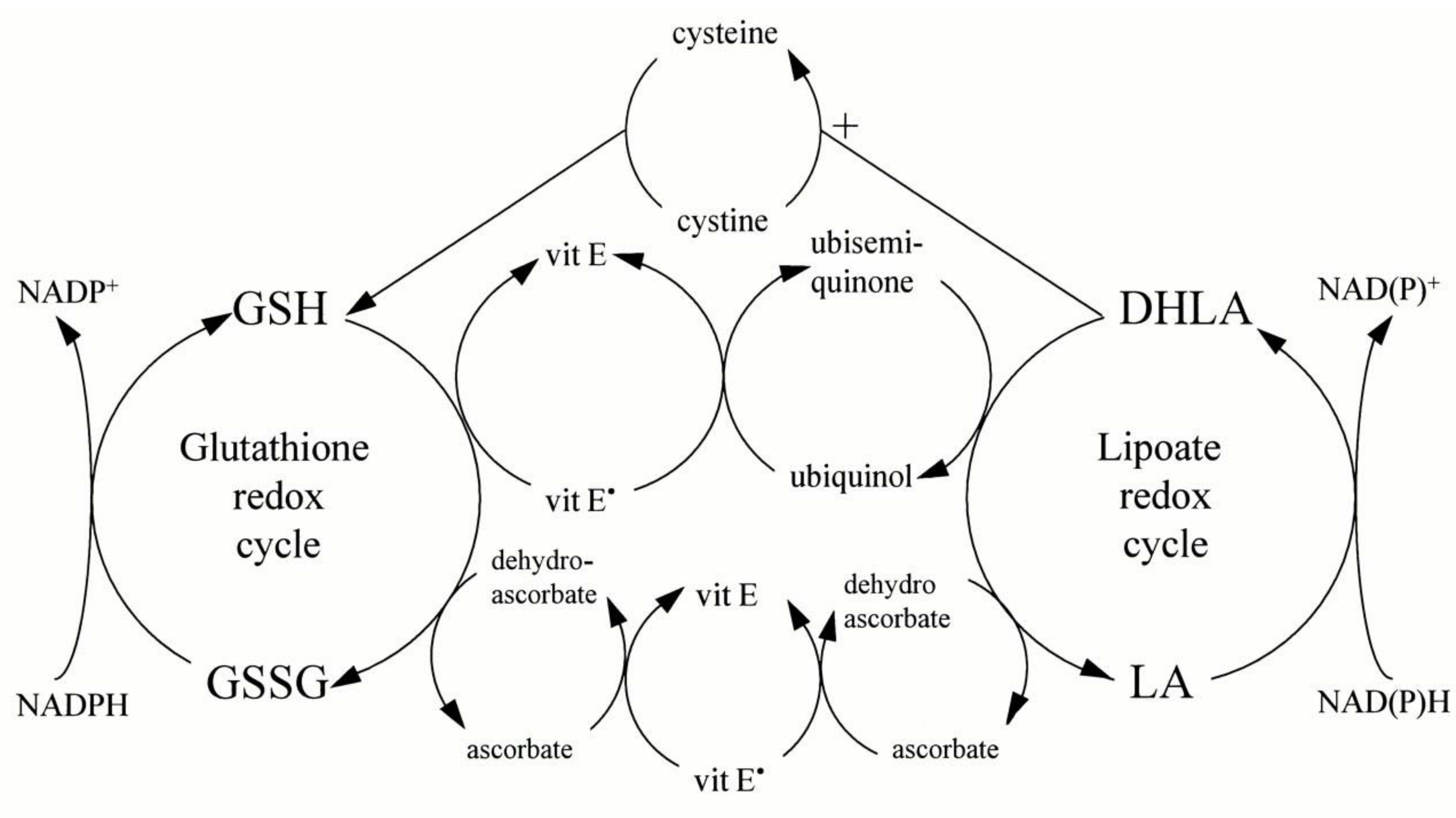
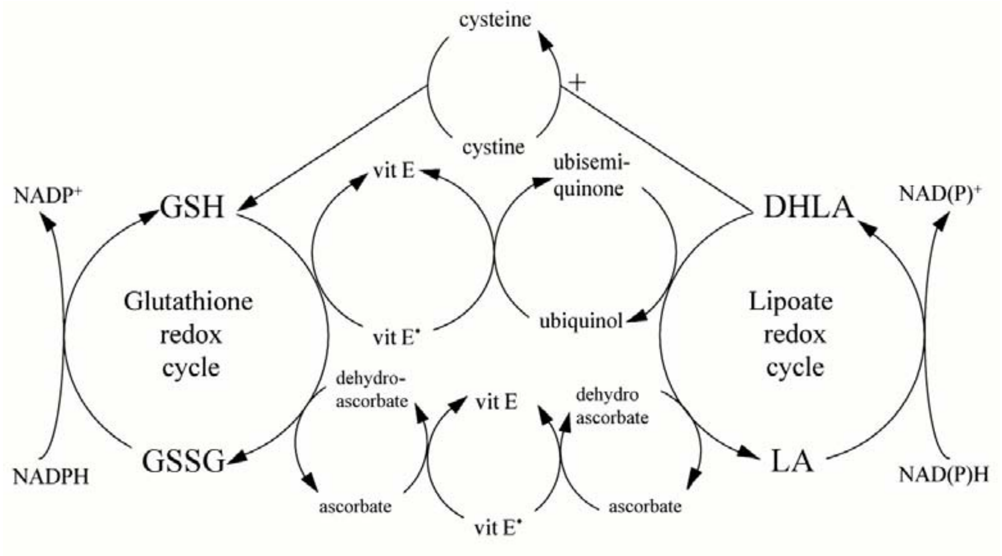
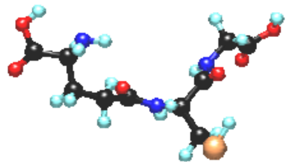


Sen C K , Packer L Am J Clin Nutr 2000;72:653S-669S

Overlapping AntiOxidant Systems

- Asc-GSH
- Asc-Vit E
- Asc-LA
- GSH-LA
- Trx-LA
- Trx-Asc
- Vit E – CoQ10
- CoQ10 as redox modulator and signaler, primarily through pro-oxidant activity

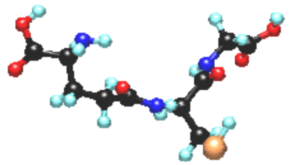




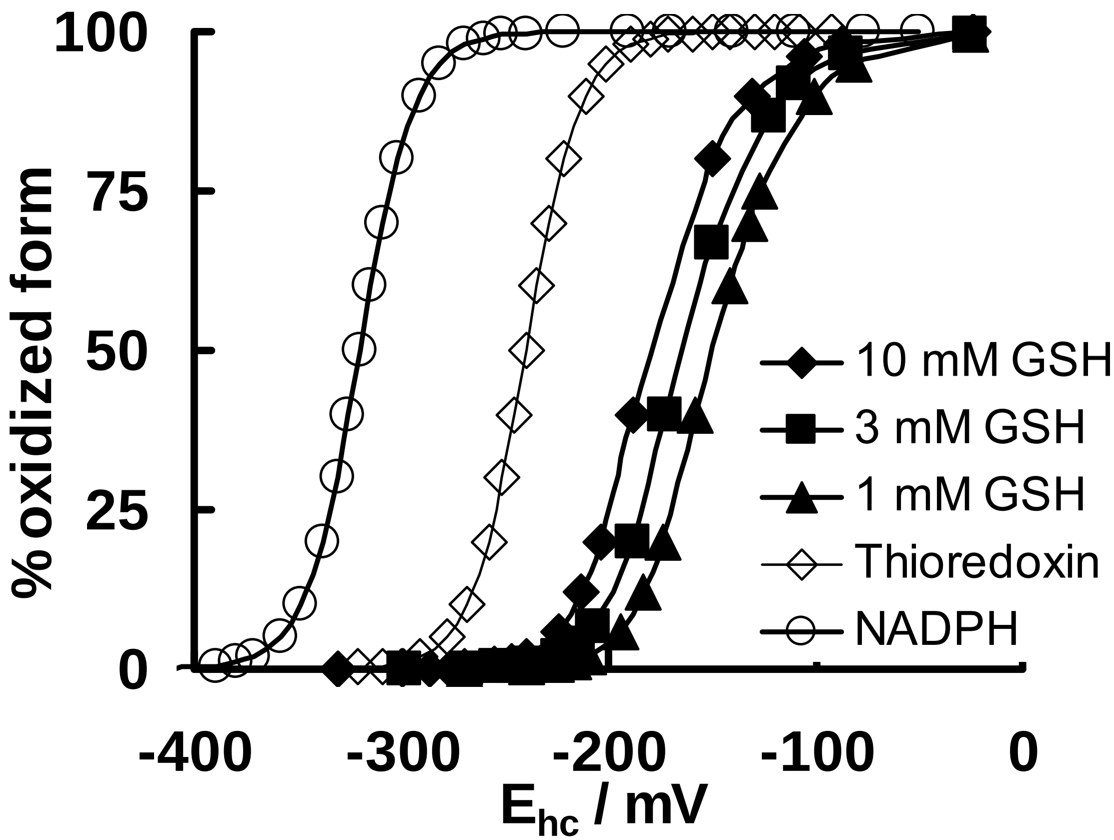
NADH and NADPH

H^- as essential fuel for the AntiOxidant and Protein Repair Systems

- -the ubiquitous coin of the realm
- -changed into local currencies by enzymes
- -carrier of the H^-, but GSH is the real bank (50x higher than NADPH)
- -H^- generated at the expense of ATP

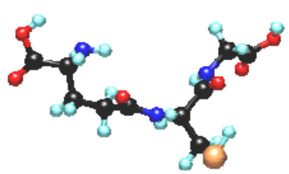


Cellular Redox Systems



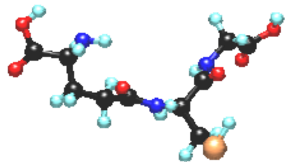
The $NADP^+$/NADPH couple provides the reducing equivalents needed for the thioredoxin and GSH system.

Schafer FQ, Buettner GR. (2001) Redox state of the cell as viewed through the glutathione disulfide/glutathione couple. *Free Radic Biol Med.* **30:**1191-1212.

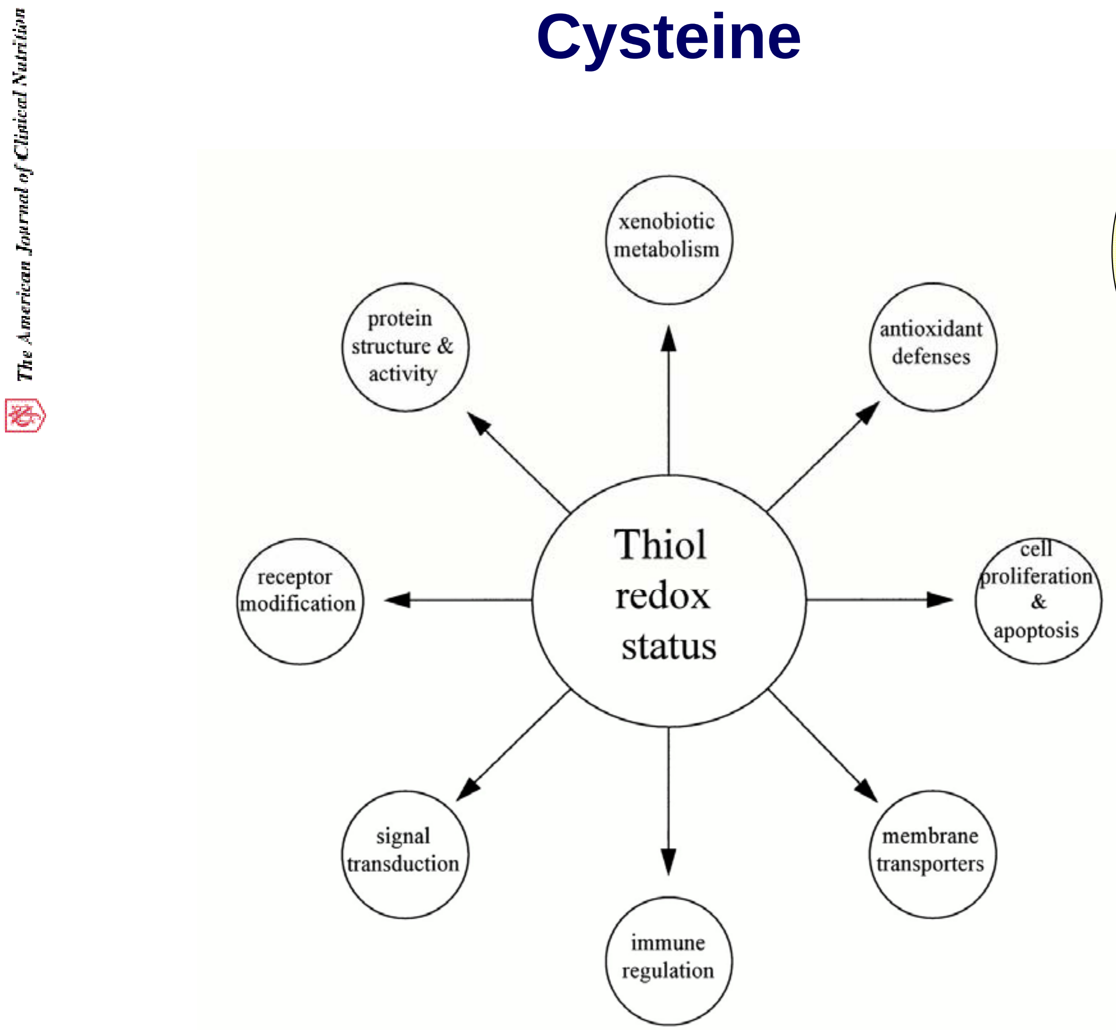


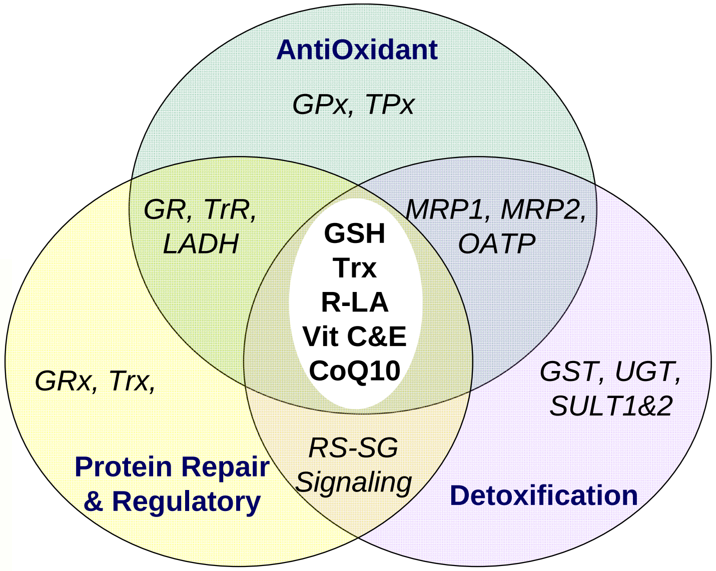
Thioredoxin

1. Powerful antioxidant
2. Reuired for RNA transcription
3. Neuron protective
4. Strong interactions in antioxidant web
    1. Interaction with ALA through lipoamide dehydrogenase
        1. Reduction of insulin disulfides by Trx – *one likely mechanism for ALA effect on diabetes*
        2. RBC's lack TrR and thus can use DHLA for regeneration with lipoamide dehydrogenase for cycling



Glutathione, Thioredoxin, Cysteine





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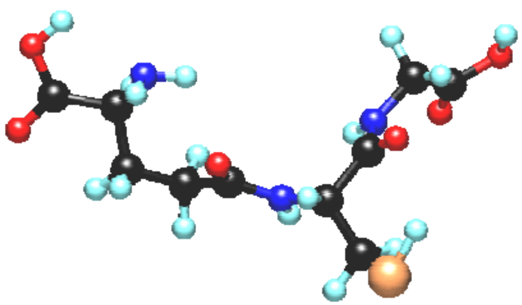
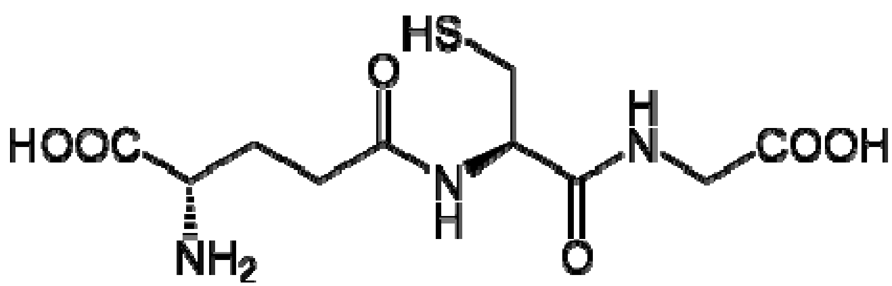
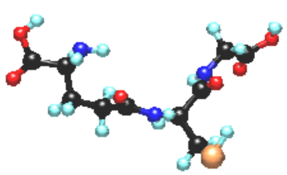
Heavy Metal Defense – Glutathione System

Antioxidant, Detoxification, Protein Repair

- Glutathione (GSH) - A thiolic tripeptide composed of glutamate, cysteine, and glycine



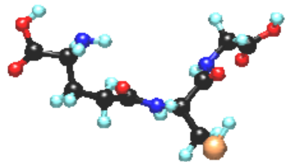




Defense – Glutathione System

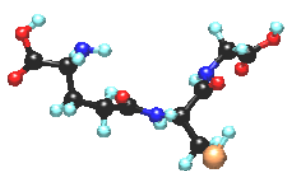
Antioxidant, Detoxification, Protein Repair

- Synthetases (synthesize GSH from precursors)
- Transpeptidases (take apart and reassemble)
- Transferases (Phase II conjugation)
- Peroxidases (radical quenching)
- Reductases (repair after quenching)
- Redoxins (using GSH as reducing equivalent for protein repair)
- ***Glutathionylation*** *– protection of Proteins*

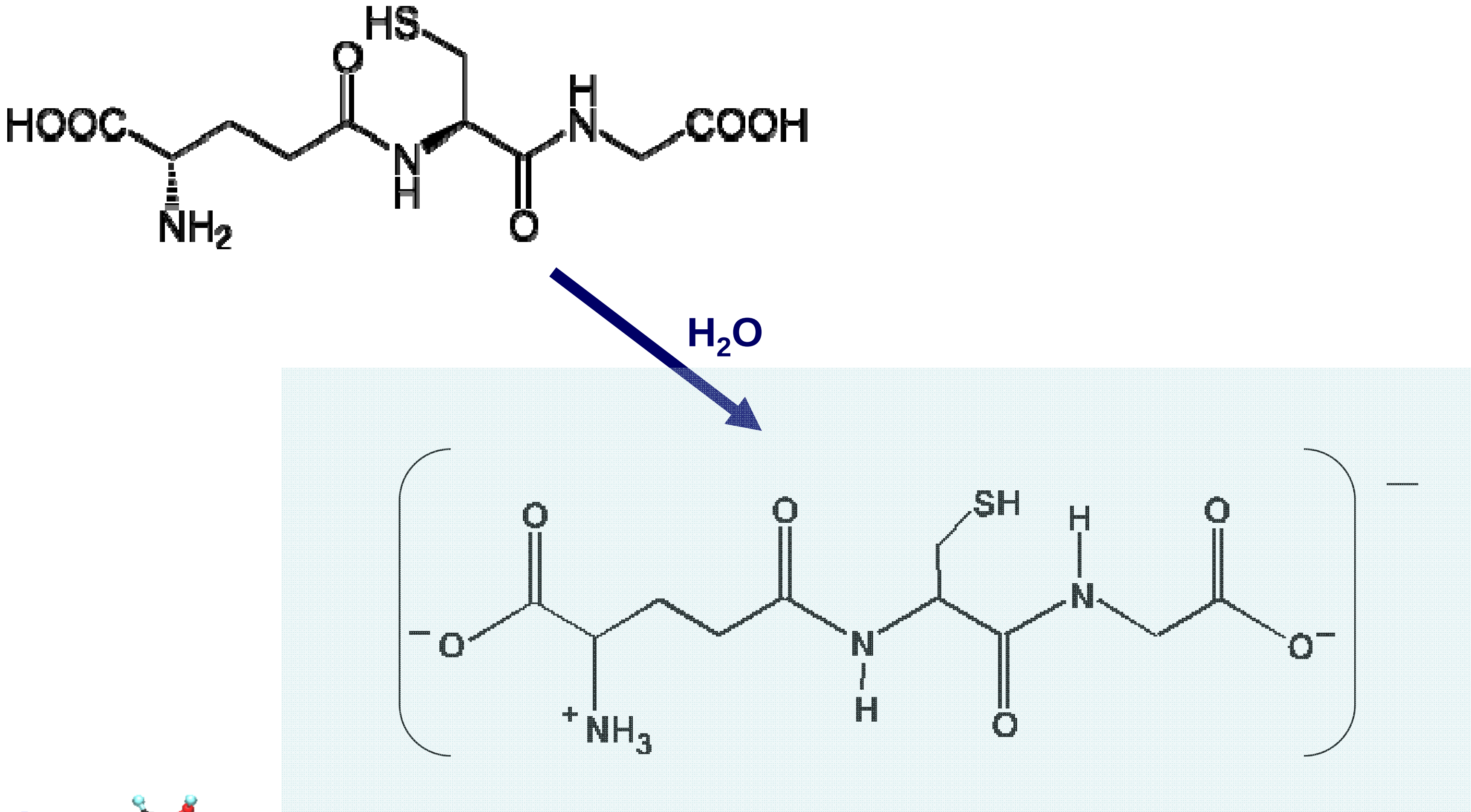


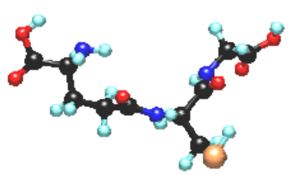
Disease and Gen Polymorphisms of GSH genes

- Hemolytic Anemia – GST (Beutler et al 1988)
- Sensitivity to DDT – GCS and GST (Hung et al. 2004)
- Bladder Cancer (Hung et al. 2004)
  - O.R. GSTM1 = 1.69
  - O.R. GSTT1 = 1.74
  - O.R. GSTM1 + Env Exposure = 2.77
- Also Leukemia, Cancers of cervix, prostate, head and neck, lung
- Cirrhosis of liver
- Type II Diabetes
- Schizophrenia



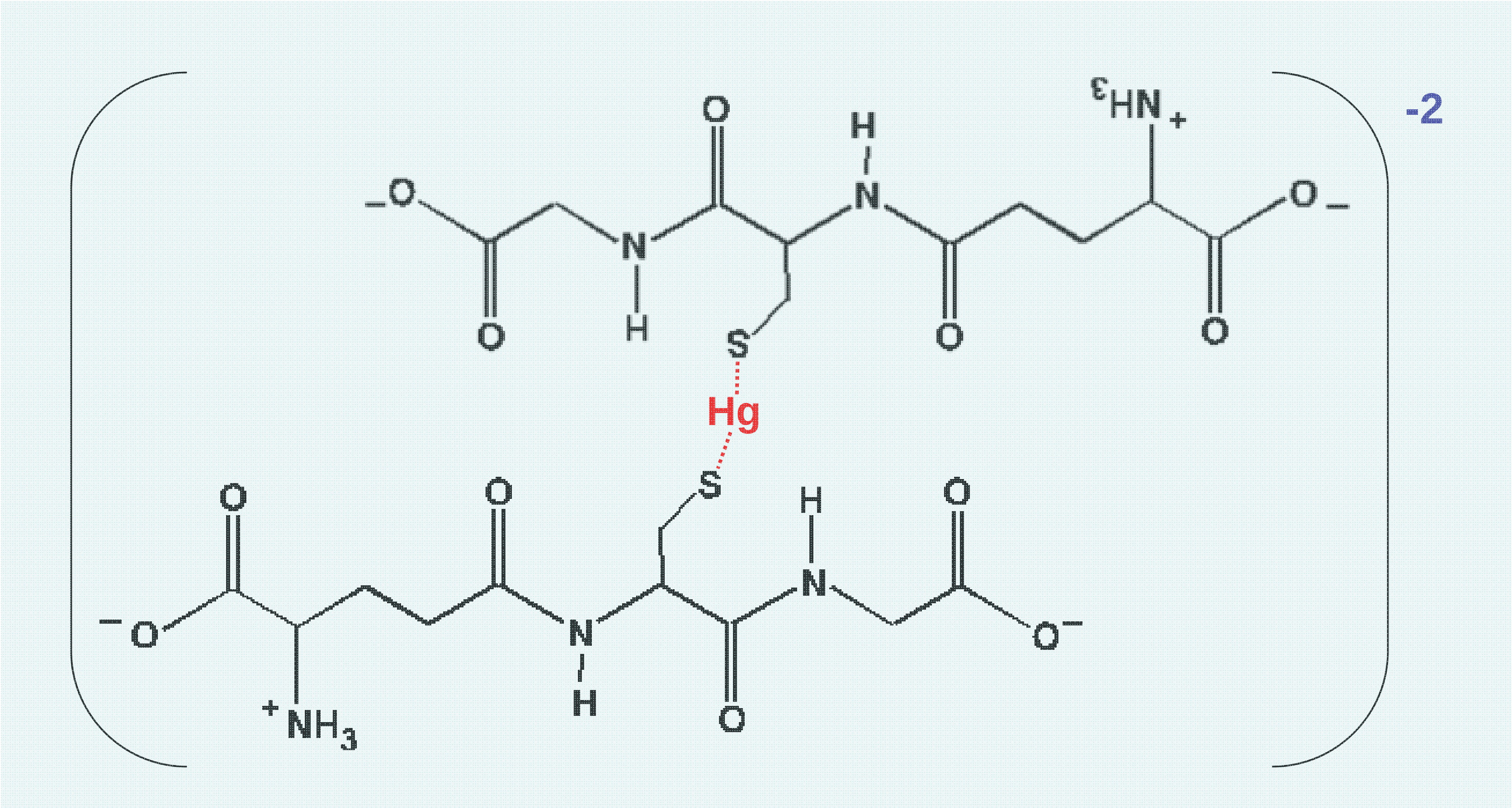
Organic Anion Transporters: GSH Ionization in Aqueous Solutions

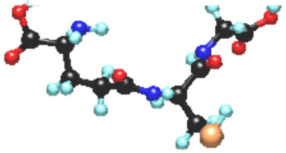




Mercuric DiGlutathione DiAnion

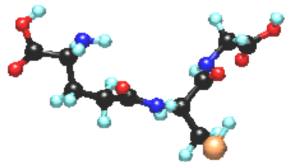
$Hg(GSH)_2^{-2}$ - Soluble, mobile, recognizable





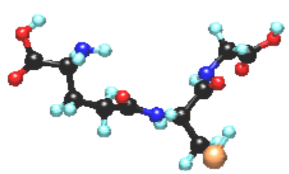
The Human Detoxification System

- Detoxification ***Phases I, II, III***
  - ***Phase I*** is an activation,
  - ***Phase II*** is conjugation (***mobilization***)
  - ***Phase III*** is transport (recently delineated; control point; ***elimination***)



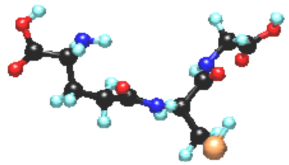
The Human Detoxification System

- ***Phase I -*** an oxidative activation, usually the Cytochrome P450 system
  - Prepares toxin for conjugation in ***Phase II*** with GSH, Glucuronic acid, Sulfate, Gycine or other amino acid, Taurine, Methyl group
  - **Not needed for metals,** but very important to have coupled to ***Phase II***
    - ***Creates Essentially Free-Radicals***



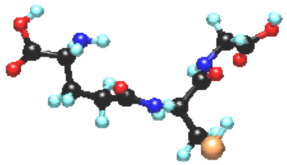
The Human Detoxification System

- ***Phase II*** – conjugation makes toxin more water soluble and recognizable by transporters
  - ***Glutathione S-Transferases (GST)*** responsible for GSH conjugation
  - Low expression in people with high MeHg and with sensitivity (allergy) to Thimerosal (EthylHg)



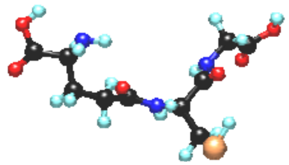
The Human Detoxification System

- ***Phase III*** **is the transport out**!
  - Several transport proteins (**cMOAT, OAT, MRP1, MRP2, GS-X**)
    - *Organic Anion Transporters*
  - Same transporters for many pathways (glucuronide, sulfate, glycinate, GSH)
  - In cells, liver, intestines, kidneys – biggest in liver then intestines



Breakdown of the defense system

- GSH deficiency –
  - Genetic (GCS polymorphisms, epigenetic dysfunction)
  - Environmental (oxidative consumption or inflammation)
- GST problems –
  - Genetic (GST polymorphisms, epigenetic dysfunction)
  - Environmental (Inflammatory cascade/ARE dysfunction)



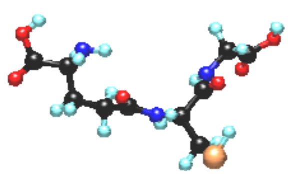
Decay in GSH levels and Enzyme Activity with Age

Table 3. Effect of *T. chebula* aqueous extract on mitochondrial non-enzymatic antioxidants in young and aged rats

	Young		Aged	
	Control	Treated	Control	Treated
Liver				
GSH (μg/mg protein)	10.66 ± 0.54	11.85 ± 0.50 [a†]	8.74 ± 0.46 [a‡]	10.67 ± 0.53 [b‡]
VIT-C (μg/mg protein)	9.17 ± 0.49	9.23 ± 0.44	6.75 ± 0.46 [a‡]	9.21 ± 0.52 [b‡]
VIT-E (μg/mg protein)	4.43 ± 0.27	4.51 ± 0.22	3.07 ± 0.26 [a‡]	4.22 ± 0.24 [b‡]
Kidney				
GSH (μg/mg protein)	9.66 ± 0.33	10.15 ± 0.24 [a§]	7.32 ± 0.26 [a‡]	9.74 ± 0.31 [b‡]
VIT-C (μg/mg protein)	8.07 ± 0.38	8.14 ± 0.31	5.75 ± 0.30 [a‡]	8.02 ± 0.35 [b‡]
VIT-E (μg/mg protein)	4.18 ± 0.32	4.26 ± 0.34	3.12 ± 0.30 [a‡]	4.12 ± 0.28 [b‡]

Each value is expressed as mean ± SD for six rats in each group. Superscript letters represent $p < 0.05$ (Tukey-Kramer Multiple comparisons Test).
[a]As compared with Young control,
[b]As compared with Aged control.
[§]$p < 0.05$; [†]$p < 0.01$ [‡]$p < 0.001$.

	Young	Old	% Change
GSH -Liv	10.66	8.74	**-18%**
GSH - Kid	9.66	7.32	**-24%**
Vit C	5.61	3.52	**-37%**
Vit E	0.98	0.64	**-35%**



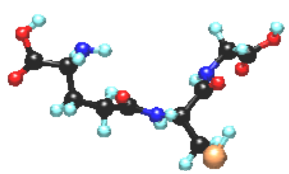
Decay in GSH levels and Enzyme Activity with Age

Table 2. Effect of *T. chebula* aqueous extract on enzymatic antioxidants in young and aged rats

	Young		Aged	
	Control	Treated	Control	Treated
Liver				
MnSOD (50% reduction of NBT/min/mg protein)	3.37 ± 0.20	3.55 ± 0.18	2.11 ± 0.24 [a‡]	3.13 ± 0.23 [b‡]
CAT (μmol H_2O_2 consumed/min/mg protein)	4.59 ± 0.31	4.51 ± 0.22	5.72 ± 0.28 [a‡]	4.54 ± 0.25 [b‡]
GPx (μmole GSH utilized/min/mg protein)	6.44 ± 0.24	6.53 ± 0.20	7.96 ± 0.26 [a‡]	6.67 ± 0.23 [b‡]
GR (nmol NADPH oxidized/min/mg protein)	5.61 ± 0.28	5.73 ± 0.31	3.52 ± 0.26 [a‡]	5.46 ± 0.25 [b‡]
GST (μmoles of CDNB-GSH conjugated/min/mg protein)	0.98 ± 0.02	1.03 ± 0.03 [a§]	0.64 ± 0.02 [a‡]	0.99 ± 0.04 [b‡]
G6PDH (Units/min/mg protein)	3.25 ± 0.18	3.54 ± 0.14 [a§]	1.64 ± 0.16 [a‡]	3.14 ± 0.19 [b‡]
Kidney				
MnSOD (50% reduction of NBT/min/mg protein)	3.35 ± 0.18	3.42 ± 0.21	2.06 ± 0.16 [a‡]	3.21 ± 0.23 [b‡]
CAT (μmol H_2O_2 consumed/min/mg protein)	4.14 ± 0.20	4.20 ± 0.15	4.92 ± 0.22 [a‡]	4.27 ± 0.22 [b‡]
GPx (μmole GSH utilized/min/mg protein)	5.18 ± 0.27	5.07 ± 0.31	6.03 ± 0.25 [a‡]	5.26 ± 0.29 [b‡]
GR (nmol NADPH oxidized/min/mg protein)	4.35 ± 0.24	4.48 ± 0.24	2.36 ± 0.26 [a‡]	4.39 ± 0.22 [b‡]
GST (μmoles of CDNB-GSH conjugated/min/mg protein)	1.32 ± 0.06	1.41 ± 0.03 [a†]	0.92 ± 0.04 [a‡]	1.35 ± 0.03 [b‡]
G6PDH (Units/min/mg protein)	1.80 ± 0.08	1.88 ± 0.06	1.11 ± 0.08 [a‡]	1.83 ± 0.05 [b‡]

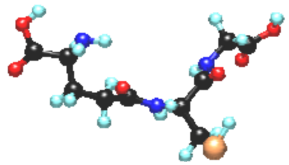
Each value is expressed as mean ± SD for six rats in each group. Superscript letters represent $p < 0.05$ (Tukey-Kramer Multiple comparisons Test).
[a] As compared with Young control,
[b] As compared with Aged control.
[§] $p < 0.05$; [†] $p < 0.01$; [‡] $p < 0.001$.

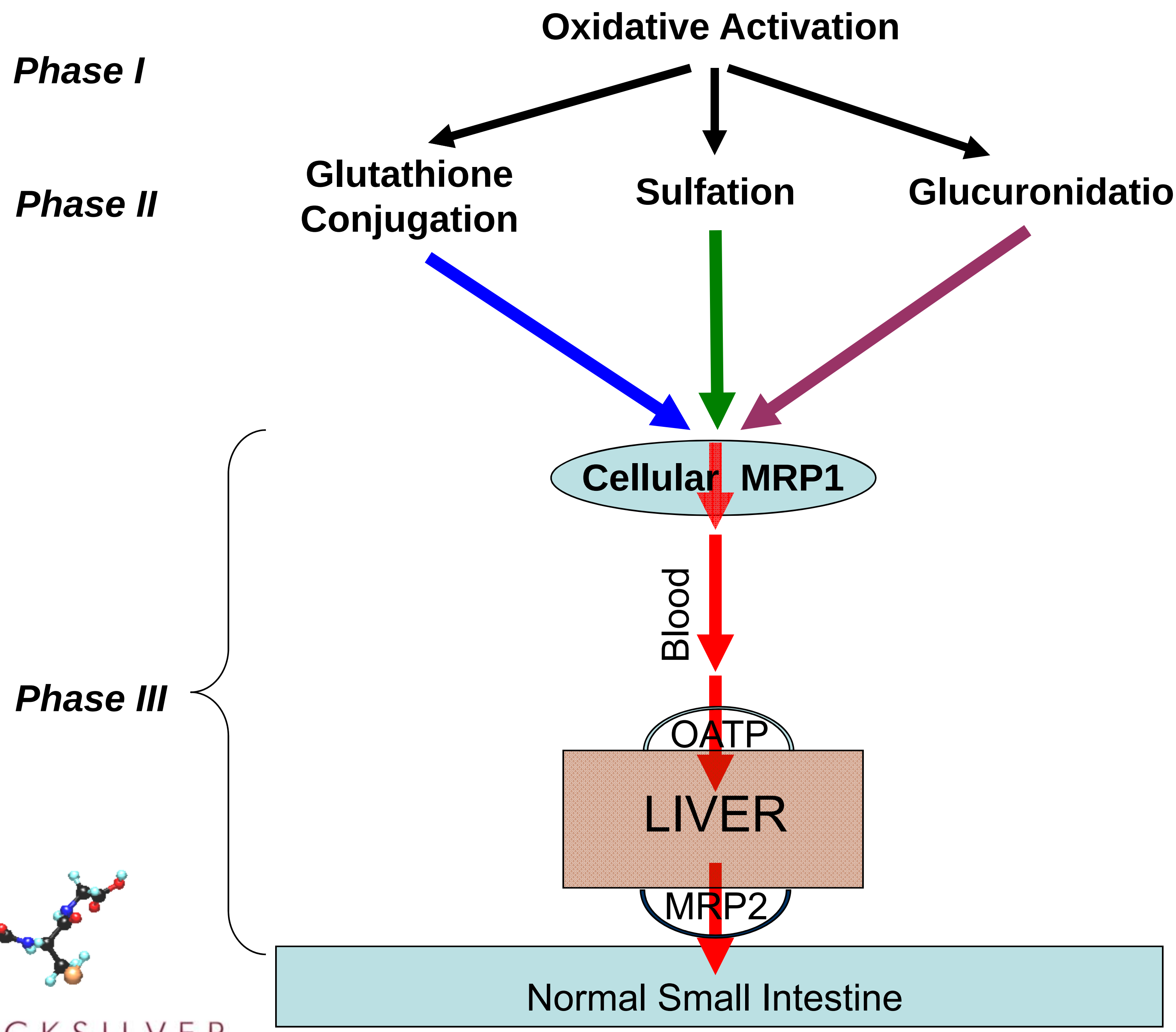
	Young	Old	% Change
MnSOD	3.37	2.11	**-37%**
GR	5.61	3.52	**-37%**
GST	0.98	0.64	**-35%**
G6PDH	3.25	1.64	**-50%**
CAT	4.59	5.72	**25%**
GPx	6.44	7.96	**24%**

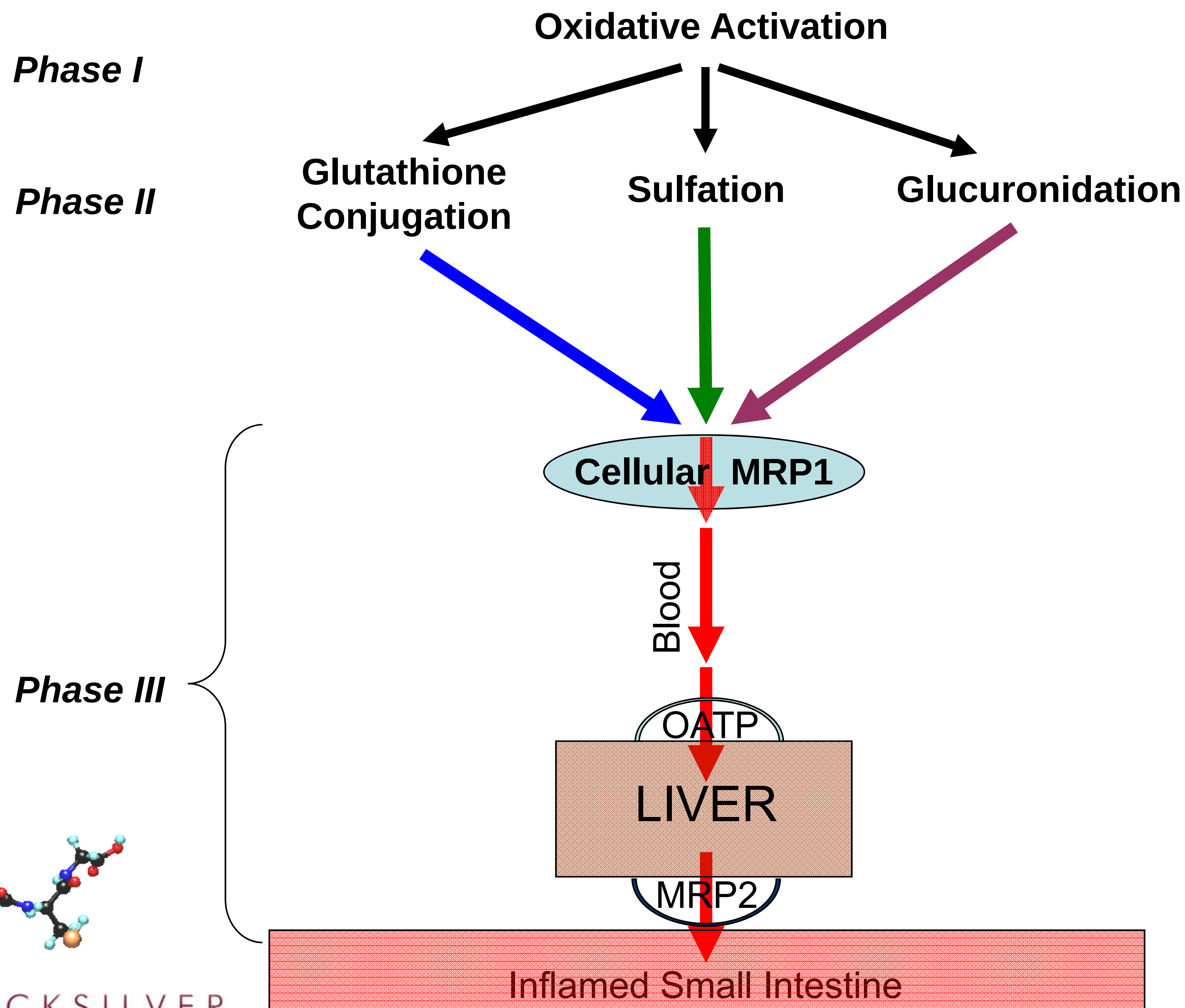


Breakdown of the defense system

- **Glutathione deficiency**
  - Genetic
  - Environmental
- **Glutathione S-Transferase (*Phase II*) problems**
  - Genetic
  - Environmental
- ***Phase III can get blocked and then downregulates Phase II enzymes***
  - ***Can stop multiple detoxification pathways and control the expression of the Glutathione system!***




Oxidative Activation
Phase I
Phase II
Glutathione Conjugation
Sulfation
Glucuronidation
Cellular MRP1
Blood
Phase III
OATP
LIVER
MRP2
Normal Small Intestine



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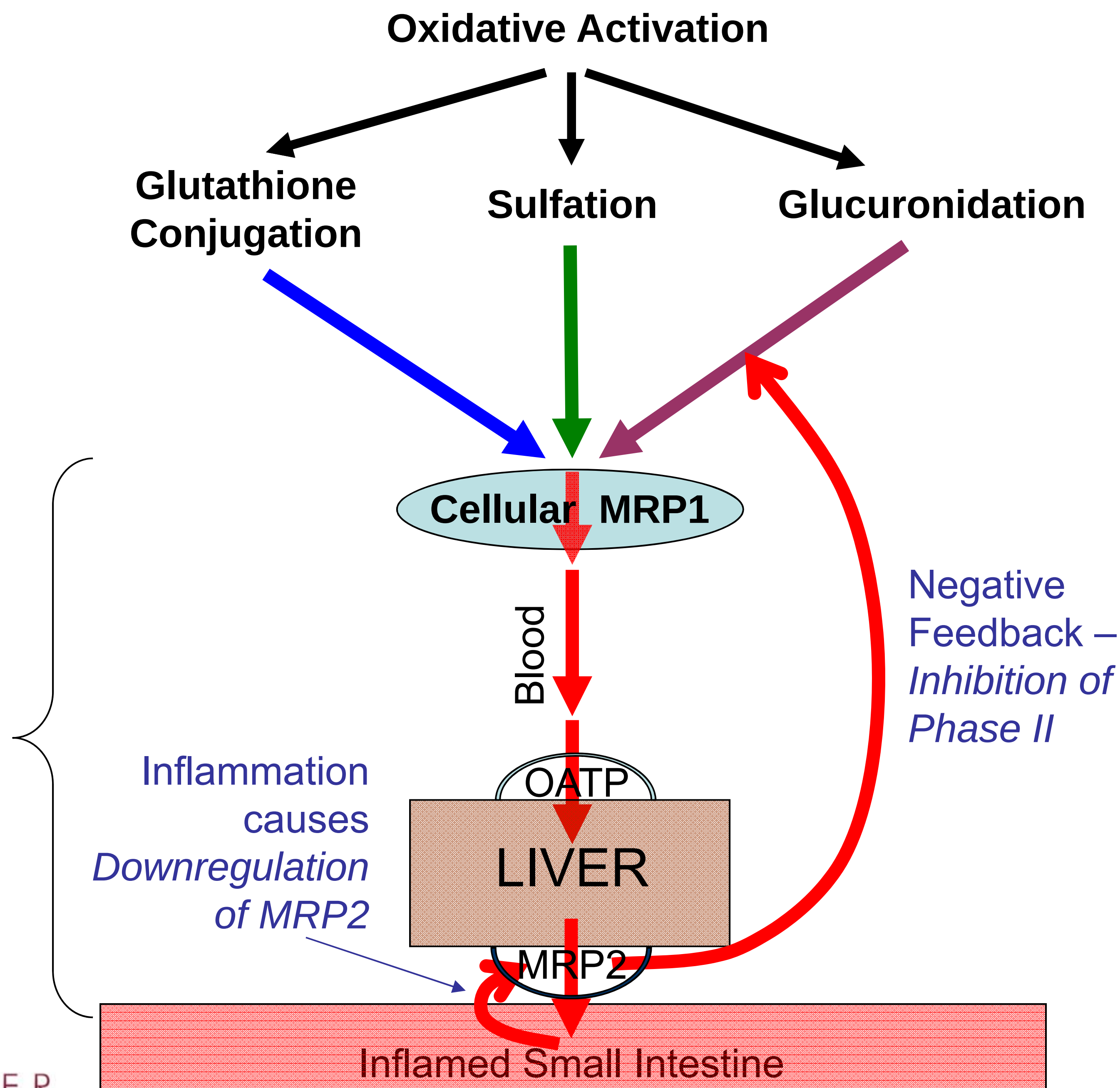

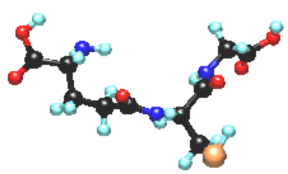

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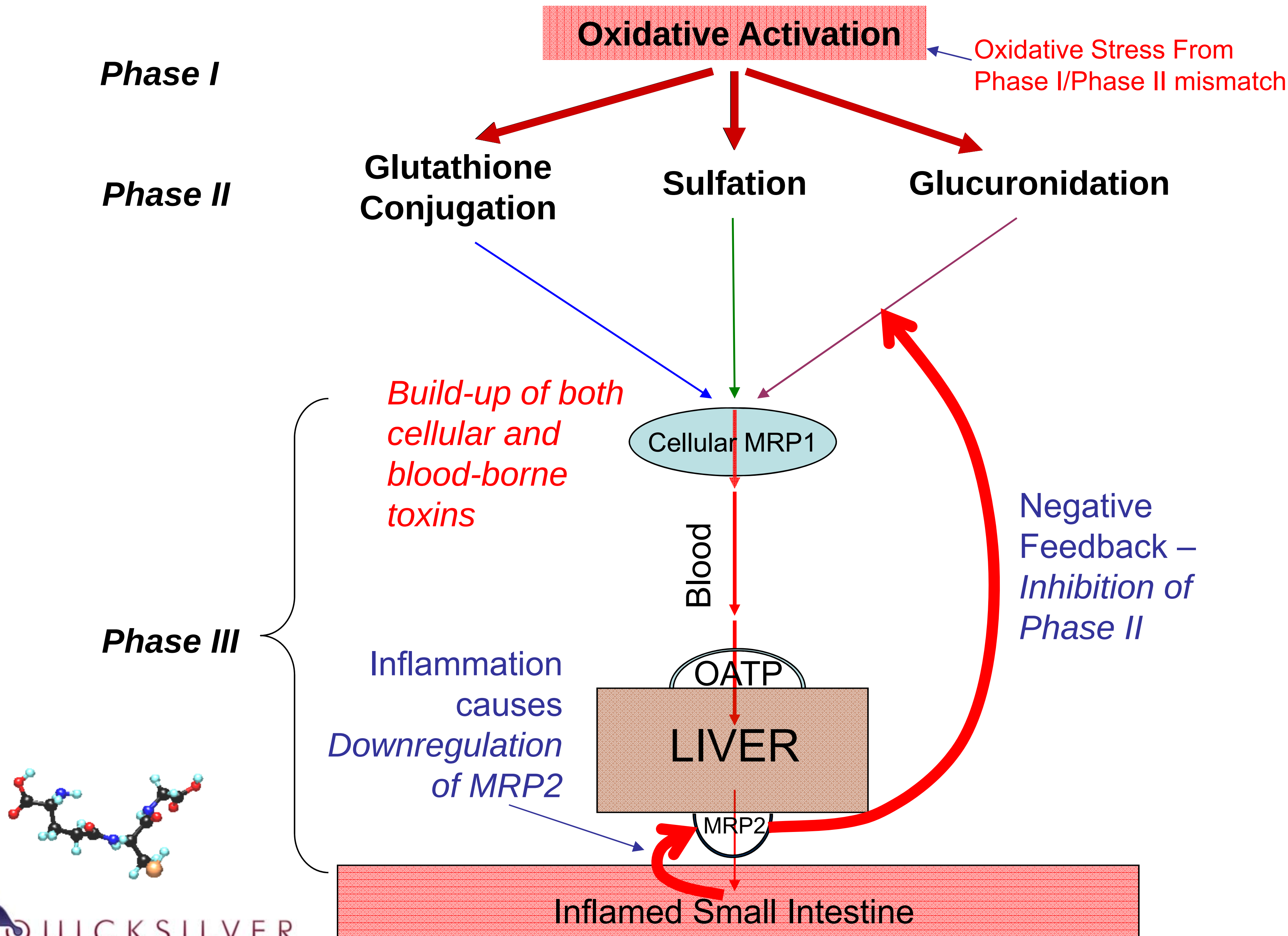
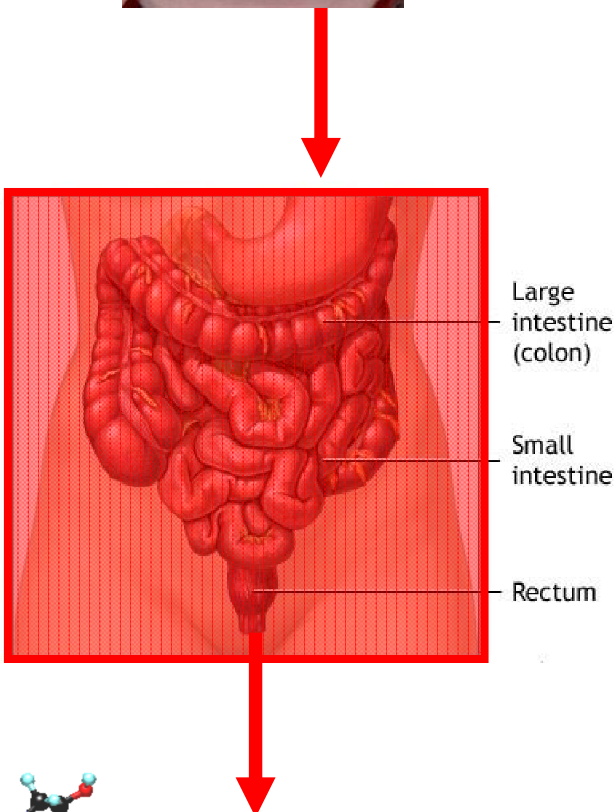
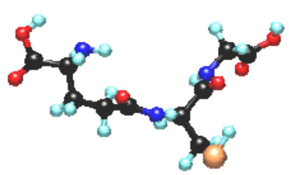


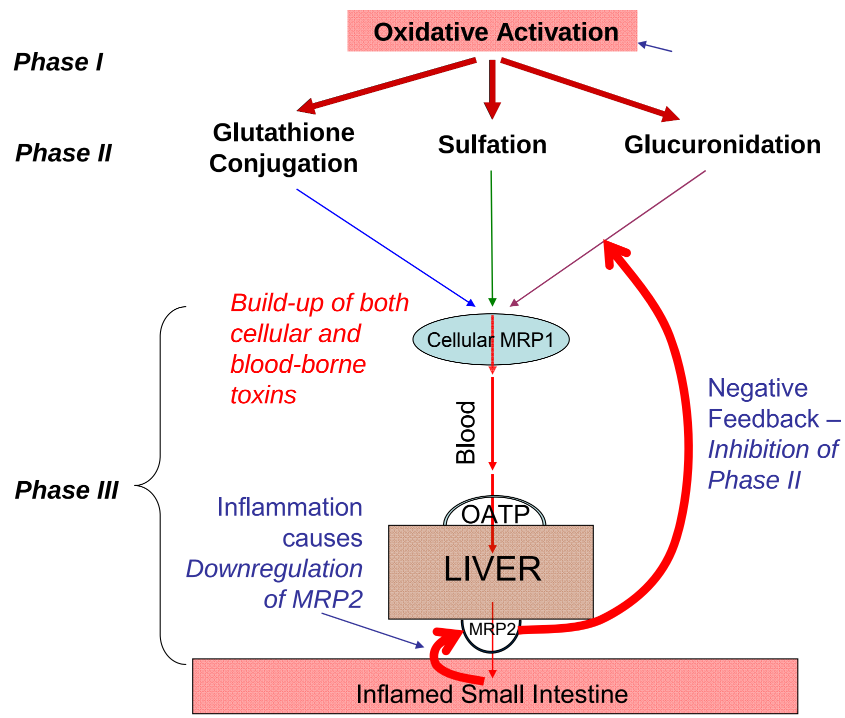
Phase I
Oxidative Activation
Oxidative Stress From Phase I/Phase II mismatch
Phase II
Glutathione Conjugation
Sulfation
Glucuronidation
Phase III
Build-up of both cellular and blood-borne toxins
Cellular MRP1
Blood
Negative Feedback – Inhibition of Phase II
Inflammation causes Downregulation of MRP2
OATP
LIVER
MRP2
Inflamed Small Intestine

Pathways Out – Amalgam Poisons Intestinal Function





Corrosion Products of Amaglam (Inorganic Hg) are swallowed with saliva and create inflammation



SUPPRESSION OF DRUG-METABOLIZING ENZYMES AND EFFLUX TRANSPORTERS IN THE INTESTINE OF ENDOTOXIN-TREATED RATS

J. Kalitsky-Szirtes, A. Shayeganpour, D.R. Brocks, and M. Piquette-Miller

Department of Pharmaceutical Sciences, University of Toronto, Toronto, Ontario, Canada (J.K.-S., M.P.-M.); and Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta, Edmonton, Alberta, Canada (A.S., D.R.B.)

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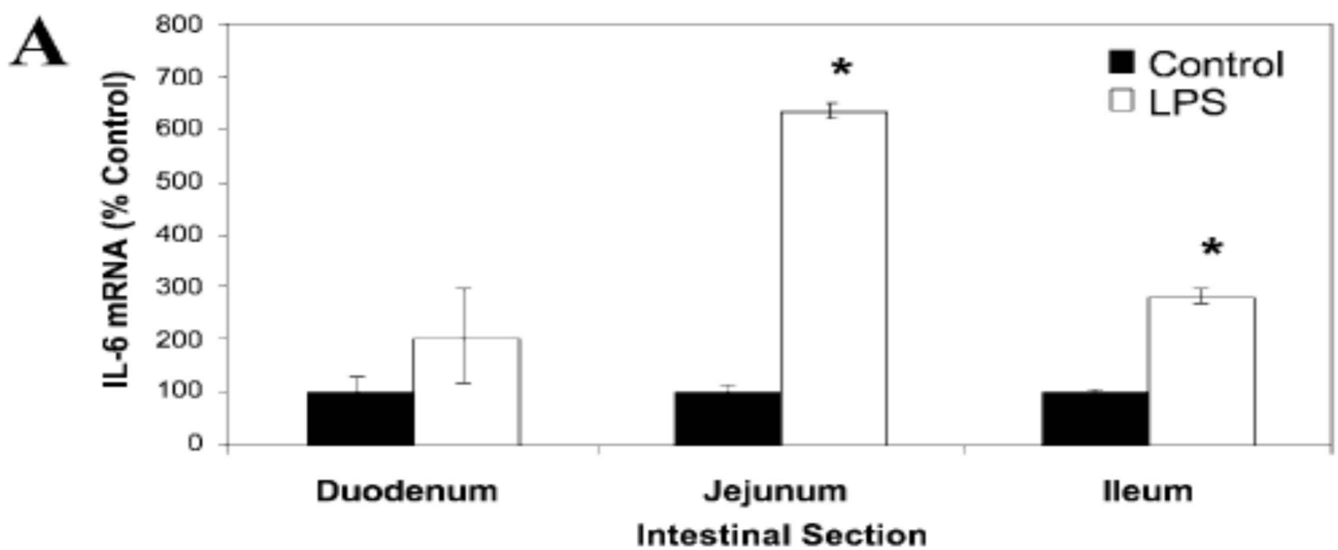

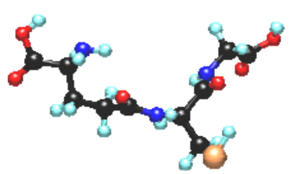

SUPPRESSION OF DRUG-METABOLIZING ENZYMES AND EFFLUX TRANSPORTERS IN THE INTESTINE OF ENDOTOXIN-TREATED RATS

J. Kalitsky-Szirtes, A. Shayeganpour, D.R. Brocks, and M. Piquette-Miller

Department of Pharmaceutical Sciences, University of Toronto, Toronto, Ontario, Canada (J.K.-S., M.P.-M.); and Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta, Edmonton, Alberta, Canada (A.S., D.R.B.)

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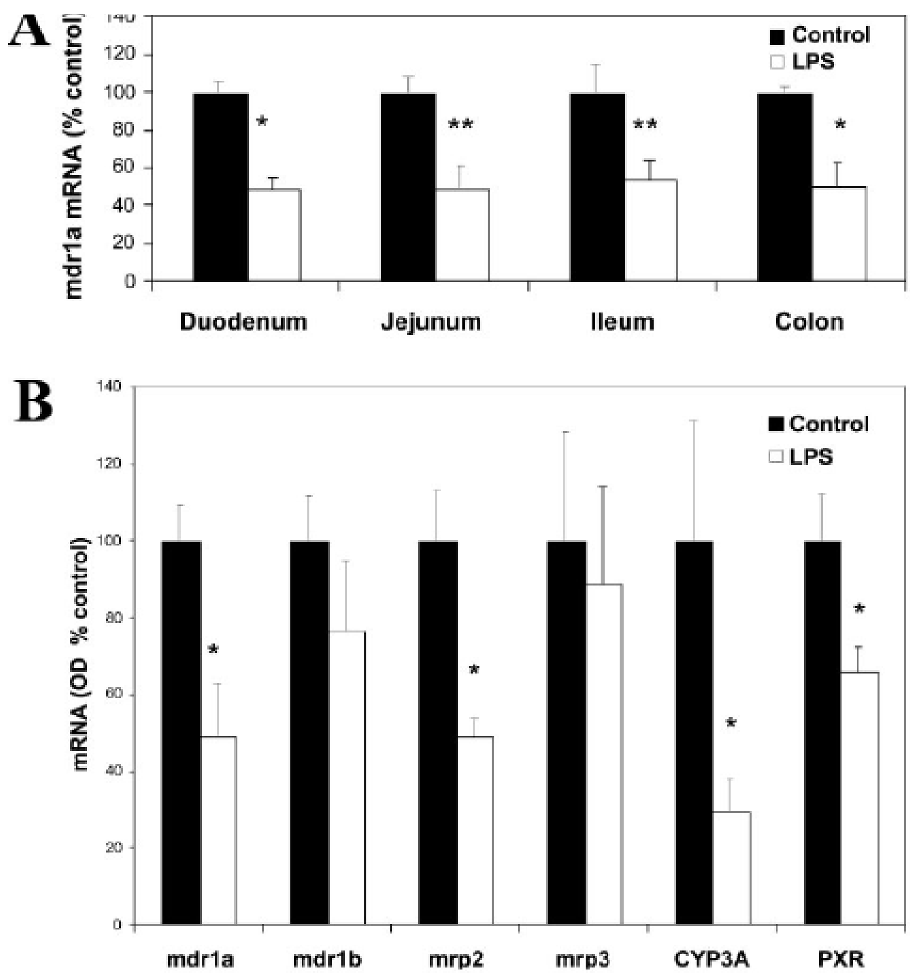
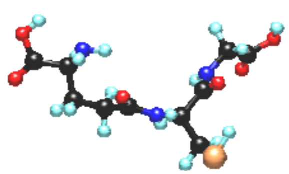
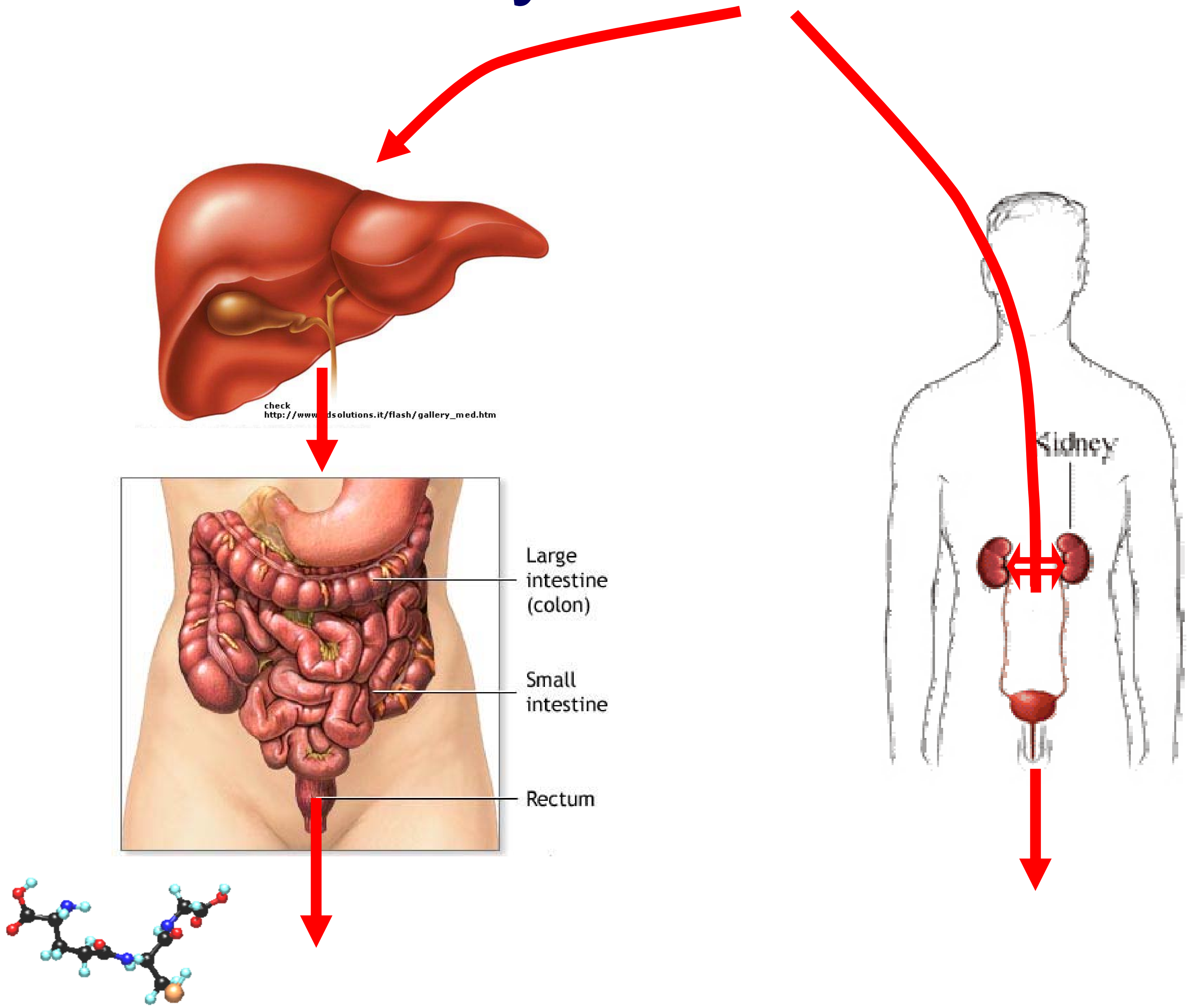
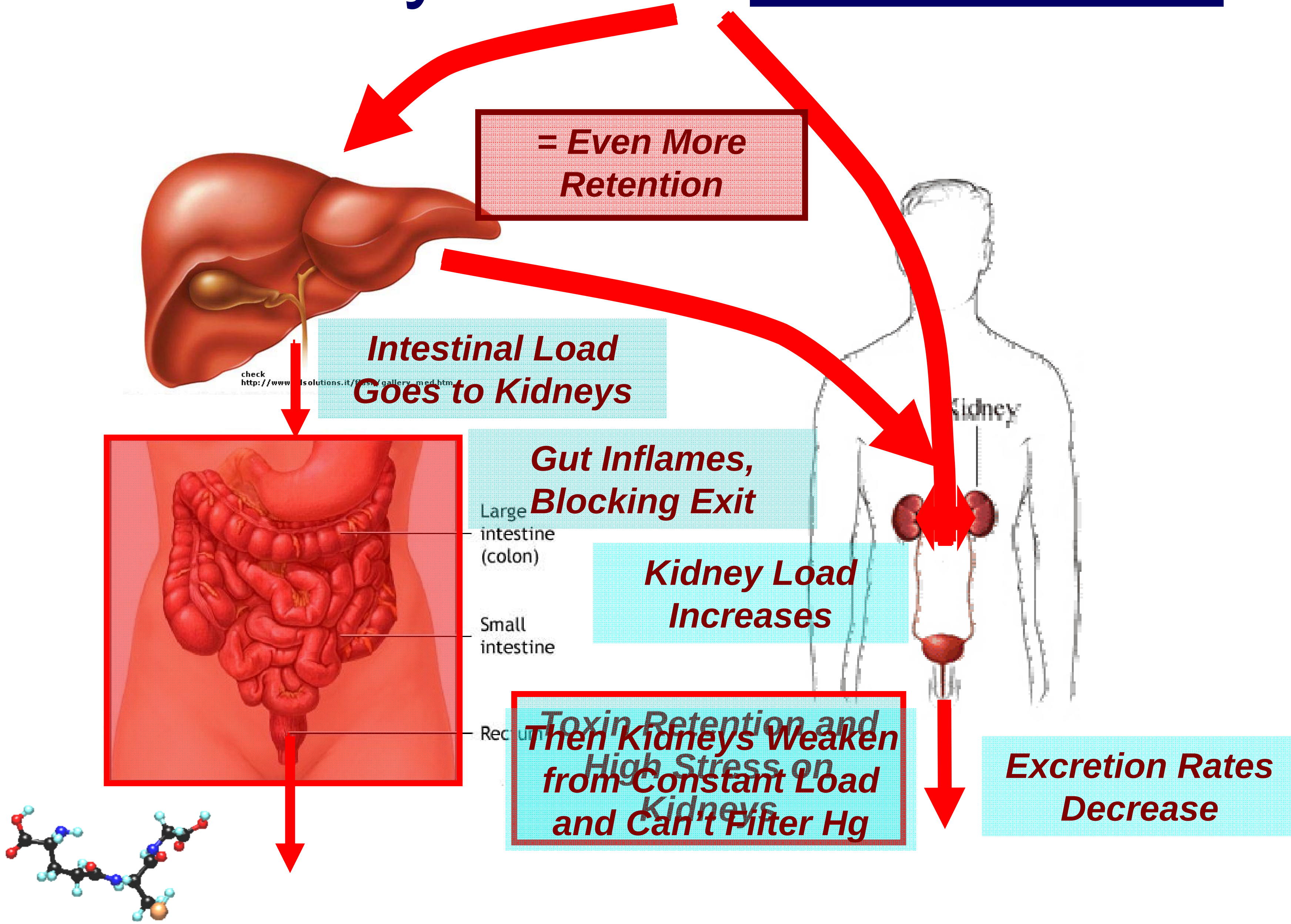




Pathways Out – Normal



Pathways Out – *Gut Blocked*

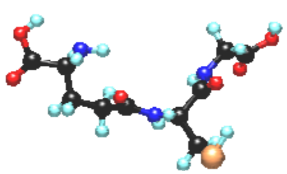


Cellular Biochemical Detoxification Requirements

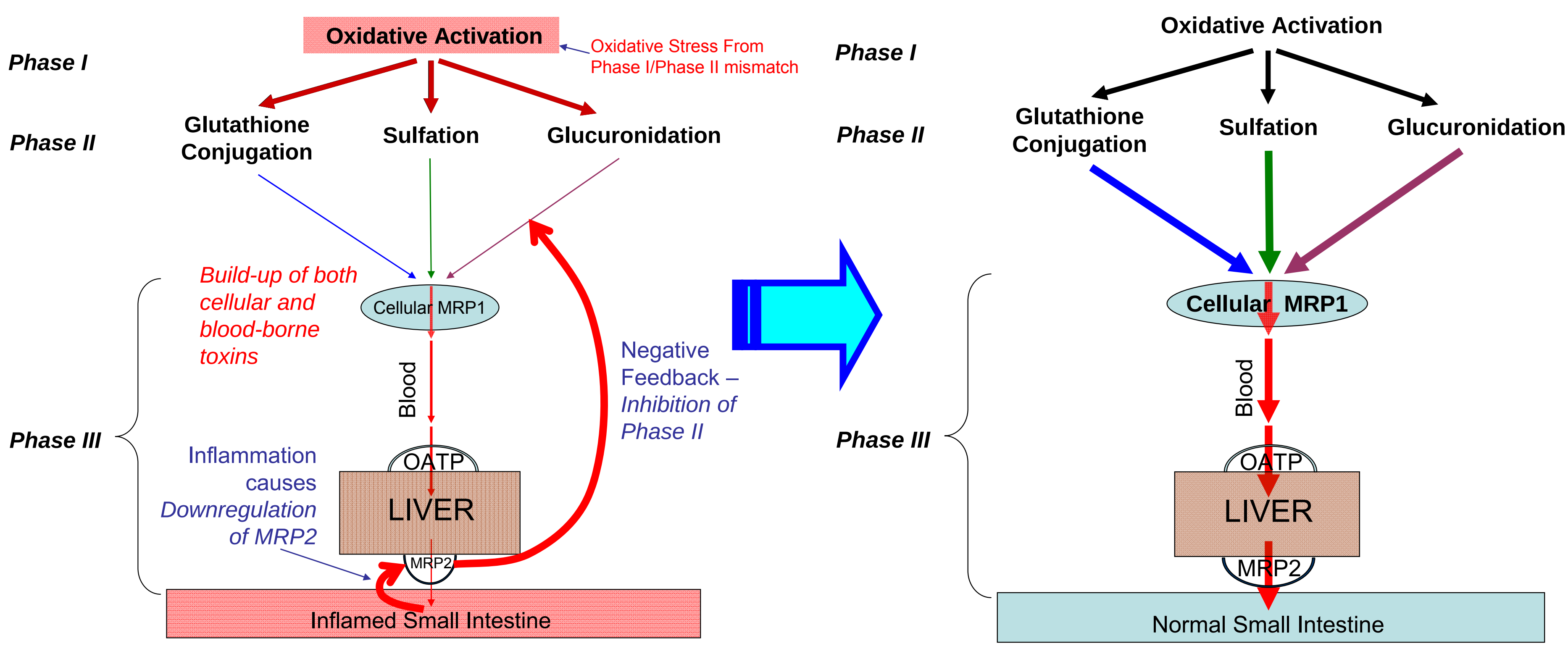
1. Effective Phase III Clearance including intestinal binding and Elimination

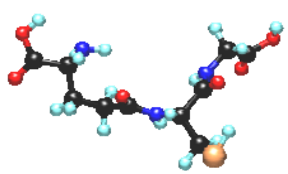
2. Effective GST Activity(Phase II-Mobilization)

3. Intracellular Antioxidant Sufficiency



Correct Phase III Elimination





QUICKSILVER SCIENTIFIC

Gut-based “Binders”

Different types of Binding – thiol binding, anion exchange, cation exchange, sorption binding

- Charcoal
- Chlorella
- Clays/Zeolites
- Pectin
- Alginates
- Thiol-functionalized solids

Phase III Enhancement

Bilirubin (unrelated to GSH) falls too

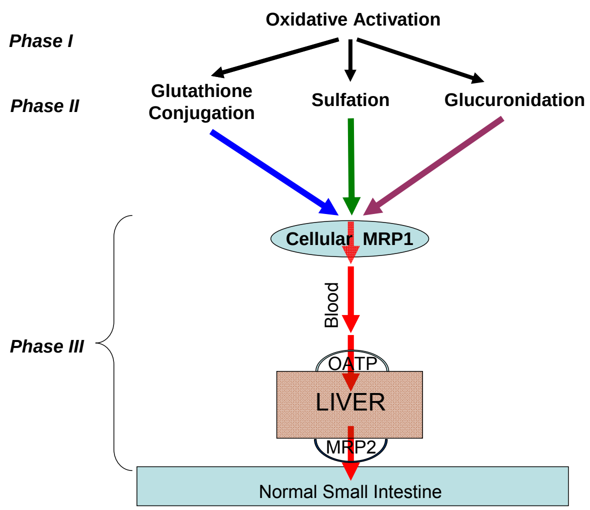
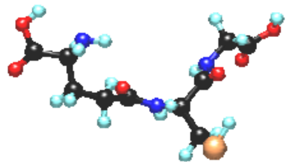


Table 2. Bilirubin Levels on Select Patients with Elevated Blood Bilirubin (From Clinics 1 & 2, 7-10 interventions)

Before	After	% Change
2	1.1	-45
1.7	0.9	-47.1
1.4	1.3	-7.1
1.2	0.9	-25.0
3.4	2.6	-23.5
1.7	1.2	-29.4



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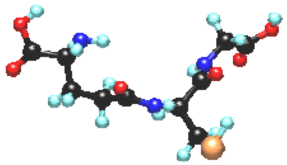
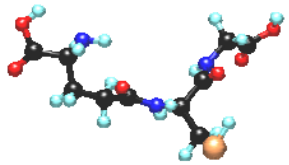
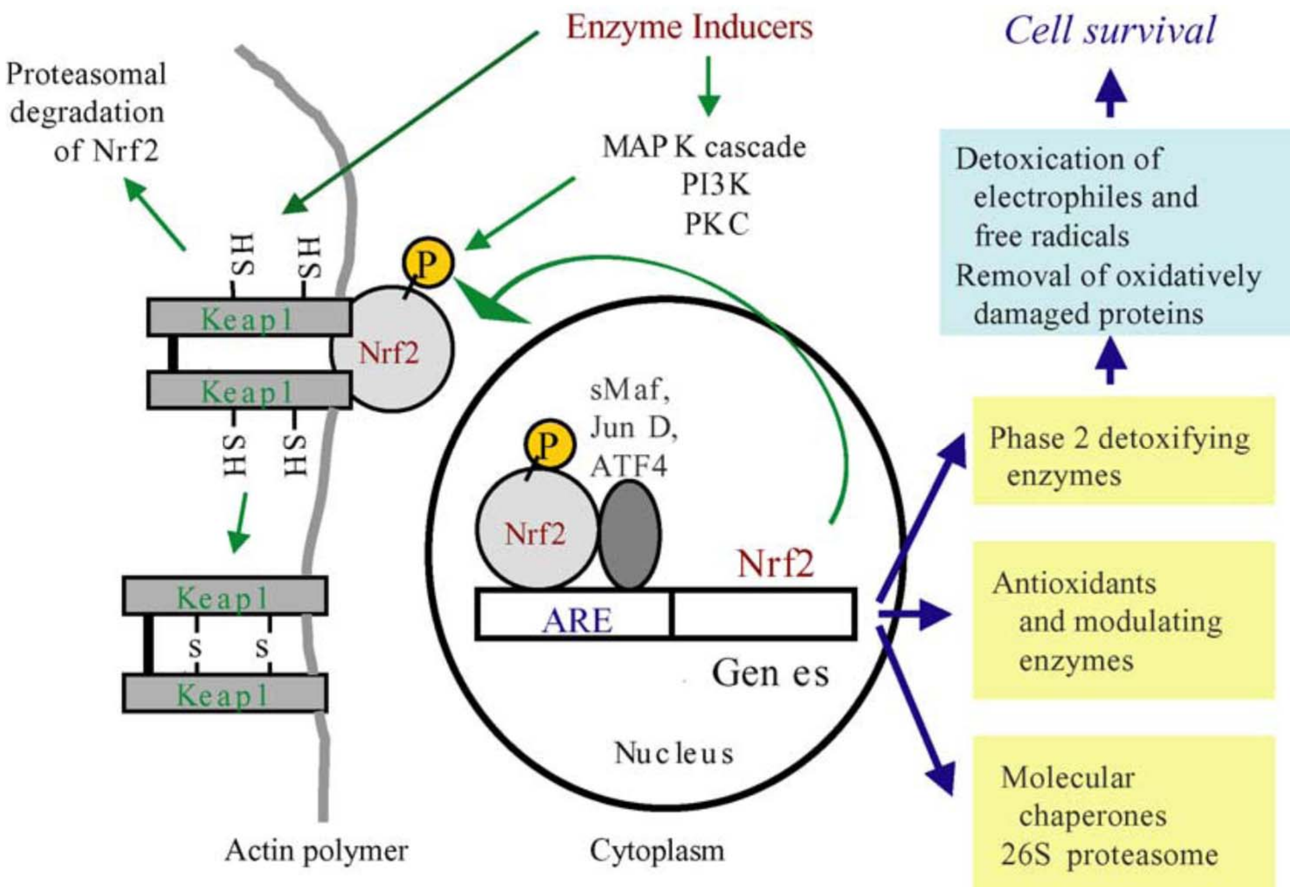


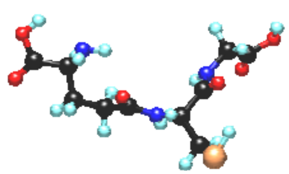
“Phytogenomics”

- Certain Phytochemicals *upregulate Phase II enzymes as well as GSH, SOD* (cellular antioxidants)
- *The Anti-Inflammatory Cascade*
- Polyphenolic Antioxidants
- Sulfur compounds
  – Alpha Lipoic Acid
  – Crucifers
  – Garlic oil



Chemoprevention by Keap1-Nrf2 Signaling pathway by Phase II Inducers

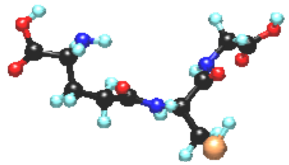




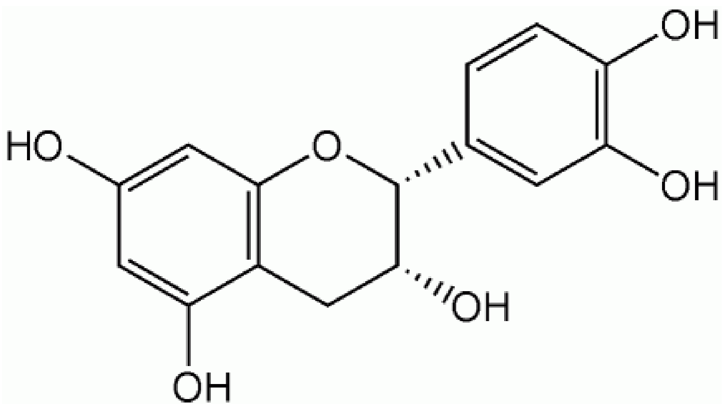
Kwak et al., **2004**, *Mutation Research,* 555:133-148

Polyphenolics

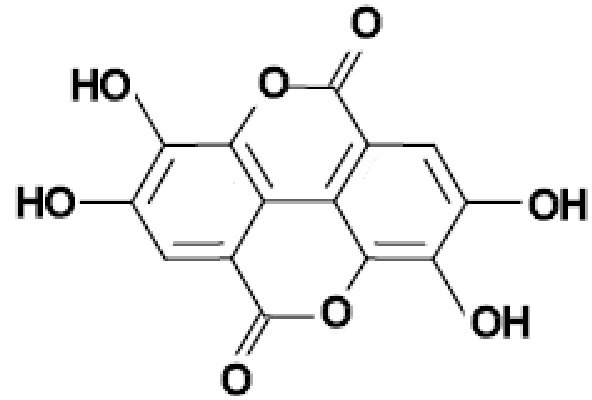
- ***Anti-inflammatory cascade***
- Upregulate **Phase II enzymes** through binding to membrane and nuclear receptors (transcription factors)
- **Vascular protective effects** (strengthen capillaries and improve oxygen delivery)
- Anti-carcinogenic
- *Cross Blood-Brain Barrier*



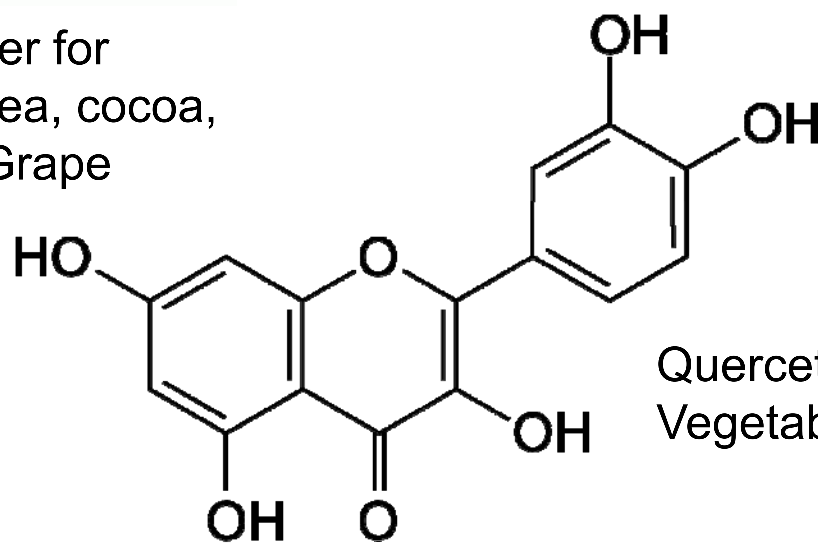
Flavanols (Polyphenolics)



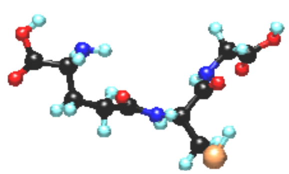
Epicatechin – monomer for OPC's from in green tea, cocoa, red wine, Pine Bark, Grape seeds



Ellagic Acid - Pomegranates



Quercetin – Fruit and Vegetable Skins



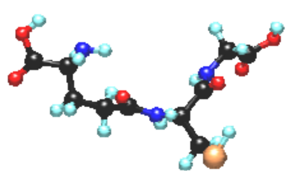
Decay in GSH levels and Enzyme Activity with Age

Table 2. Effect of *T. chebula* aqueous extract on enzymatic antioxidants in young and aged rats

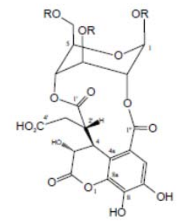
	Young		Aged	
	Control	Treated	Control	Treated
Liver				
MnSOD (50% reduction of NBT/min/mg protein)	3.37 ± 0.20	3.55 ± 0.18	2.11 ± 0.24 [a‡]	3.13 ± 0.23 [b‡]
CAT (μmol H_2O_2 consumed/min/mg protein)	4.59 ± 0.31	4.51 ± 0.22	5.72 ± 0.28 [a‡]	4.54 ± 0.25 [b‡]
GPx (μmole GSH utilized/min/mg protein)	6.44 ± 0.24	6.53 ± 0.20	7.96 ± 0.26 [a‡]	6.67 ± 0.23 [b‡]
GR (nmol NADPH oxidized/min/mg protein)	5.61 ± 0.28	5.73 ± 0.31	3.52 ± 0.26 [a‡]	5.46 ± 0.25 [b‡]
GST (μmoles of CDNB-GSH conjugated/min/mg protein)	0.98 ± 0.02	1.03 ± 0.03 [a§]	0.64 ± 0.02 [a‡]	0.99 ± 0.04 [b‡]
G6PDH (Units/min/mg protein)	3.25 ± 0.18	3.54 ± 0.14 [a§]	1.64 ± 0.16 [a‡]	3.14 ± 0.19 [b‡]
Kidney				
MnSOD (50% reduction of NBT/min/mg protein)	3.35 ± 0.18	3.42 ± 0.21	2.06 ± 0.16 [a‡]	3.21 ± 0.23 [b‡]
CAT (μmol H_2O_2 consumed/min/mg protein)	4.14 ± 0.20	4.20 ± 0.15	4.92 ± 0.22 [a‡]	4.27 ± 0.22 [b‡]
GPx (μmole GSH utilized/min/mg protein)	5.18 ± 0.27	5.07 ± 0.31	6.03 ± 0.25 [a‡]	5.26 ± 0.29 [b‡]
GR (nmol NADPH oxidized/min/mg protein)	4.35 ± 0.24	4.48 ± 0.24	2.36 ± 0.26 [a‡]	4.39 ± 0.22 [b‡]
GST (μmoles of CDNB-GSH conjugated/min/mg protein)	1.32 ± 0.06	1.41 ± 0.03 [a†]	0.92 ± 0.04 [a‡]	1.35 ± 0.03 [b‡]
G6PDH (Units/min/mg protein)	1.80 ± 0.08	1.88 ± 0.06	1.11 ± 0.08 [a‡]	1.83 ± 0.05 [b‡]

Each value is expressed as mean ± SD for six rats in each group. Superscript letters represent $p < 0.05$ (Tukey-Kramer Multiple comparisons Test).
[a]As compared with Young control,
[b]As compared with Aged control.
§$p < 0.05$; †$p < 0.01$; ‡$p < 0.001$.

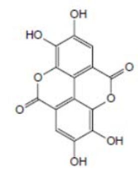
	Young	Old	% Change
MnSOD	3.37	2.11	**-37%**
GR	5.61	3.52	**-37%**
GST	0.98	0.64	**-35%**
G6PDH	3.25	1.64	**-50%**
CAT	4.59	5.72	**25%**
GPx	6.44	7.96	**24%**



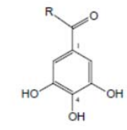
Decay in GSH levels and Enzyme Activity with Age



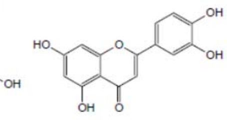
R = galloyl, chebulinic acid (1)
R = H, galloyl-free chebulinic acid (2)



ellagic acid (3)



R = radical, galloyl group
R = OH, gallic acid (4)
R = OCH_2CH_3, ethyl gallate (5)



luteolin (6)

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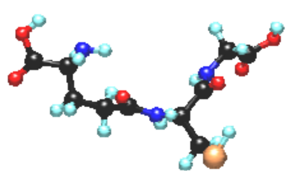
GST	0.98	0.64	**-35%**	0.99
G6PDH	3.25	1.64	**-50%**	3.14
CAT	4.59	5.72	**25%**	4.54
GPx	6.44	7.96	**24%**	6.67

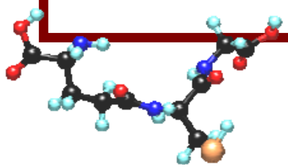


Defense – Glutathione System

Antioxidant, Detoxification, Protein Repair

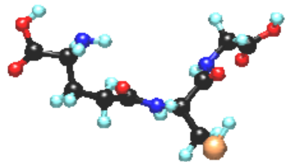
NEED WHOLE ENZYME SYSTEM!!!

- Synthetases (synthesize GSH from precursors)
- Transpeptidases (take apart and reassemble)
- Transferases (Phase II conjugation)
- Peroxidases (radical quenching)
- Reductases (repair after quenching)
- Redoxins (using GSH as reducing equivalent for protein repair)
- ***Glutathionylation*** *– protection of Proteins*



Sulfur Compounds

- ***Anti-inflammatory cascade***
- Upregulate Phase II (and Phase III) enzymes through binding to membrane and nuclear receptors
- Vascular protective effects
- Anti-carcinogenic



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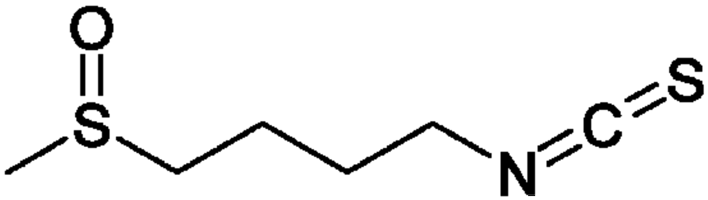
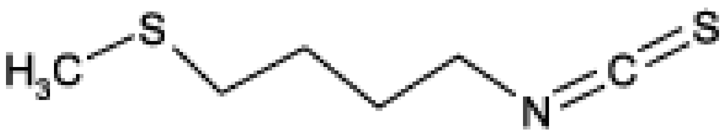
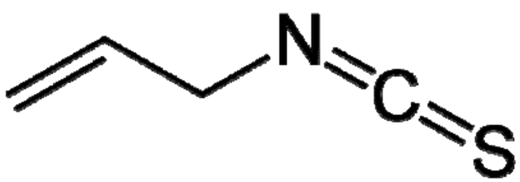


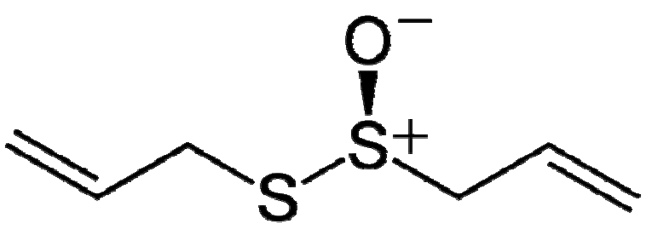
Sulforaphane – the famous crucifer compound



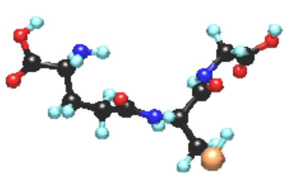
Erucin – from crucifers; not as strong as SF



Allyl-isothiocyanate – “Oil of mustard”, horseradish



Allicin – from garlic



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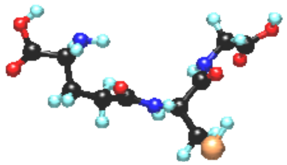
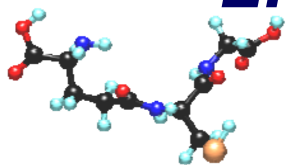


Nutritional Glutathione Support

1. ***Vitamin C*** supports antioxidant system and Glutathione synthesis
2. Antioxidant Phytonutrients (***Polyphenolics***) and ***Alpha Lipoic Acid*** upregulate Glutathione production
3. Glutathione Precursors feed Glutathione synthesis
    1. ***N-Acetyl Cysteine***
    2. ***Whey Protein***



Biochemical Hg Removal Requirements – GSH Sufficiency

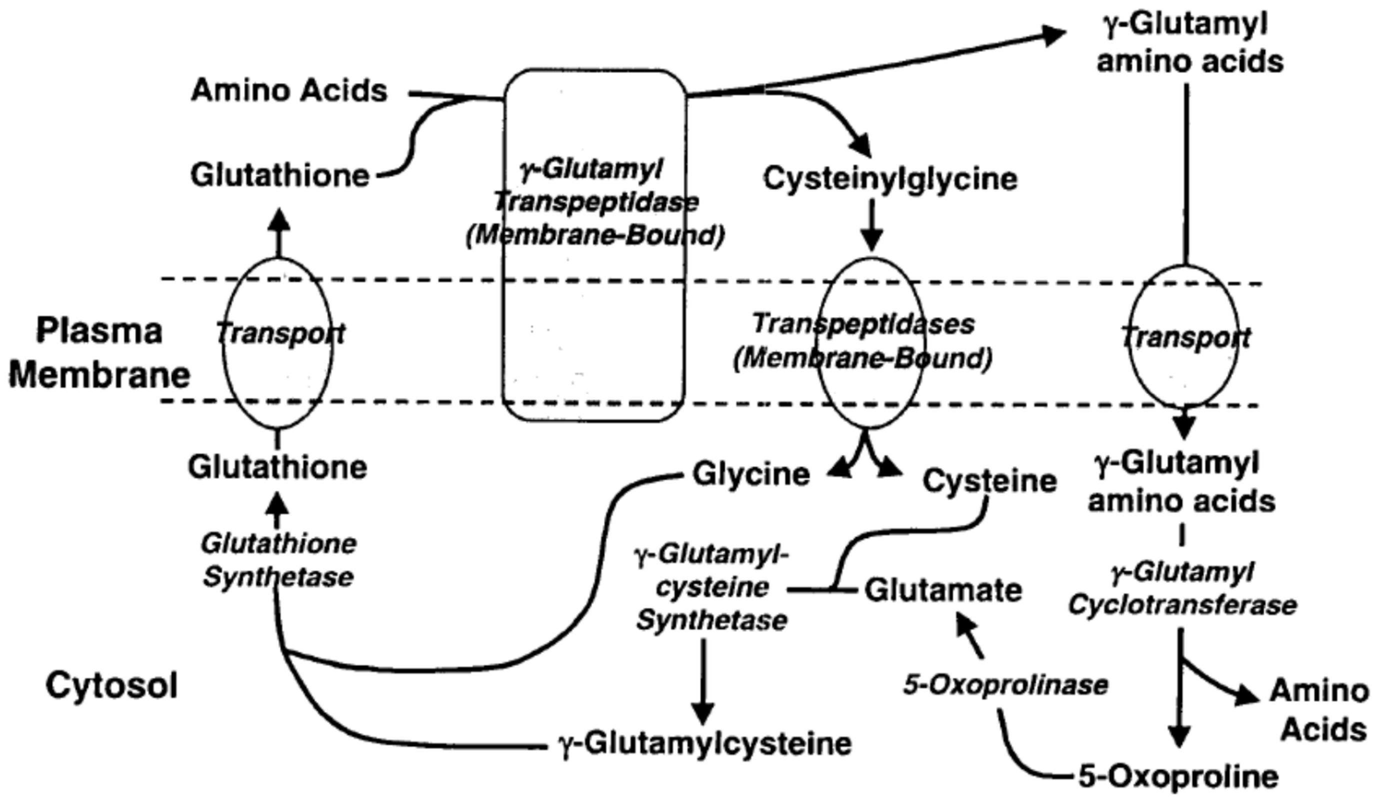
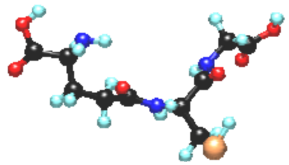
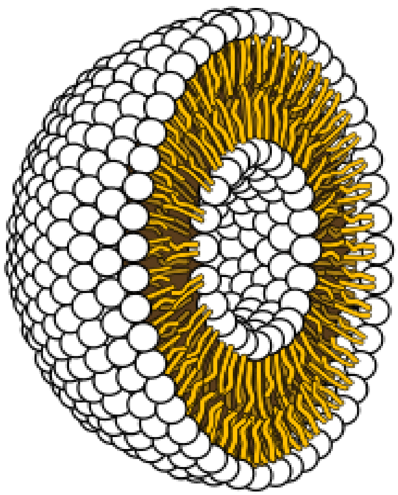


FIGURE 1 The glutathione cycle.



Nanosphere Encapsulation/Liposomes



Liposomal Encapsulation

•Phospholipid bilayer

•Bypasses peptidases that break down GSH and barrier to EDTA and high-dose Vitamin C

•Direct absorption in upper intestine

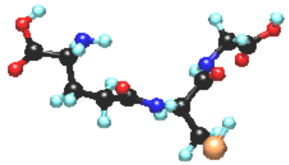
•Proven enhanced intracellular delivery

Nanosphere Encapsulation

•Same principles - Phospholipid shell, etc

•Smaller size and more structural integrity – missed by reticulo-endothelial filtration system

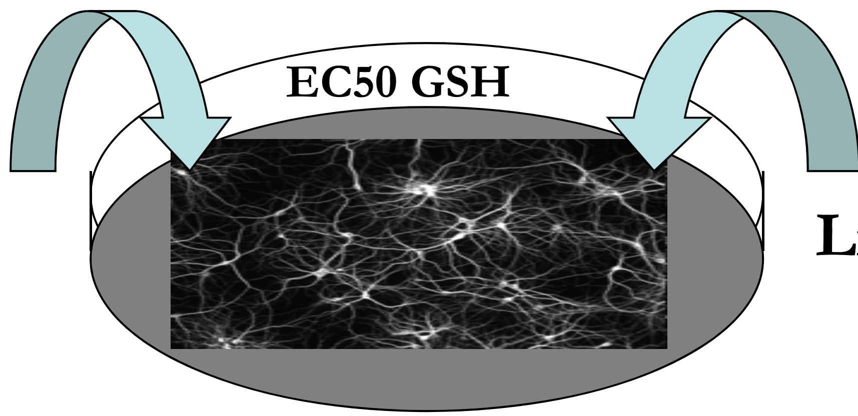
•Small size = intraoral absorption



Can L-GSH Improve GSH Concentration across Membranes?

- **Neuron cell culture depleted of GSH by DEM (diethyl maleate)**
- **L-GSH was > 100-fold more potent than plain GSH in solution (non-L-GSH) in replenishing intracellular GSH**

Mol Wt. GSH - 304



Plain GSH
575 µM

Liposomal GSH
4.75 µM

Zeevalk G, Guilford F, Bernard L. Liposomal glutathione for replenishment and maintenance of intracellular glutathione in mesencephalic cultures. Abstract Neuroscience 2009: Soc. for Neuroscience 2009 Chicago, Oct 17, 2009

Coordinated Expression of Phase II and III

Coordinated nuclear receptor regulation of the efflux transporter, Mrp2, and the phase-II metabolizing enzyme, GSTπ, at the blood–brain barrier

Björn Bauer[1,2], Anika MS Hartz[1,3], Jonathan R Lucking[1], Xiaodong Yang[4], Gary M Pollack[4] and David S Miller[1]

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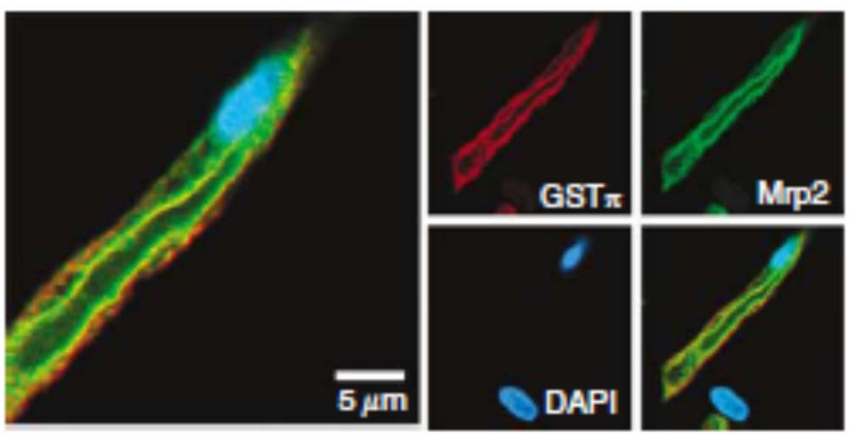

MRP2 and GSTπ coregulated

Review

- See the whole interconnected System
- Supplement with all the players: GSH, Vit C, Vit E, CoQ10, Vit A
- Supplement the phytochemicals that bring up the enzyme system to make the whole system work together
- Clear the gut transport system