Dr Gary Samuelson PhD

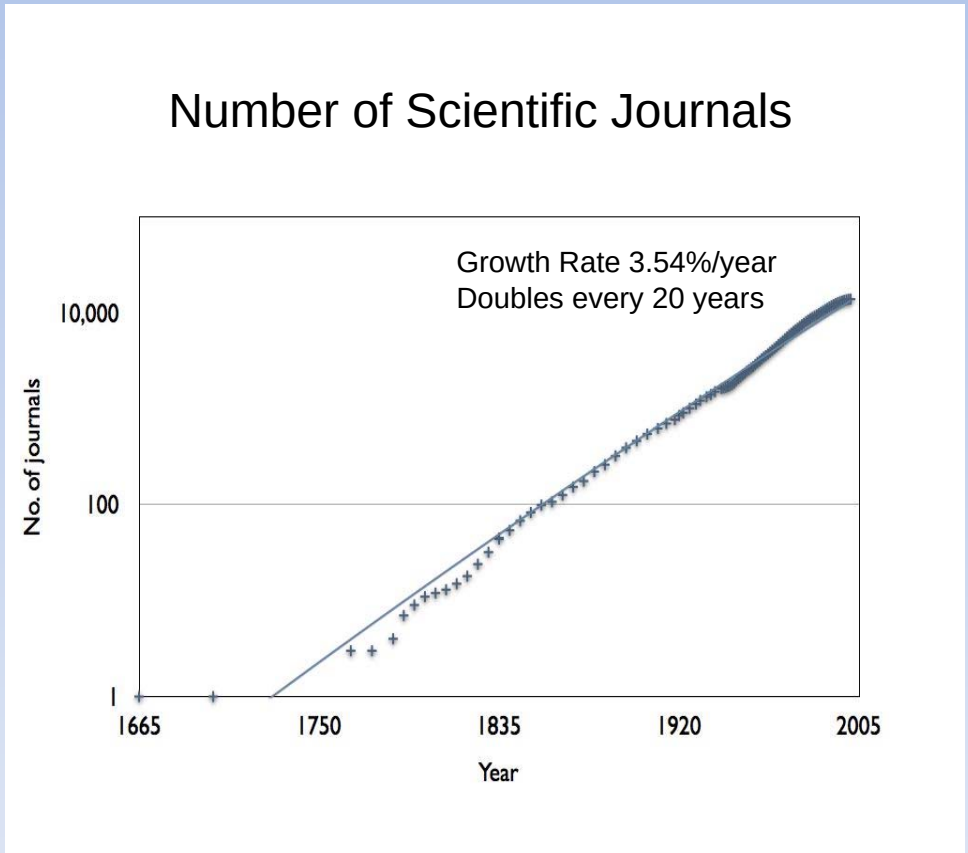
Applications of Redox Biochemistry in Health and Ageing

Gary L. Samuelson, Ph.D.
Atomic/Medical Physics

Independent Science Advisor/Investigator representing a variety of commercial interests

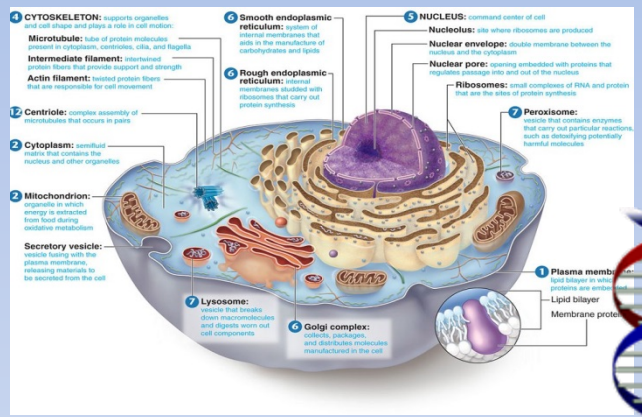
The Journey into Redox Biochemistry

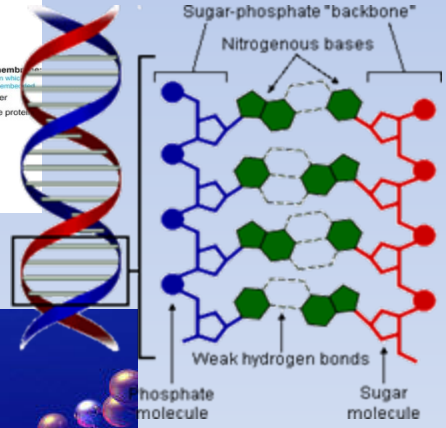
- 1665-1780 (10 Generations)
  - Philosophical Transactions of Royal Society of London
- 1900 (5 Generations)
  - 250 journals, 7000 articles
  - Einstein, Max Plank
- 2013
  - 23,000 journals, 1.4M articles
  - Representing over 10 million months of science published per month



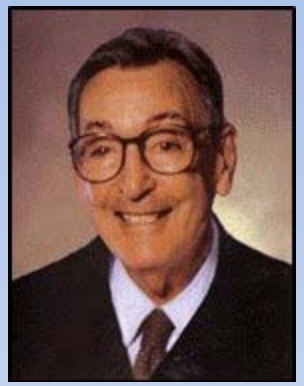
The Journey into Redox Biochemistry

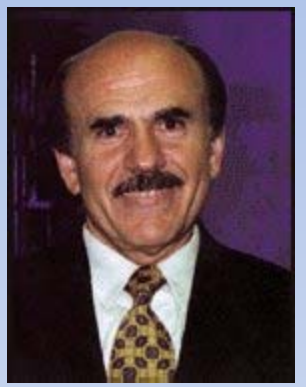
- 1940 (3 Generations)
  - Fundamental Cell Structure
  - Mitochondrial Function
- 1970 (2 Generations)
  - DNA Structure
  - Protein Synthesis
- 2000
  - Human Genome Sequenced
  - Stem Cells, Epigenetics
- 2013
  - Redox Biochemistry
  - Redox Signaling



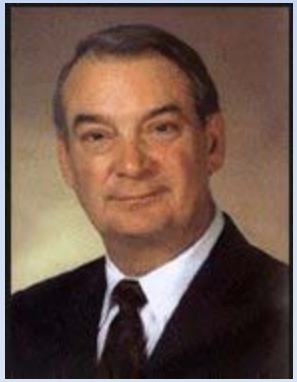


The Journey into Redox Signaling





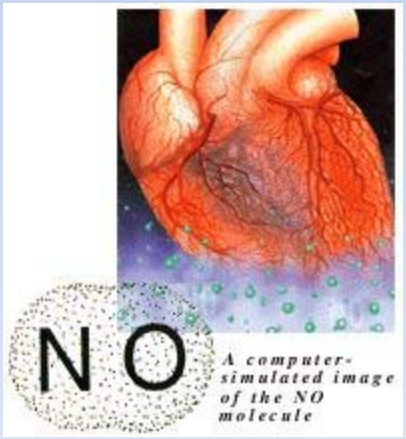
The Nobel Assembly at the Karolinska Institute in Stockholm, Sweden, has awarded the Nobel Prize in Physiology or Medicine for 1998 to **Robert F Furchgott, Louis J Ignarro** and **Ferid Murad** for their discoveries concerning "the nitric oxide as a signalling molecule in the cardiovascular system".

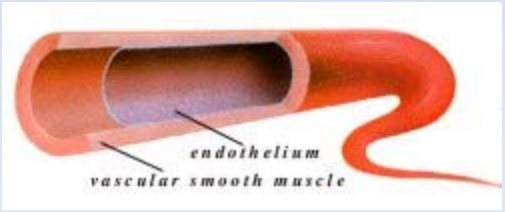


Robert F Furchgott, born 1916
Dept. of Pharmacology,
SUNY Health Science Center
New York

Louis J Ignarro, born 1941
Dept. of Molecular and Medical Pharmacology
UCLA School of Medicine
Los Angeles

Ferid Murad, born 1936
Dept. of Integrative Biology Pharmacology and Physiology
University of Texas Medical School, Houston





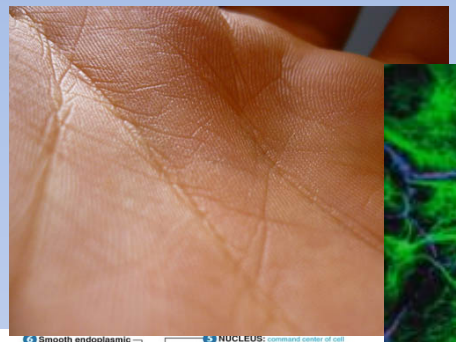
The Journey into Redox Signaling

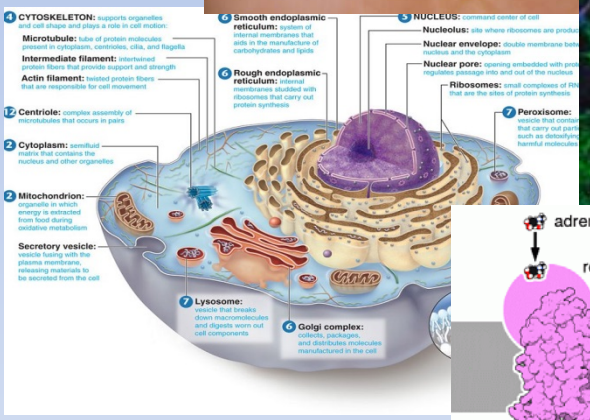
The Royal Swedish Academy of Sciences award the Nobel Prize in Chemistry for 2012 to

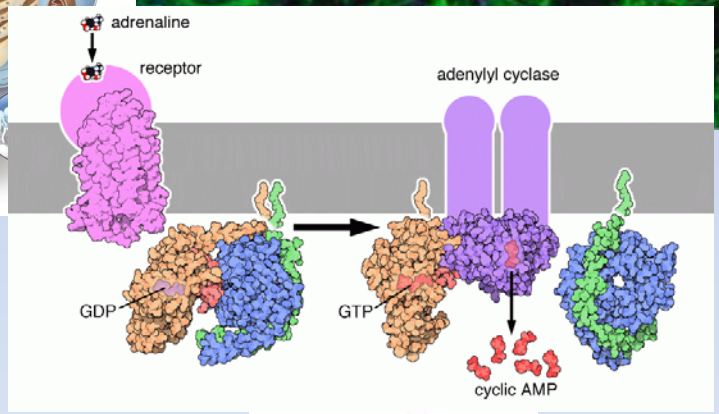
Robert J. Lefkowitz
Howard Hughes Medical Institute and Duke University Medical Center, Durham, NC, USA
and
Brian K. Kobilka
Stanford University School of Medicine, Stanford, CA, USA
"for studies of G-protein–coupled receptors"

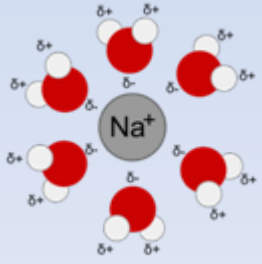
Redox Signaling: The Secret of Life

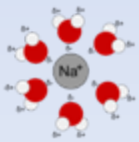
- Diverse and Complex Structures of Life
  - Interconnected Tissues
- Tissues
  - Interconnected Cells
- Cells
  - Interconnected Molecules
- ComplexMolecules
  - Interconnected atomic fields
- Water
  - Interconnects all fields

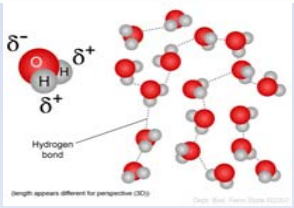


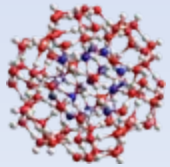






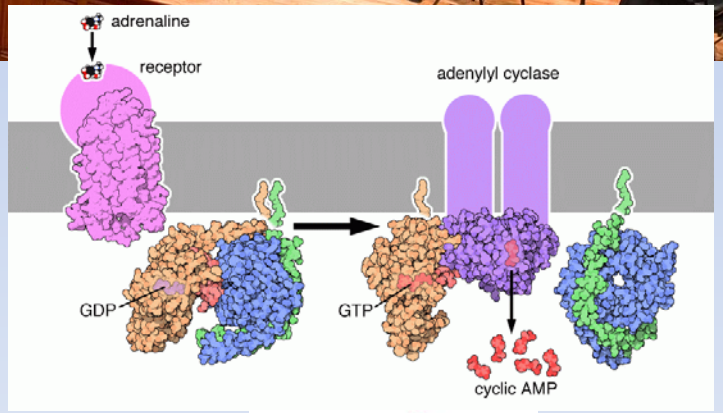


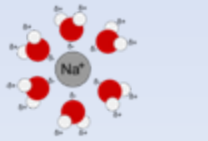


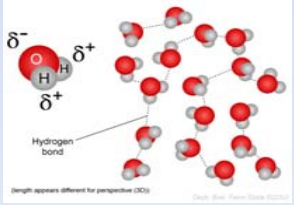


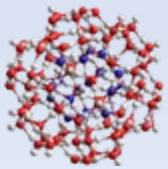
Redox Signaling: The Secret of Life

- Harmonious Orchestration of Life
- Interconnected Molecular Players
- Who/What Conducts the Orchestration?
- Interconnecting Ocean of vital fluids

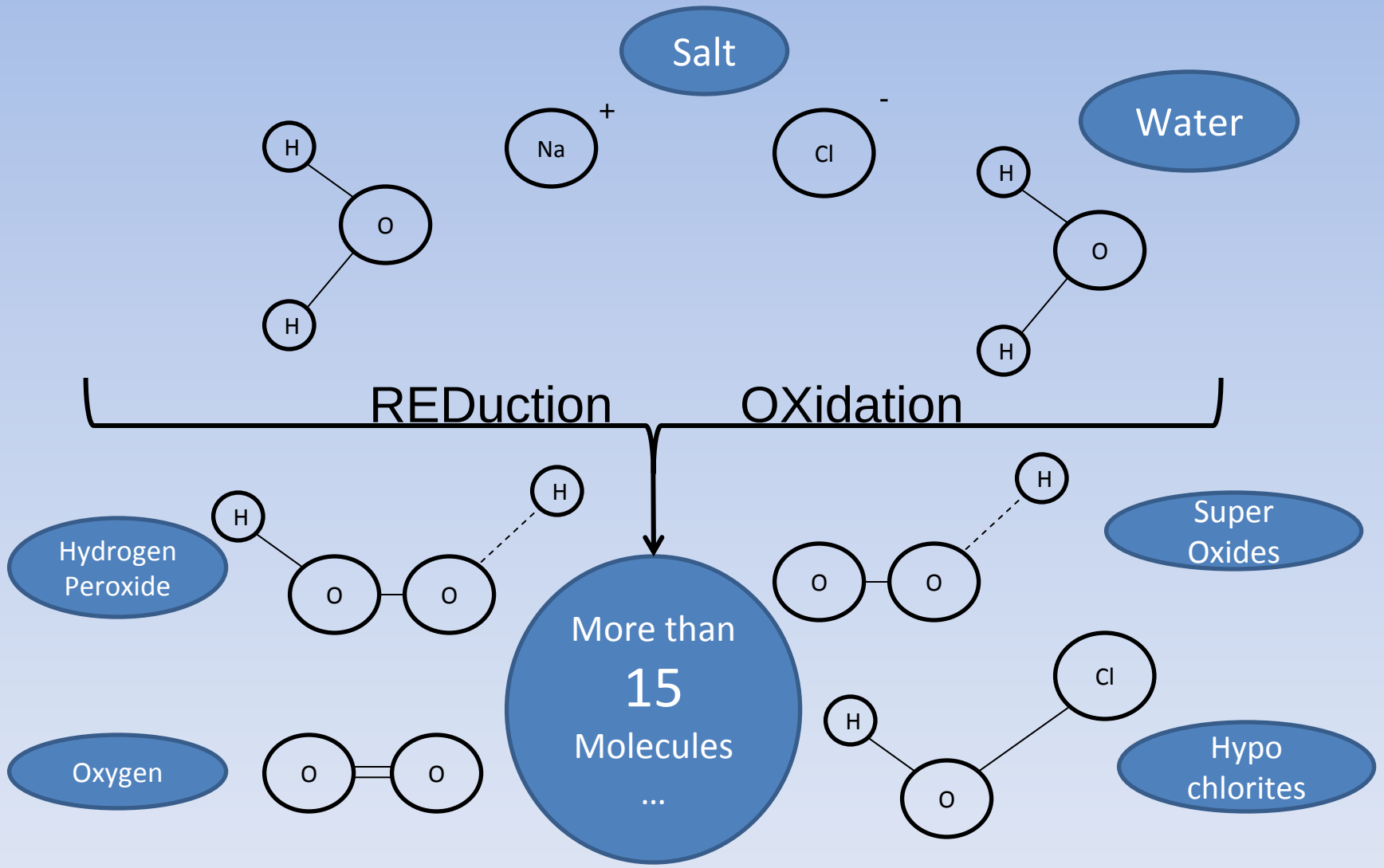








Redox Signaling: Molecules in the Ocean of Life

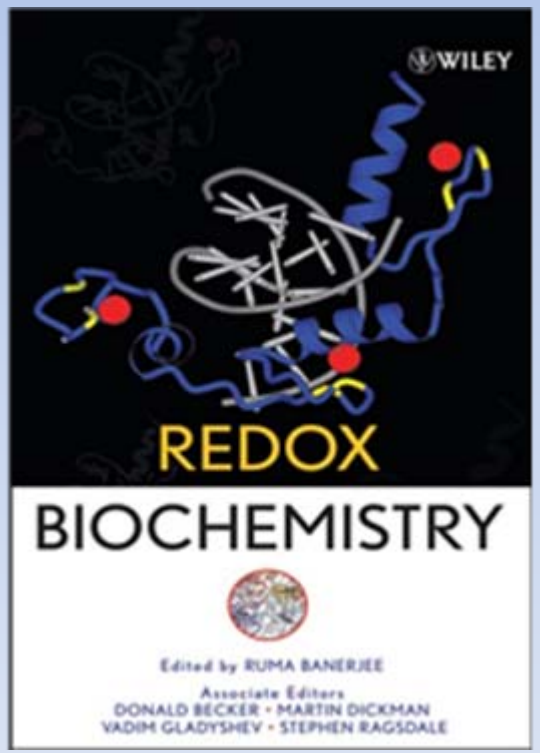


Dr. Britton Chance Lays the Foundations to The Field of Redox Signaling

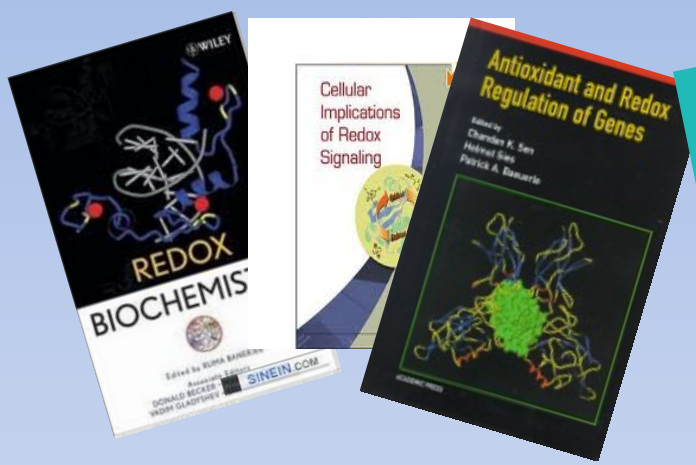
- 1986-1996 Helps Establish Foundational Science that Oxidants are Beneficial Signaling Molecules

Redox Biochemistry, Edited by Ruma Banerjee, is an Excellent Reference

Dr. Ruma Banerjee



Redox Signaling is an Exploding Field of Science



Books

- Redox Biochem
  - Ruma Banerjee
- Antioxidant and Redox Regulation of Genes
  - Chandan K Sen
- Redox Signaling in Biology and Medicine
  - Claus Jacob
- Redox-Mediated Signal Transduction: Methods and Protocols
  - John T Hancock
- Redox Protemics
  - Nick Lane
- And More

Journals

- Antiox & Redox Signaling
- Physiol Genomics
- Am. J. of Physiology
- The J. of Immunology
- PNAS
- Arteriosclerosis, Thromb. Vasc.
- Hundreds More

Conferences

- The Gordon Research *Conference* on Thiol-based *Redox* Regulation and *Signaling*
- 4th International Conference on Oxidative/Nitrosative Stress and Disease
- International Symposium on the Pathophysiology of Reactive Oxygen and Nitrogen Species
- Many more

Redox Signaling: Molecules in Vital Fluids

	Redox Signaling Molecule	Chemical Symbol	Major Sources	Lifetime (half-life) in Cells : Tissues
ROS	Superoxide Free Radical	O_2*-	Mitochondria, NADPH Complexes	15 millionths of a second : 10 thousandths of a second
ROS	Hydroperoxyl Free Radical	HO_2*	Mitochondria, NADPH Complexes	60 millionths of a second : 30 thousandths of a second
ROS	Hydroxyl Free Radical	HO*-	Fenton Reaction, H_2O_2	1 millionth of a second
ROS	Hydrogen Peroxide	H_2O_2	O_2*-	20 thousanths of a second : 10 seconds
RNS	Nitric Oxide Free Radical	NO*	NOS, NADPH	0.5 seconds : 4 seconds
ROS	Hypochlorite ion	OCl-	MPO	1 – 10 minutes
ROS	Hypochlorous acid	HOCl	MPO, acid	30 seconds
RSS	Hydrogen Sulfide	H_2S	CysBSynthase, Sulfides	?
ROS	Singlet Oxygen	$^1O_2$	O_2*-, Mitochondria	?
ROS	Carbon Monoxide	CO	Environment, HO	minutes

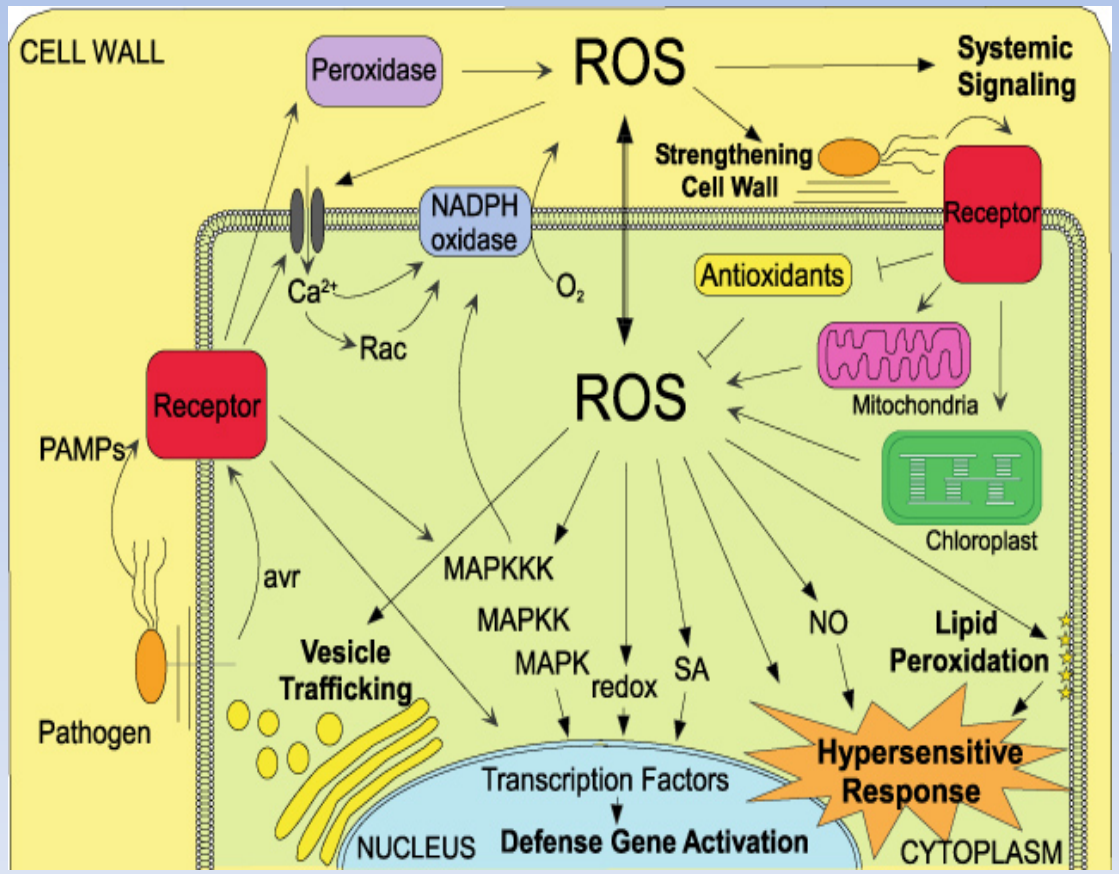
ROS - Reactive Oxygen Species

RNS - Reactive Nitrogen Species

RSS – Reactive Sulfur Species

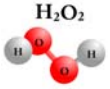
Redox Signaling: Controlling the Pathways Through Vital Fluids

- Redox Signaling Pathways active in plant cells
- Attack from pathogens Stimulates ROS production
- ROS activates defense measures

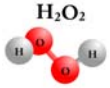


Redox Signaling: Molecules in Vital Fluids Oxidize "Semaphore molecules" that Control Pathways

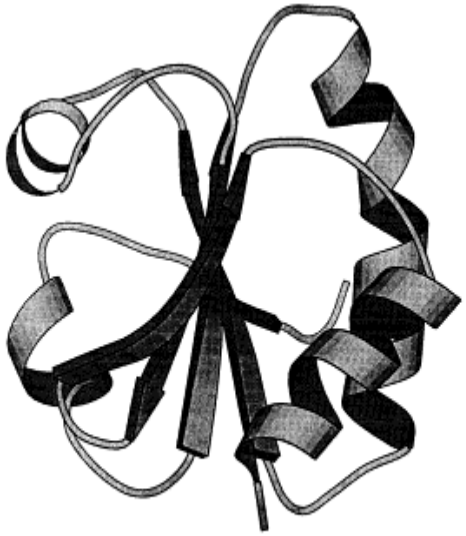
- H_2O_2 Releases Disulfide Bonds



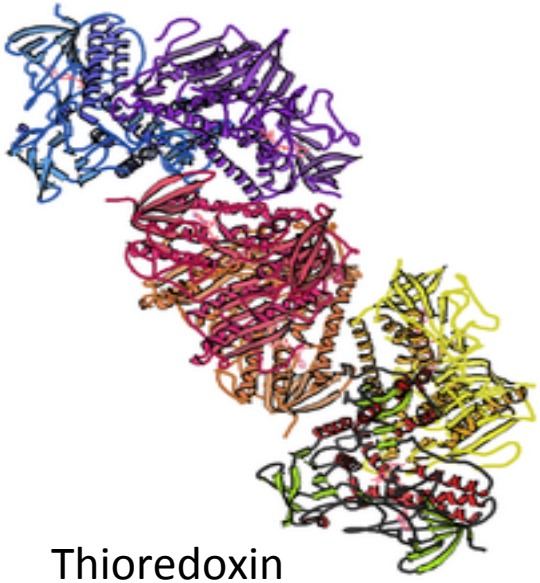
- Causing Conformational Change



Hydrogen Peroxide (ROS)

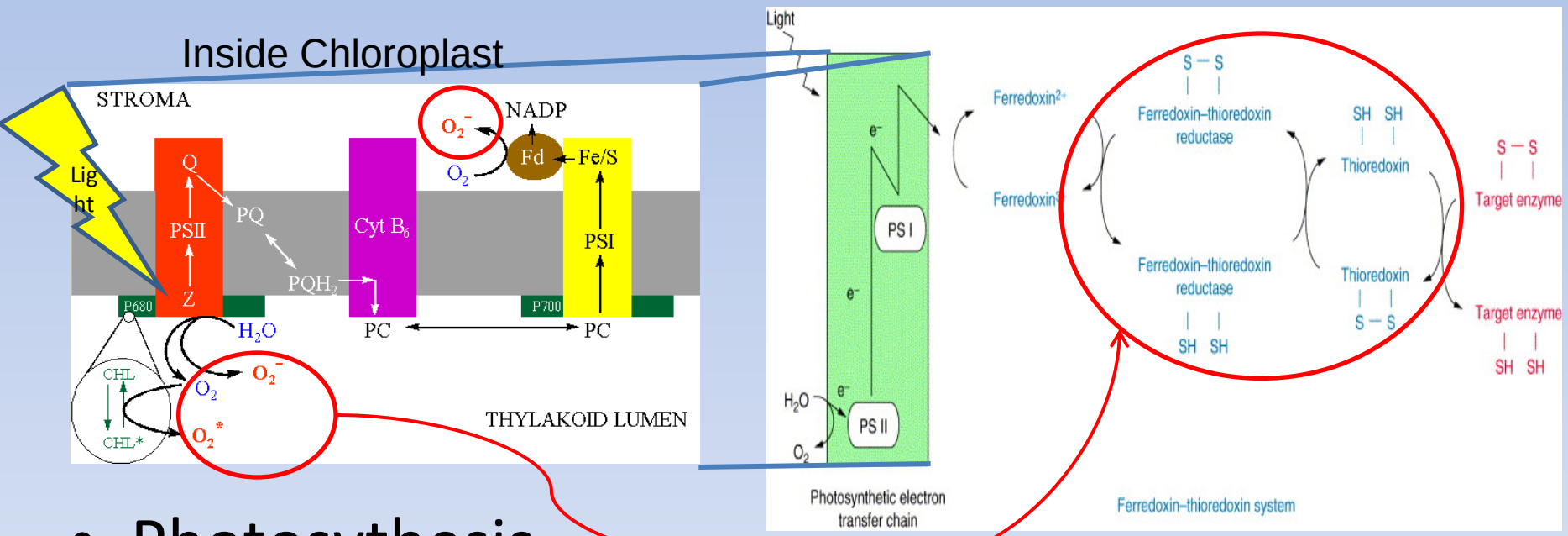


Thioredoxin (Trx)



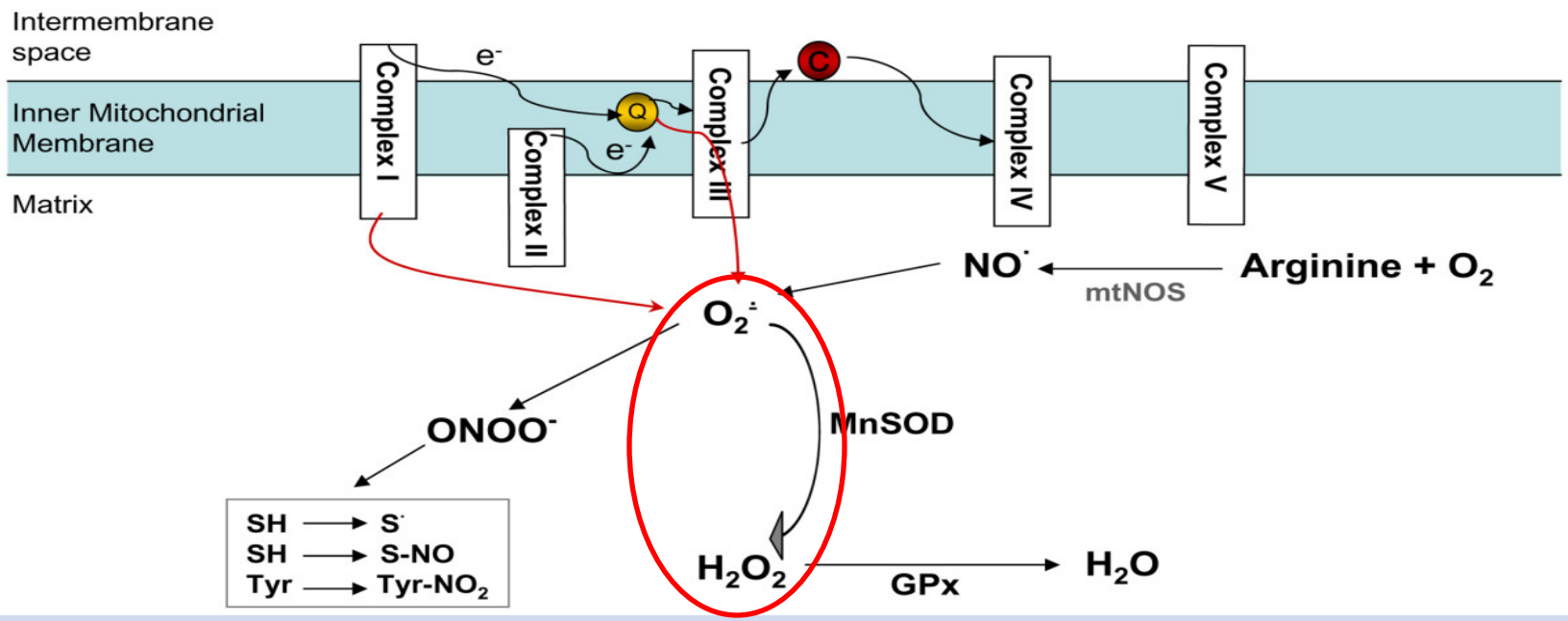
Thioredoxin Reductase (TrxR)

Redox Signaling Regulates Photosynthesis in plants



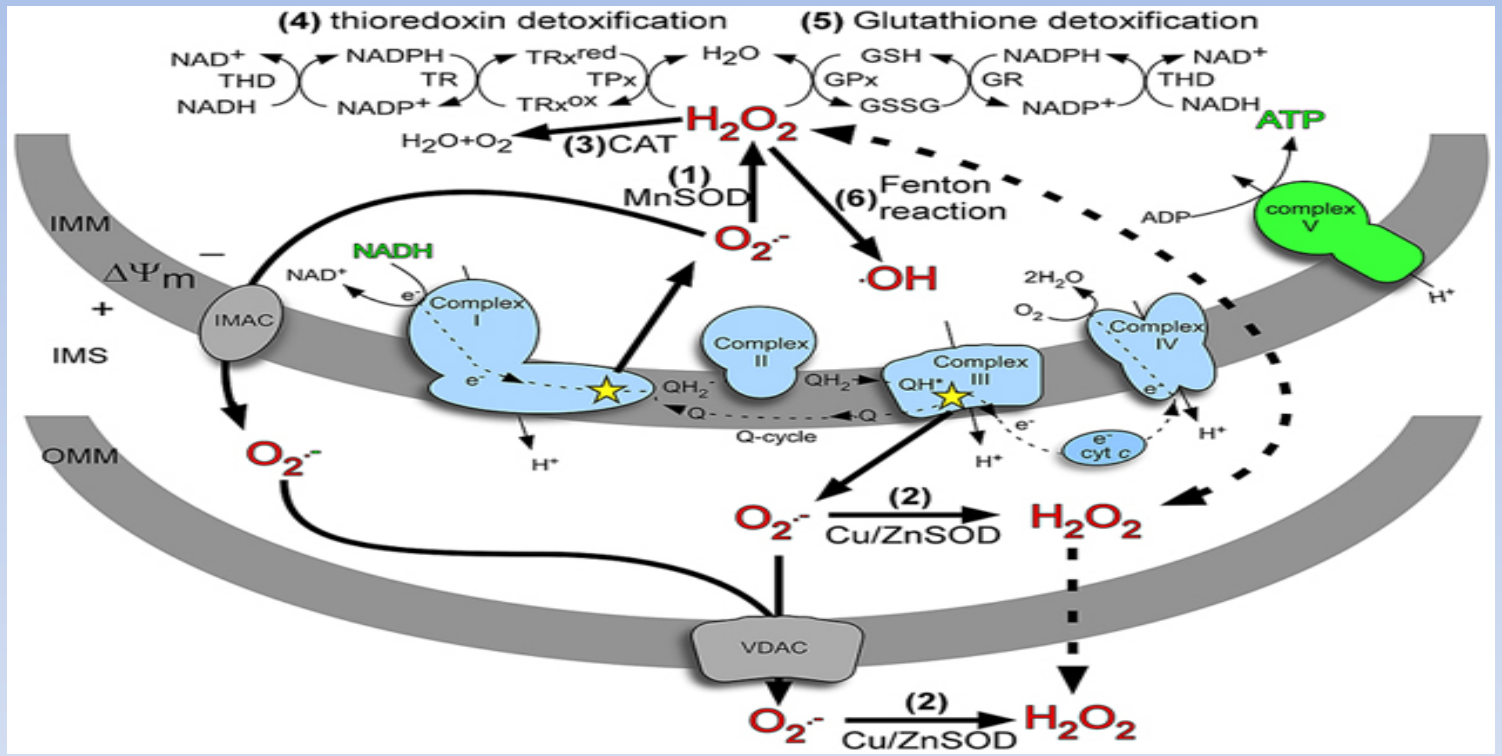
- Photosythesis
  - H_2O+Light→ O_2*-→H_2O_2+O_2→NADPH→Carbs & Fats
  - Thioredoxin regulates photosynthesis
  - H_2O_2 regulates thioredoxin

Redox Signaling Regulates Mitochondrial Respiration in Humans



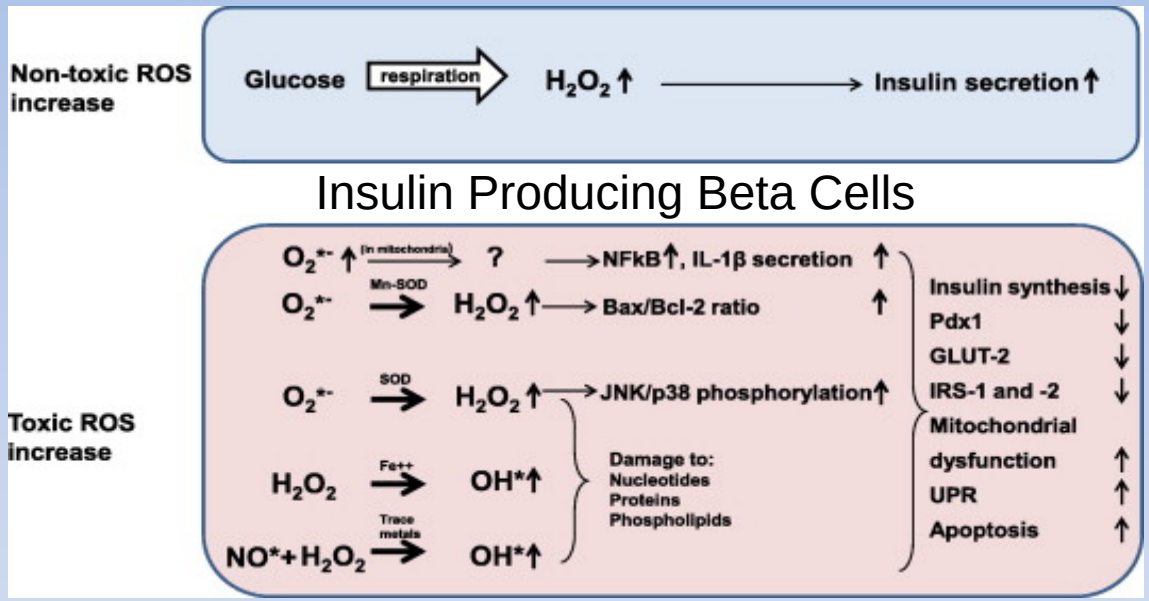
- Respiration
  - Carb & Fats →NADH→O_2→O_2*-→H_2O_2+H_2O+ATP
  - 3% of Inspired oxygen results in production of ROS (H_2O_2/O_2*-)
  - ROS facilitates production of RNS (NO*/ONOO-)

ROS (H_2O_2/O_2*-) Regulates Mitochondrial Metabolism



- Redox Signaling in Metabolism
  - H_2O_2/O_2*- → Conformational Change
  - H_2O_2/O_2*- → Pathway redirection

ROS (H_2O_2/O_2*-) Regulates Insulin Production and Insulin Resistance



- Redox Signaling in Glucose Regulation
  - H_2O_2/O_2*- beta cells → Insulin → down regulates glucose
  - H_2O_2/O_2*- → DNA Damage
  - H_2O_2/O_2*- → Apoptosis

In Healthy Cells, Redox Signaling Messengers are in Homeostatic Balance

Redox Signaling Messengers

ROS (Smoke)

Electron Donors

Healthy Cell

Anti-oxidant Shield

Nucleus

DNA

Mitochondria

mtDNA

Salt Water

Redox Messengers

Anti-Oxidants

In Damaged Cells, Redox Signaling Messengers are Out of Balance

Redox Signaling Messengers

ROS (Smoke)

Electron Donors

Damaged Cell

Nucleus

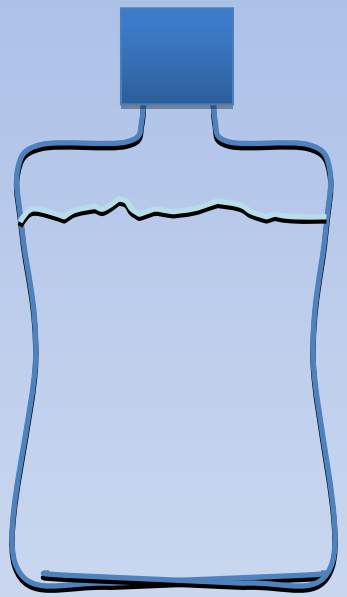
DNA

Mitochondria
mtDNA

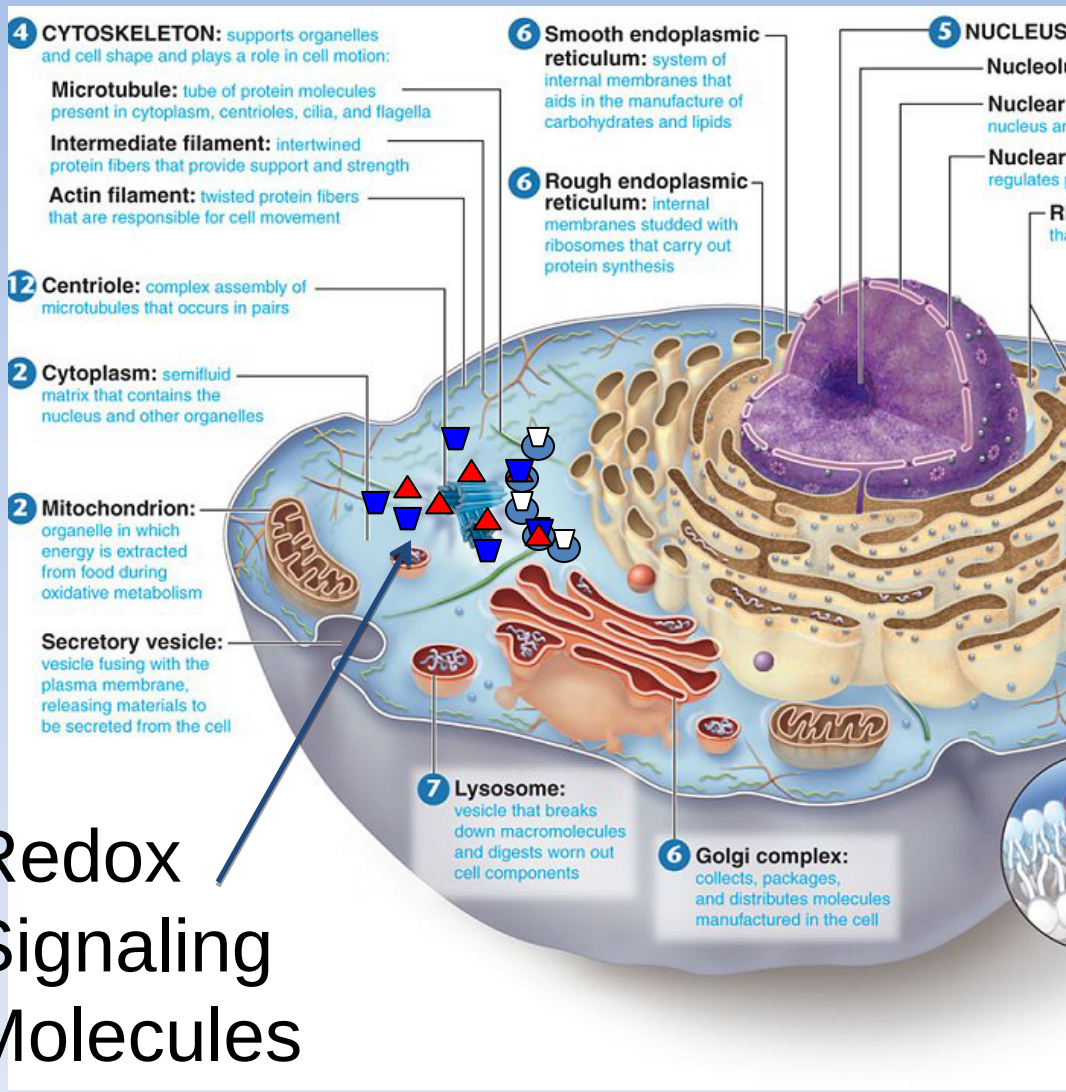
Oxidative Stress

Anti-Oxidants

Can we bottle redox signaling molecules?



- A perfectly balanced set of the same redox signaling molecules produced by the mitochondria in cells



Challenges for Utilization of Redox Signaling Molecules

- Stability
  - Half life of H_2O_2/O_2^{*-} in cells: microseconds
  - Half life of H_2O_2/O_2^{*-} in plasma: milliseconds
  - Half life of H_2O_2/O_2^{*-} in complexes: ???
- Toxicity
  - H_2O_2/O_2^{*-} have known toxic effects
  - H_2O_2/O_2^{*-} are components of oxidative stress
  - H_2O_2/O_2^{*-} side effects are unknown

Stability Issues Resolved – Confirmed by

- ESR Spin Trapping
- EPR Spin Trapping
- NMR Spin Trapping
- Established Fluorospectroscopy techniques
- Multiple Laboratories

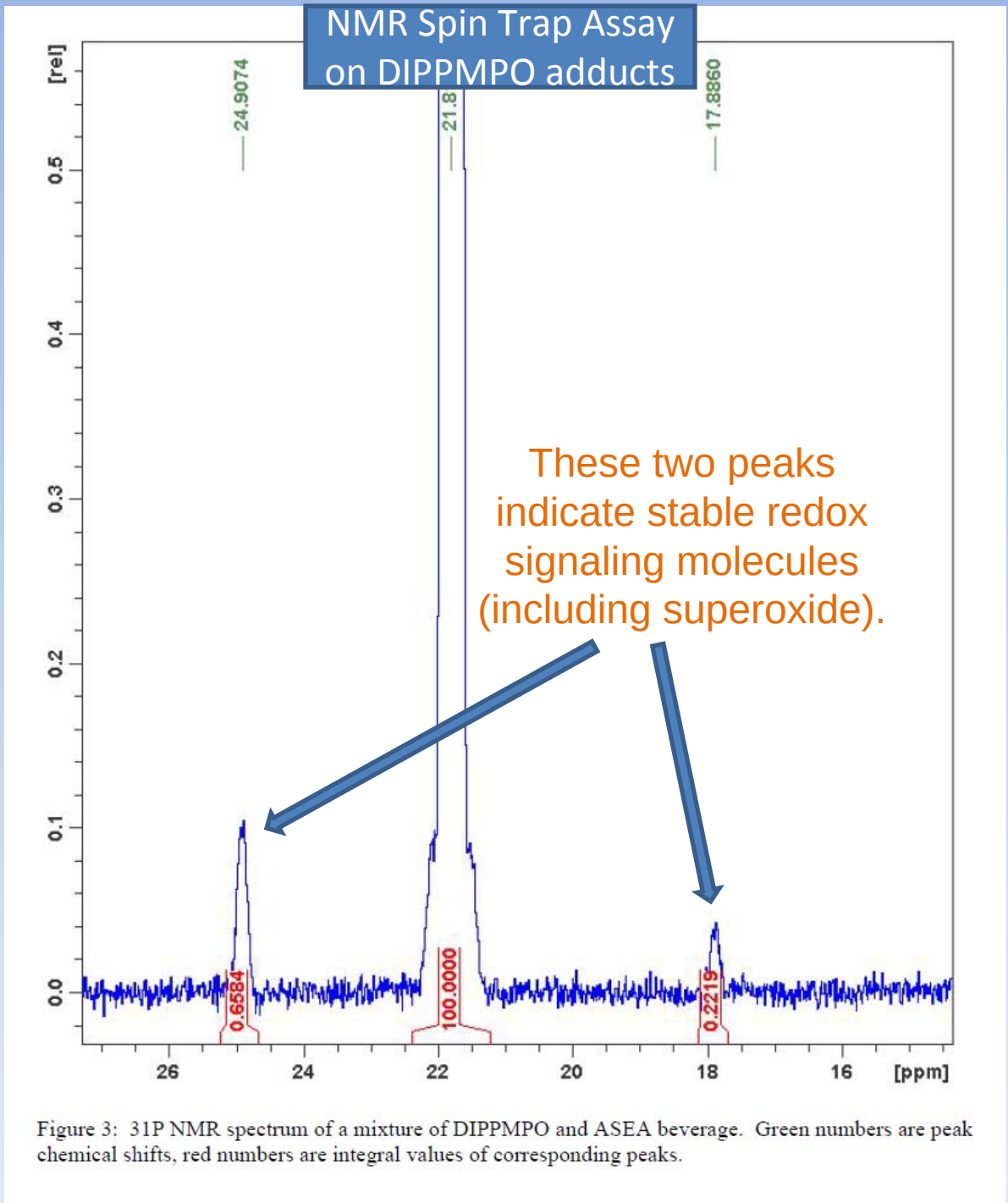
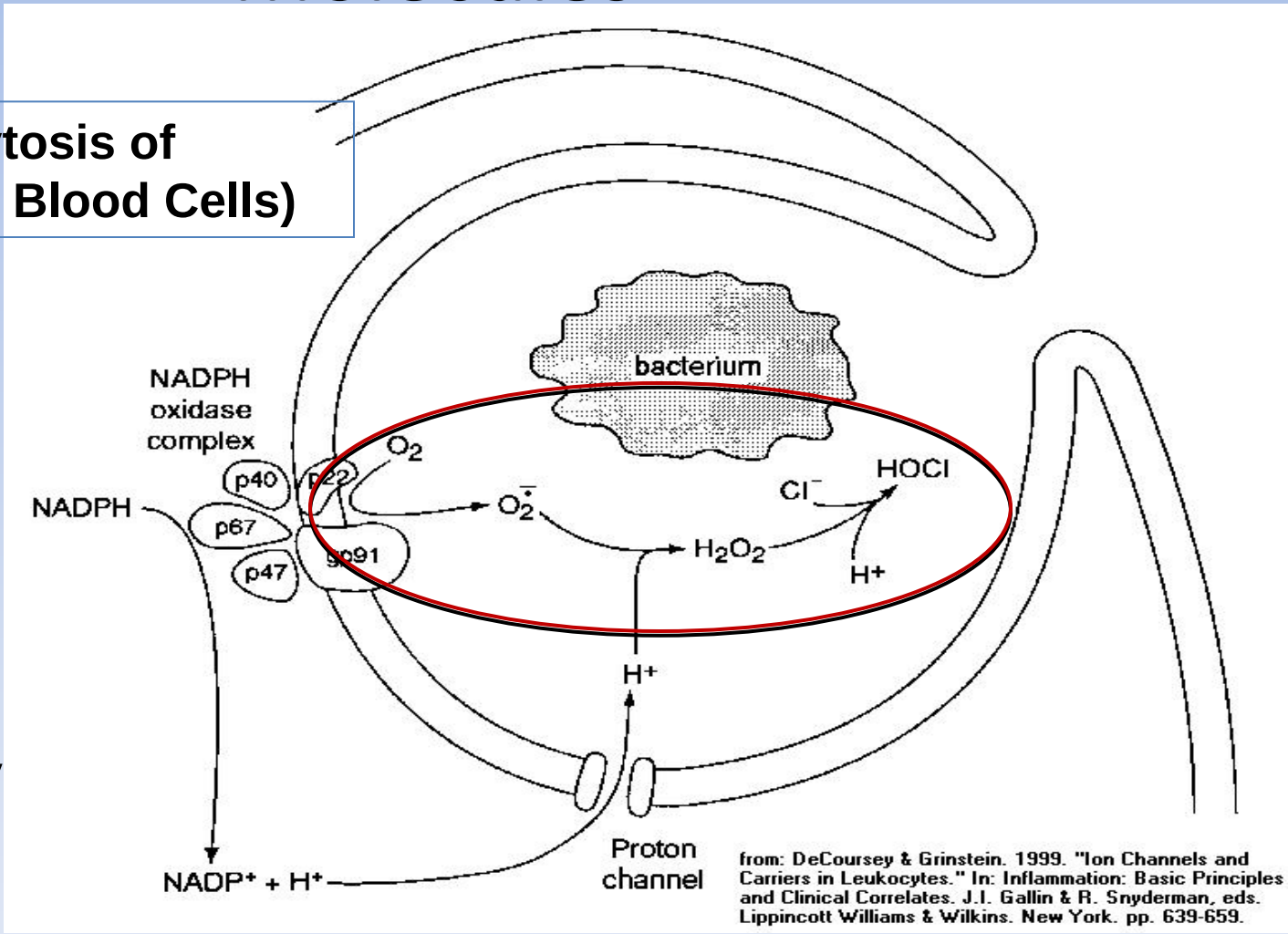


Figure 3: 31P NMR spectrum of a mixture of DIPPMPO and ASEA beverage. Green numbers are peak chemical shifts, red numbers are integral values of corresponding peaks.

The Weapon of Choice of Immune System are Redox Signaling Molecules

Example: Phagocytosis of Leukocytes (White Blood Cells)

- **Immune cells envelope bacteria**
- **Chemical Weaponry deployed to kill bacteria without harming healthy cells**



from: DeCoursey & Grinstein. 1999. "Ion Channels and Carriers in Leukocytes." In: Inflammation: Basic Principles and Clinical Correlates. J.I. Gallin & R. Snyderman, eds. Lippincott Williams & Wilkins. New York. pp. 639-659.

Stability Requires Chlorine Containing Species

- NaCl Salt is not present
- Chlorine Species probably is a hypochlorite complex

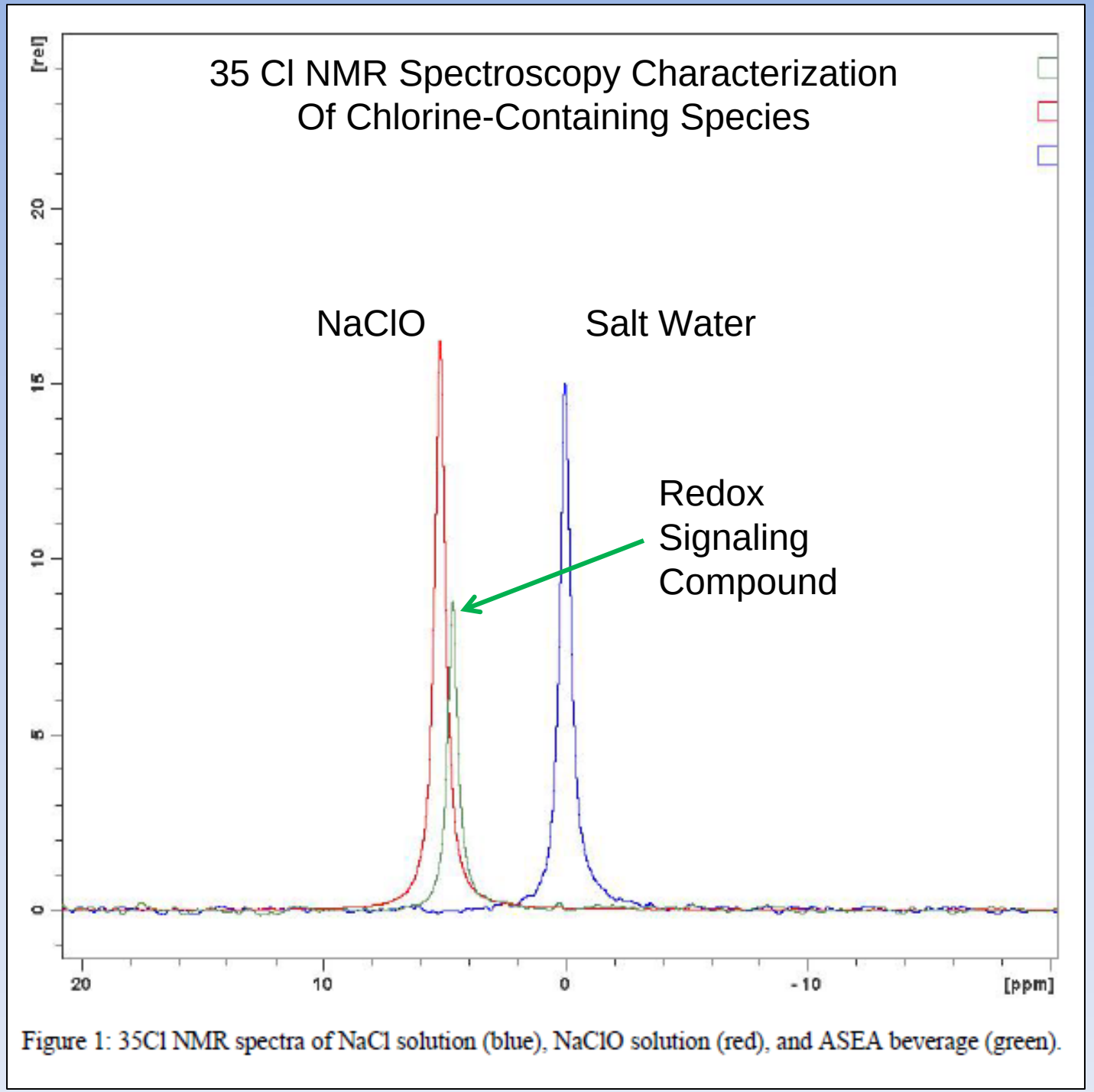
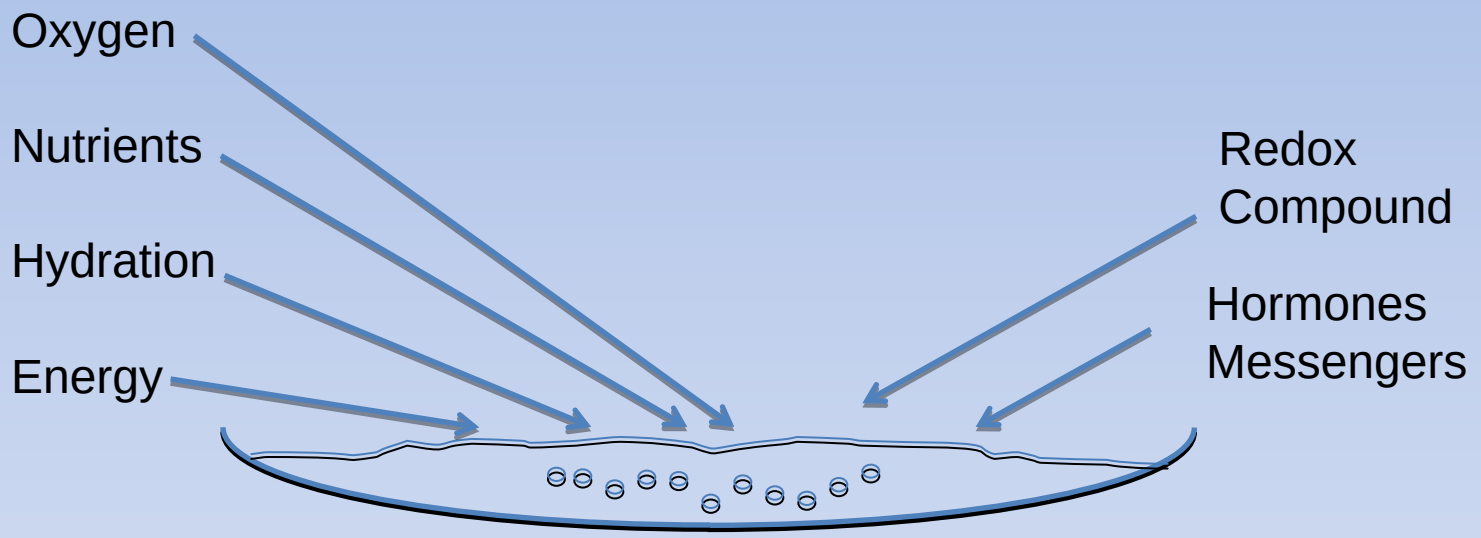


Figure 1: 35Cl NMR spectra of NaCl solution (blue), NaClO solution (red), and ASEA beverage (green).

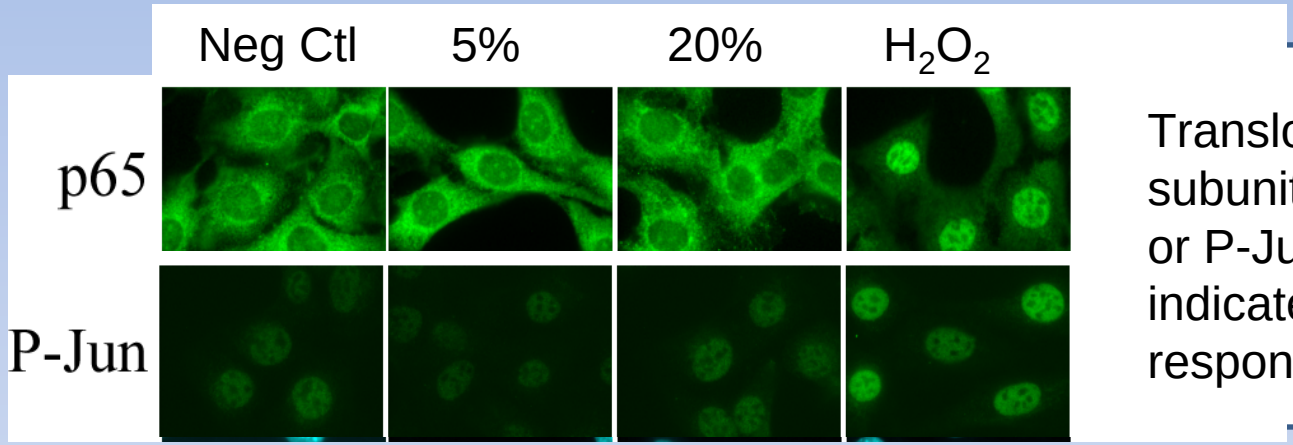
Effect of Redox Signaling Compound on In Vitro Cell Cultures



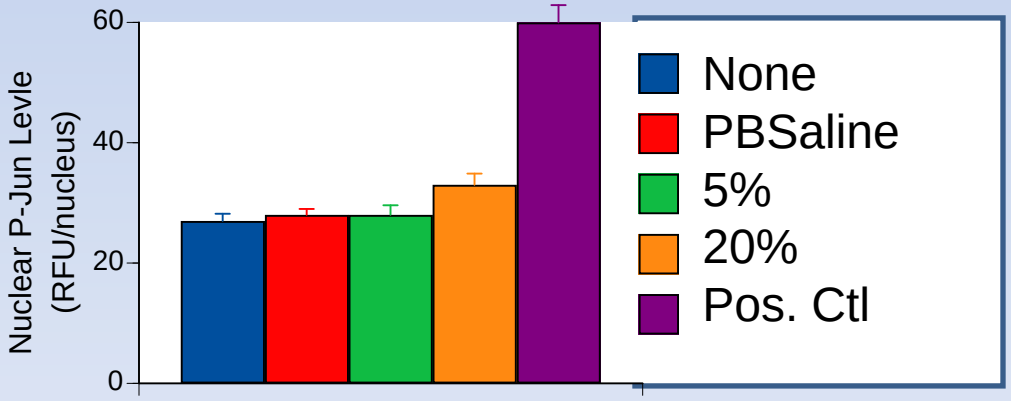
Results:

- No indication of toxicity or inflammatory markers in healthy cultures
- Enhances Protective Mechanisms (GPx & SOD) in healthy cultures
- Accelerates death of damaged cells by apoptosis in irradiated cultures
- Increases hormonal sensitivity
- Reverses senescence in ageing cell cultures

National U.S. Laboratory: In Vitro Toxicity of Oral Formulation

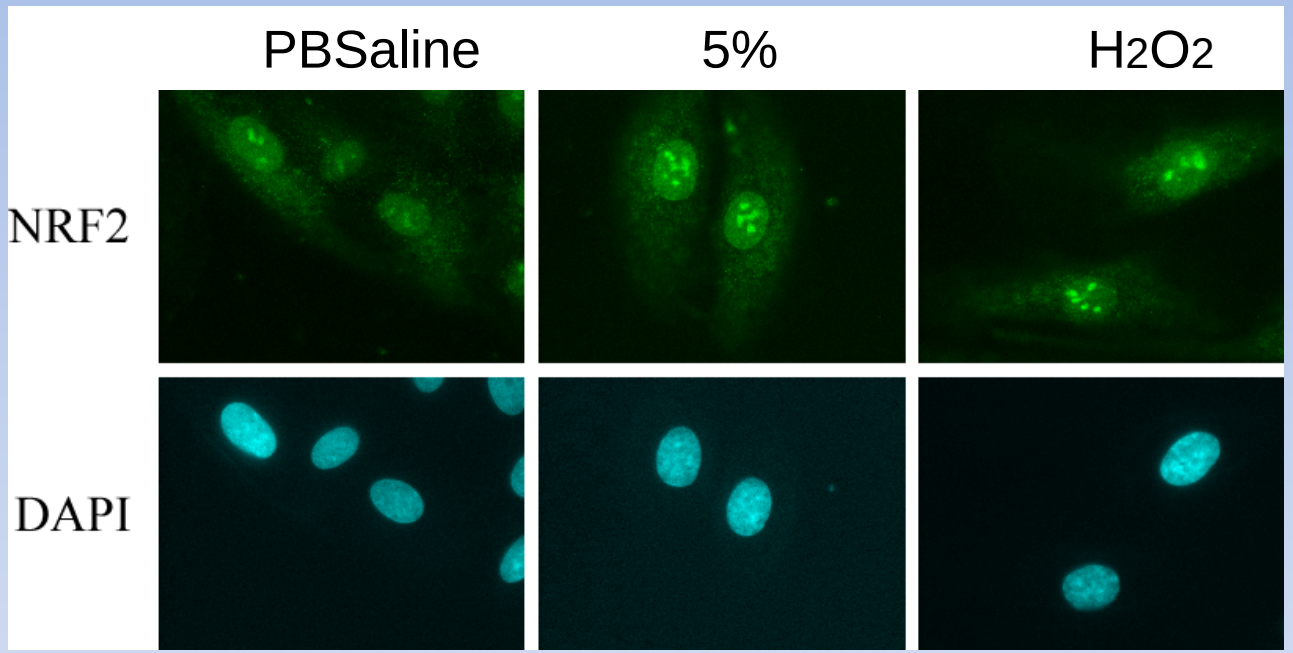


Translocation of P65 subunit of Nf-kappaB or P-Jun into nucleus indicates a toxic response



No toxicity Observed even in high concentrations

In Vitro Antioxidant Efficacy for Oral Formulation



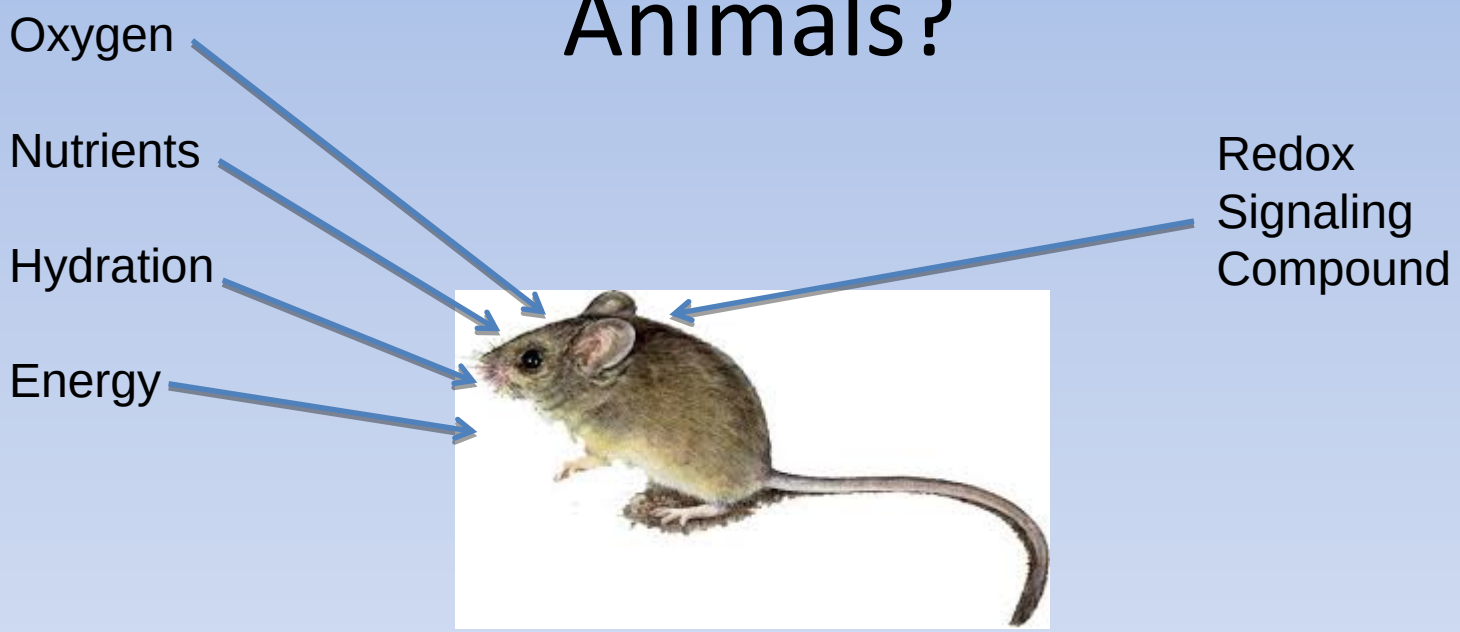
Effects lasted for 120 minutes
(100 Endothelial Cells)

- Nuclear translocation of NRF2 indicates activation of antioxidant up-regulation
- Western Blot verifies GPx and SOD production
- Antioxidant efficacy more than 400% increase

Preclinical Trials

- Microbes
  - 100% kill in under 30 seconds for all tested bacteria and viruses (over 30 resistant strains)
  - No effect on in sitio beneficial flora microbiome
- Cell Cultures
  - No inflammatory markers stimulated
  - Antioxidant efficacies increased by 500%
  - Accelerates kill rate only in damaged cells
  - Increases sensitivity to some hormones
  - Reverses cell ageing

What is the Effect of Redox Signaling Supplementation in Animals?

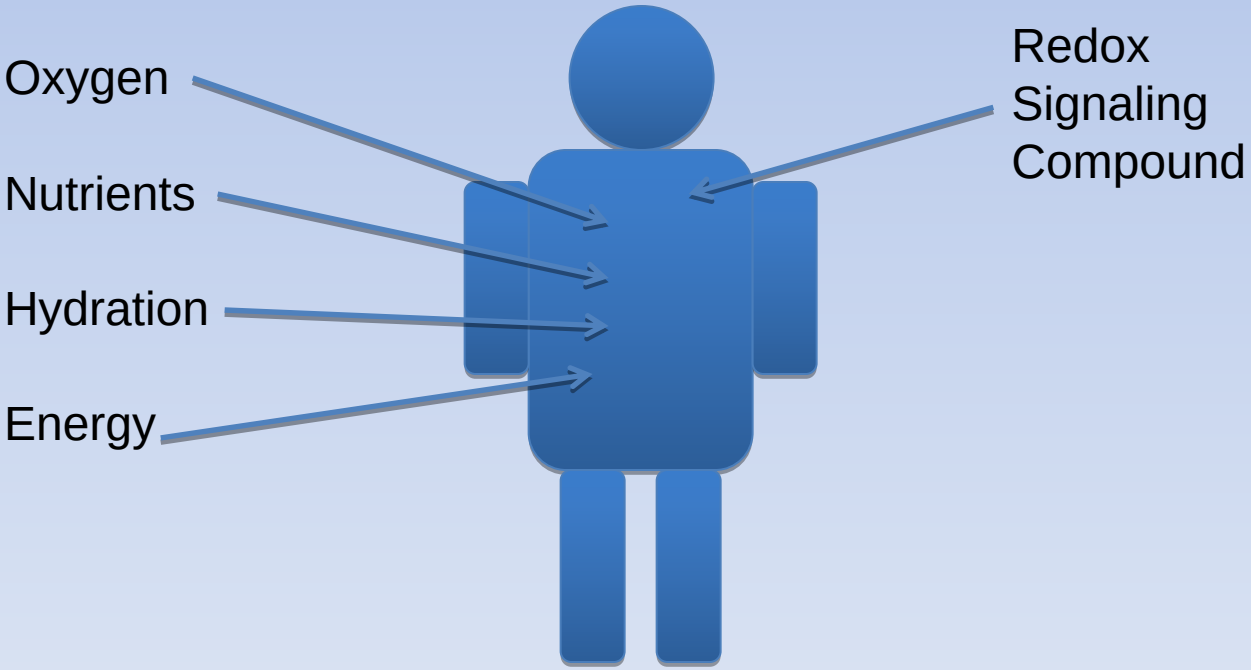


- 20 years of data in mice, rabbits, dogs
- Zero toxicity exhibited, normal histopathologies in all tissues and organs
- Mice Run 29% Longer to Exhaustion (Controlled studies)
- Antioxidant capacities increase
- Lipid peroxidation and glutathione oxidation (GSSG/GSH) decreased
- Metabolism markers normalized

18 Years of In Vivo Toxicity Research on a Perfectly balanced Redox Signaling Compound

- No endotoxicity, cytotoxicity, genotoxicity found in GLP preclinical, reverse mutigenic, genemaps and clinical blood-based studies
- No LD50 found, complete lack of side effects at any dose or form of administration
- No abnormal histopatological effects observed – All Tissues and Major Organs

Clinical Studies of Redox Signaling Supplementation in Humans

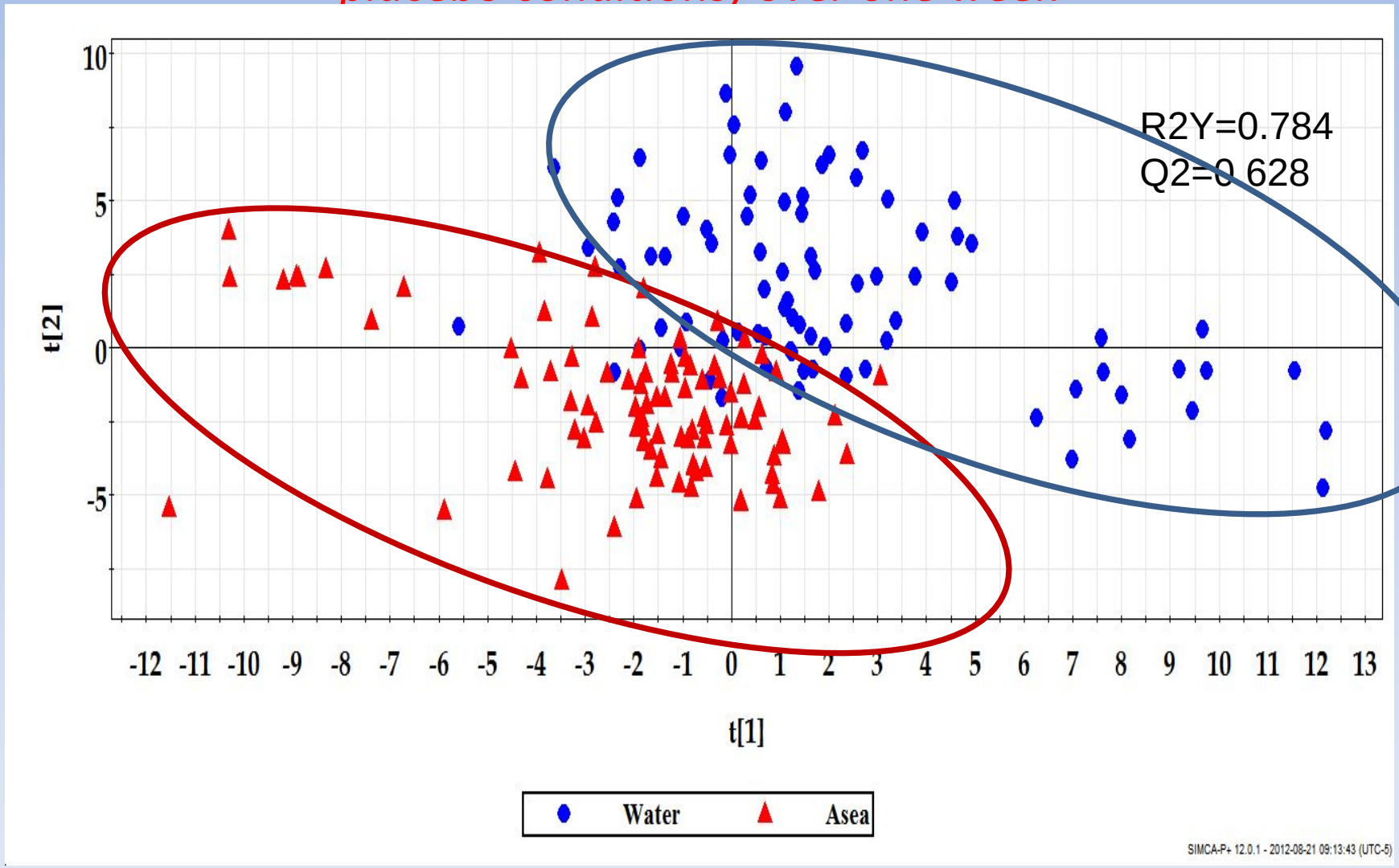


3rd Party Independent Study Results

- All 5 major clinical studies were controlled randomized double-blind placebo-based
- No adverse affects in all (160+) human subjects
  - Complete blood work, comprehensive analysis
- Metabolomic results were striking
  - 263 metabolites monitored, 60+ changed ($p<0.05$)
  - Lipids (fatty acids), sugars, metabolic intermediates
- Pharmacokinetics results verified absorption
  - Shifts in metabolites between 30 min. and 24 hrs.

PLS-DA All Data

9 samples collected in 12 subjects twice (Test Compound or placebo conditions) over one week



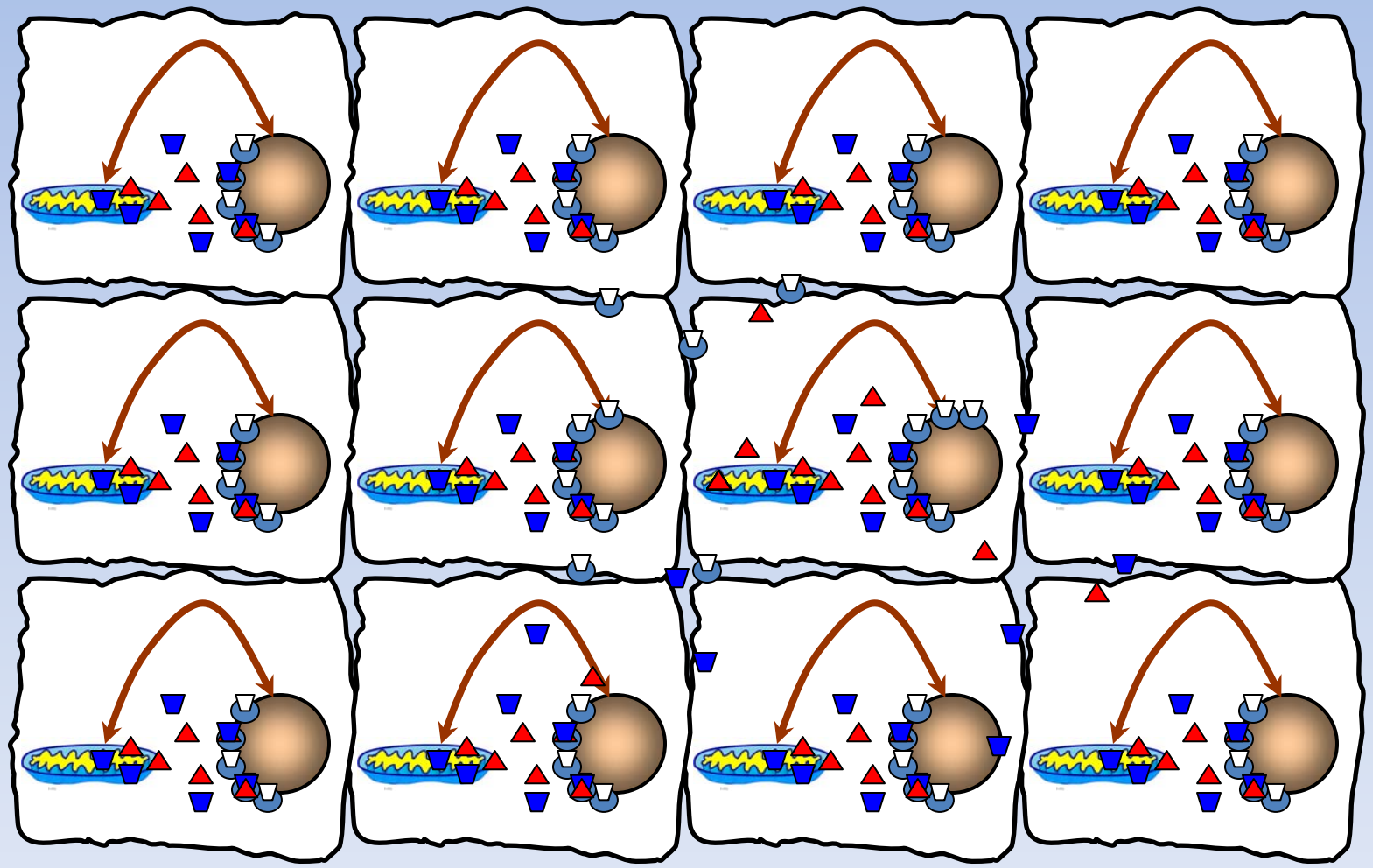
Metabolites, ASEA PK Shifts

30-min metabolites	90-min metabolites	150-min metabolites	3.5 h metabolites	6h-metabolites	24-h metabolites
d-Fructose	Proline	Fumaric acid	Threitol	Aminomalonic acid	Glycine
d-Xylose	Mannose	3-amino-2-methyl-propanoic acid	Nonanoic acid	Succinic acid	L-Methionine
Glycerol 2-phosphate	L-Valine	L-Aspartic acid	Salicylic acid	Threitol	Alanine
2-oxo-4-methylvaleric acid	Allo-isoleucine	Ethanolamine	L-Glutamine	Pyruvic acid	L-Lysine
Sorbose	Glycine	1,2-Propanediol-1-phosphate	Nona-decanoic acid	alpha-Hydroxyiso-butyric acid	Ribitol
Octadecanoic acid	Citrulline	Aminomalonic acid	Hexadecanoic acid	L-Cysteine	L-Tyrosine

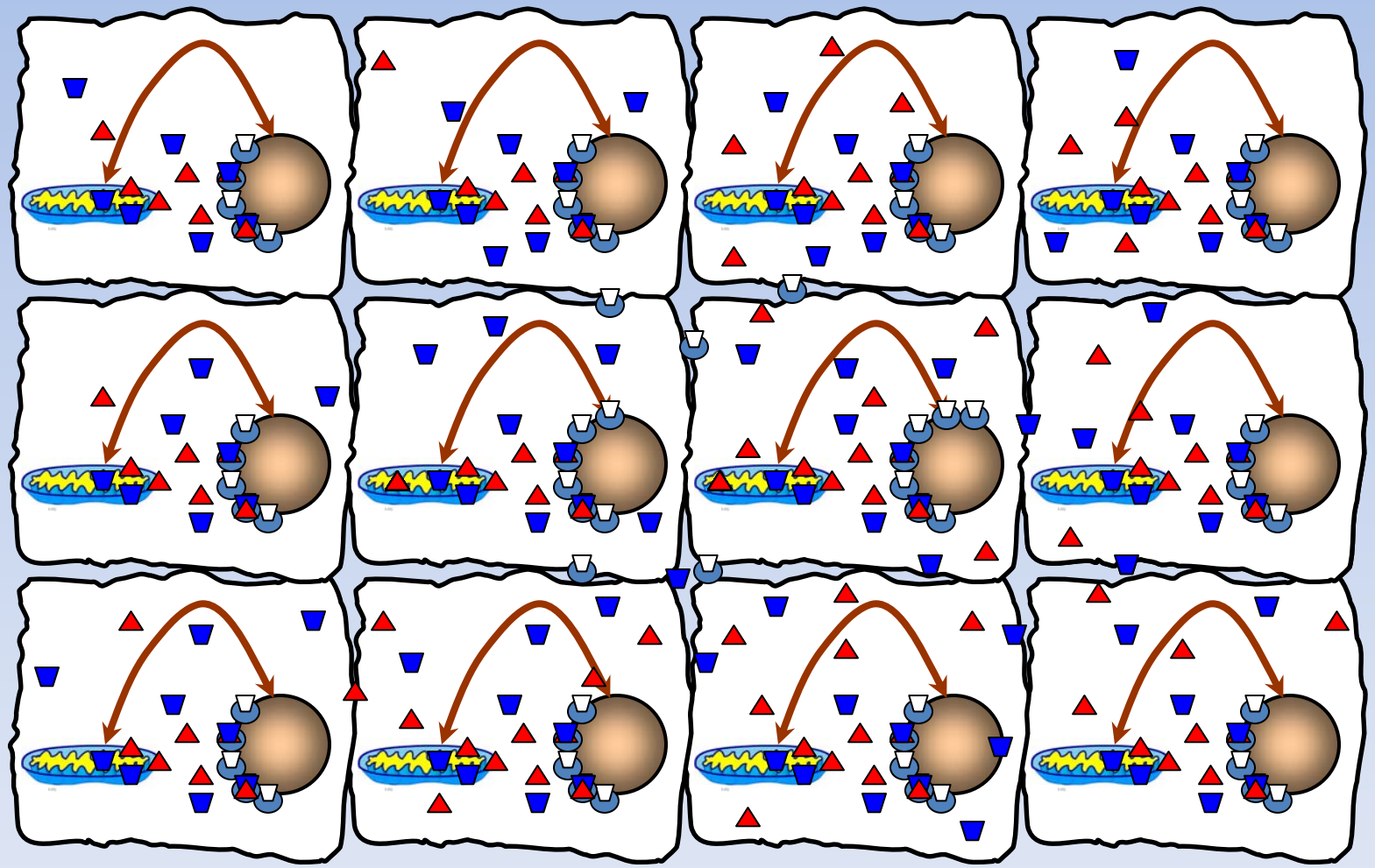
Pathway into the Future

- Data from ongoing studies still not available
- Research budget is rapidly expanding to finance multiple human clinical studies
- World renowned scientists showing interest
  - Dr. David Nieman, DrPH, FACSM (DHMRI-NCRC)
  - Dr. A. C. Naidu, Ph.D. (Fellow RSM, FLS, FACN)
- Widespread distribution as a supplement
  - Over 100,000 people with experience

Redox Imbalance (Oxidative Stress) Signals Protect-Repair-Replace Response



Innovation: Infuse Balanced Redox Signaling Molecules back into Tissues



"REDOX SIGNALING TECHNOLOGY IS DESTINED TO FUEL THE GREATEST ADVANCES IN MEDICAL SCIENCE THIS CENTURY"

- Redox Signaling is fundamental to all biological multi-cellular processes.
- It is non-specific, non-toxic and applicable to a broad range of processes and pathways.
- It is at the very core of mediating and regulating the mechanisms that restore functionality and health.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