Quantum Coherence in Reactive Oxygen Species Biology

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When you come to a fork in the road, take it!

---Yogi Berra

Singlet -Triplet Mixing: |S> ↔ |T>

A radical pair of two electron spins must be in a coherent superposition of the quantum states.

What is Quantum Biology?

Quantum biology may be thought of as the signatures of molecular-level quantum phenomena observed in biological systems at functional, cellular, or organism levels.

Quantum Biologist
-capture signatures of quantum phenomena at the molecular level and correlate them with their effects persisting at the cellular level.

Quantum Biology

- Photosynthesis – Light Energy Conversion (1980s)
- Olfaction – Vibrational Frequencies (1990s)
- Magneto-reception – Avian Navigation (2000s)

Redox biology - ROS production, oxidative signaling, and cellular outcomes (2013).

Modulate ROS partitioning by altering coherent spin dynamics via the radical pair mechanism.

Why is ROS (redox) Biology Important?

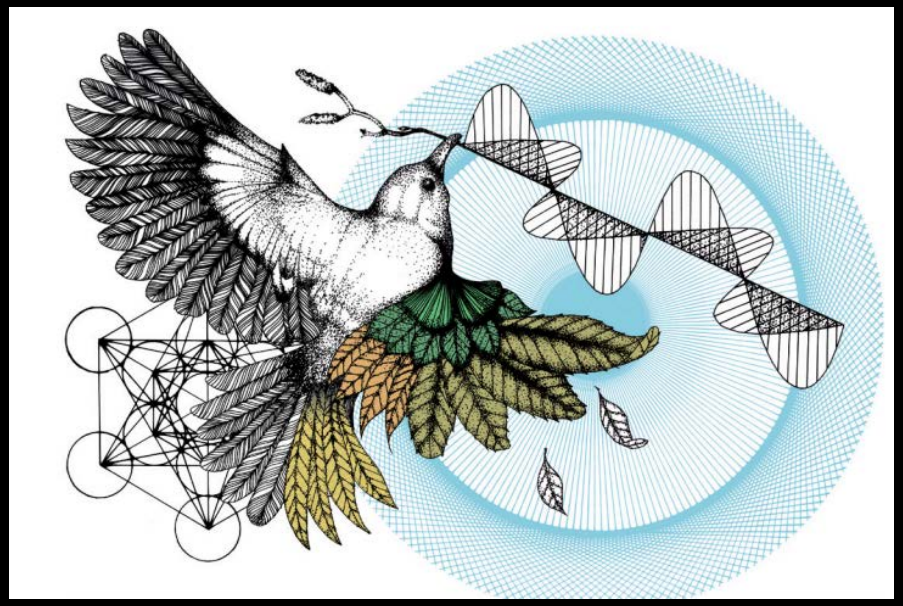
ROS are known to exert a wide range of biological effects from deleterious oxidative stress in pathogenesis to beneficial regulatory functions.

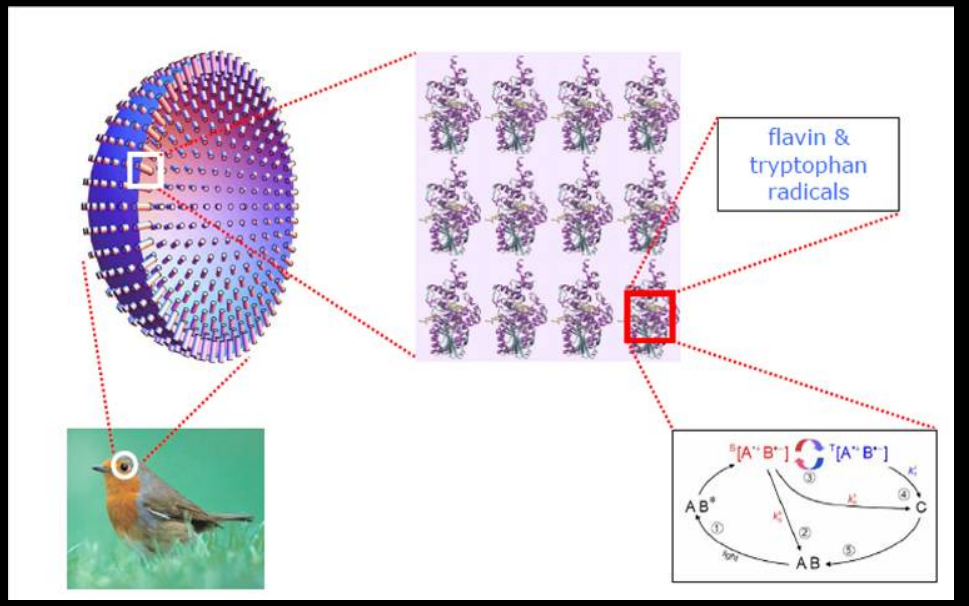
Departure from mainstream quantum biology

Transition from magneto-reception i.e. cryptochrome paradigm

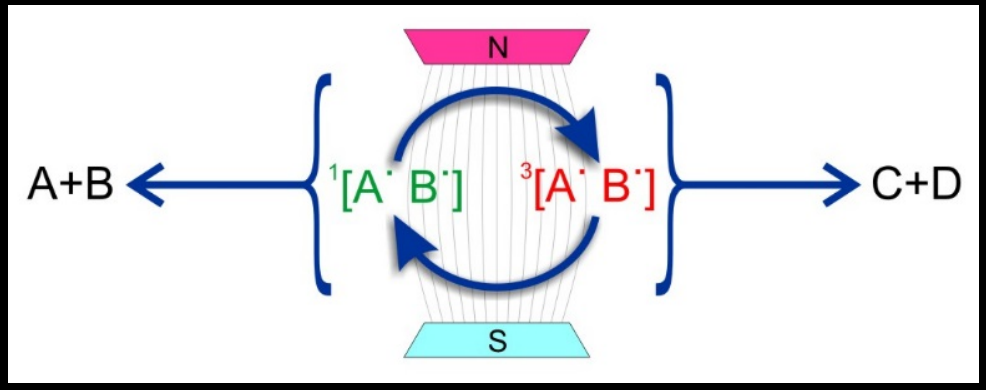
Reactive oxygen species produced by reduced flavins

Avian Navigation





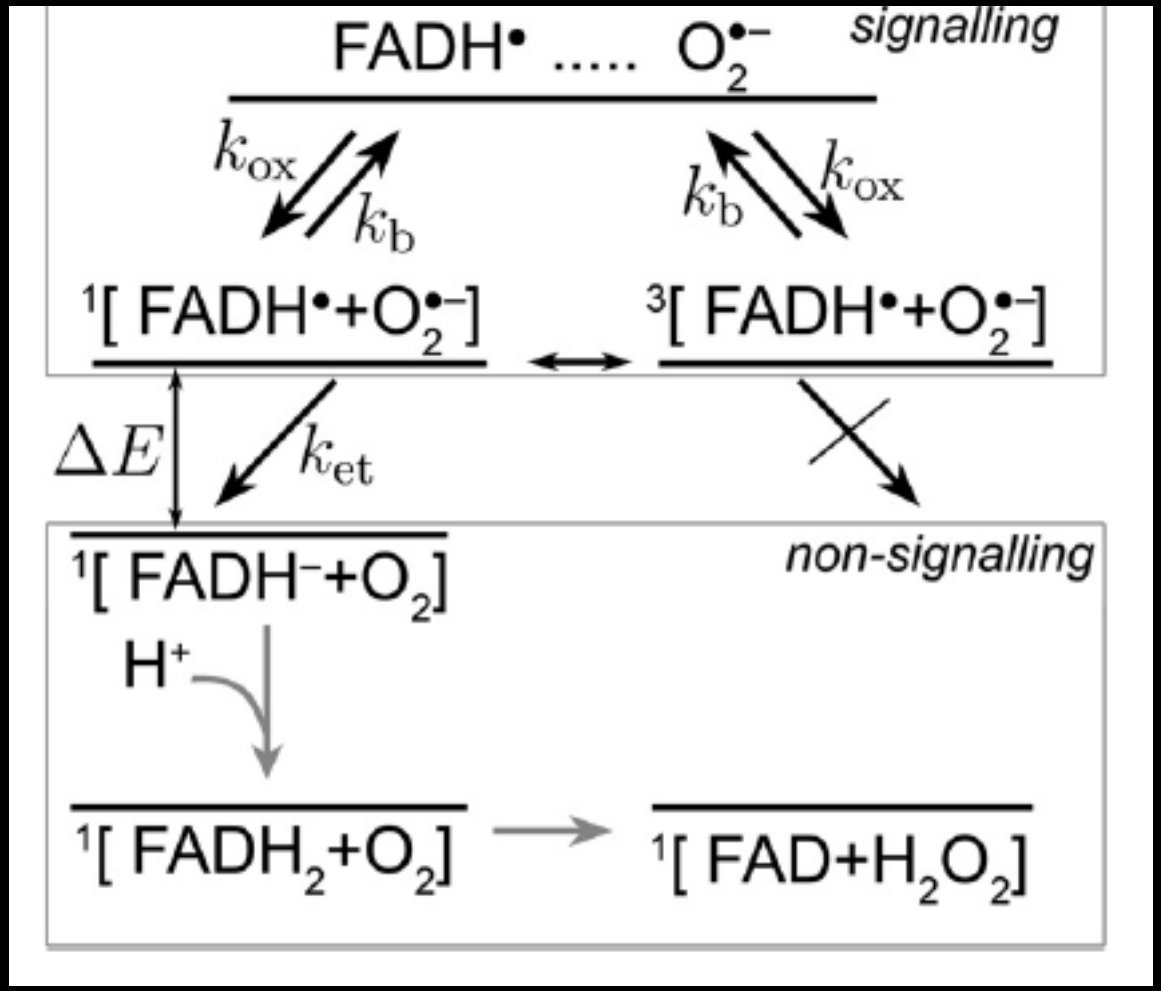
Hypothesis: cryptochrome flavoenzymes are magnetosensors that exhibit quantum coherence for avian navigation

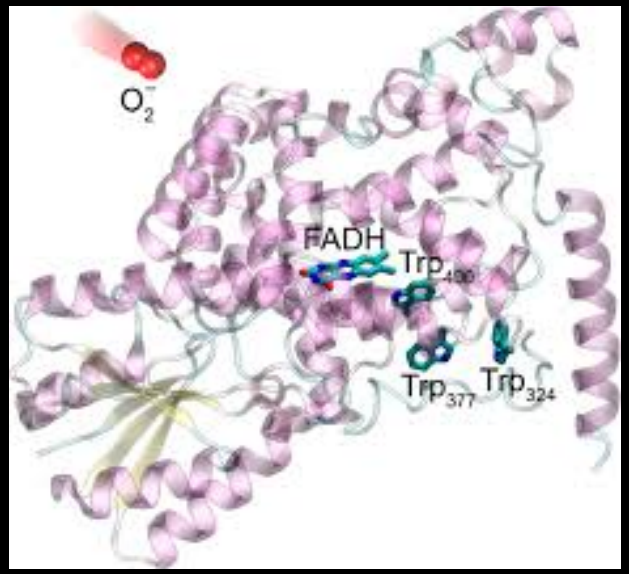


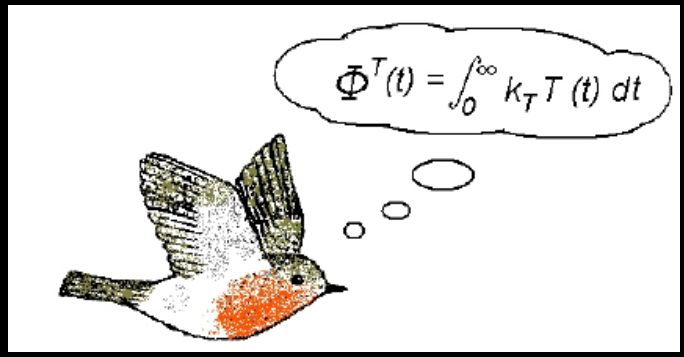
Theory:
Radical Pair
Mechanism

Magnetoreception May Involve Superoxide and Cryptochrome Flavoenzyme

Schulten Model







A need for experimental validation of RPM in biology.

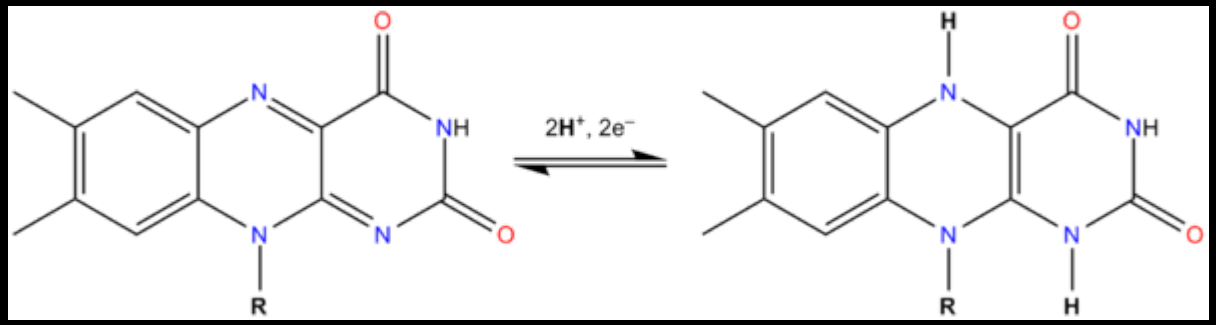
Solov'yov and Schulten *Biophysical Journal* 96 (12) 4804-4813 (2009)

ROS and The Radical Pair Mechanism

Necessary and Sufficient Conditions:

- **Singlet and triplet states must have different chemical fates**
- **The reactivity of the radical pair must be spin dependent**
- **At least one radical must have magnetic electron-nuclear hyperfine interaction**

flavin isoalloxazine ring molecule



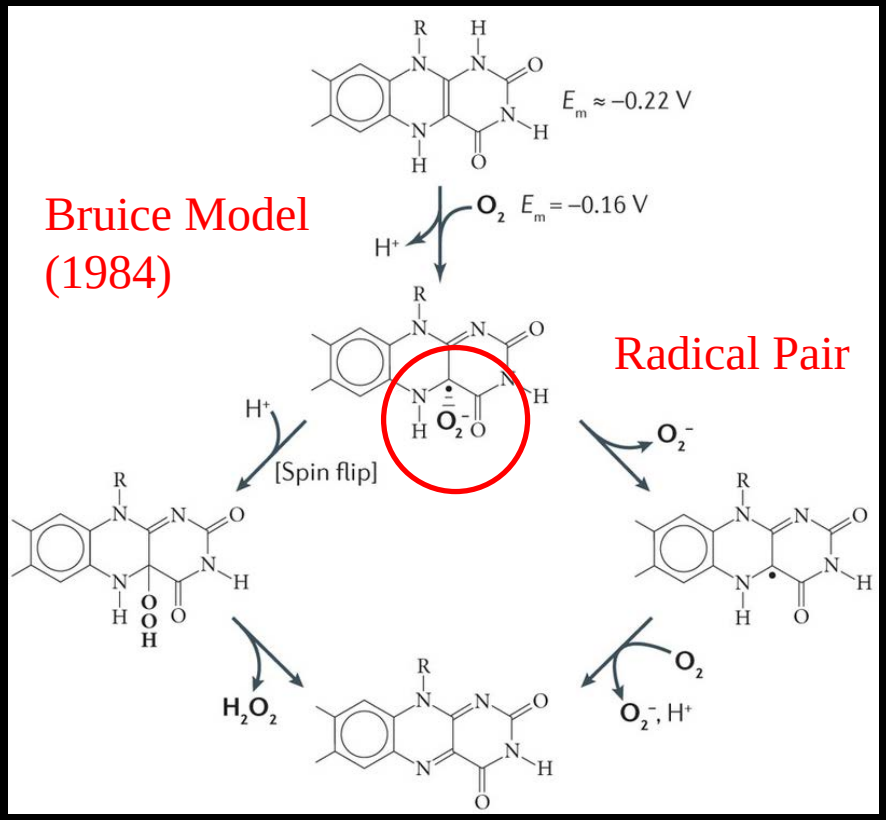
hyperfine couplings from neighboring ring protons

superoxide

$O_2^{-\bullet}$

no hyperfine couplings

Reactive Oxygen Species Produced by Reduced Flavins



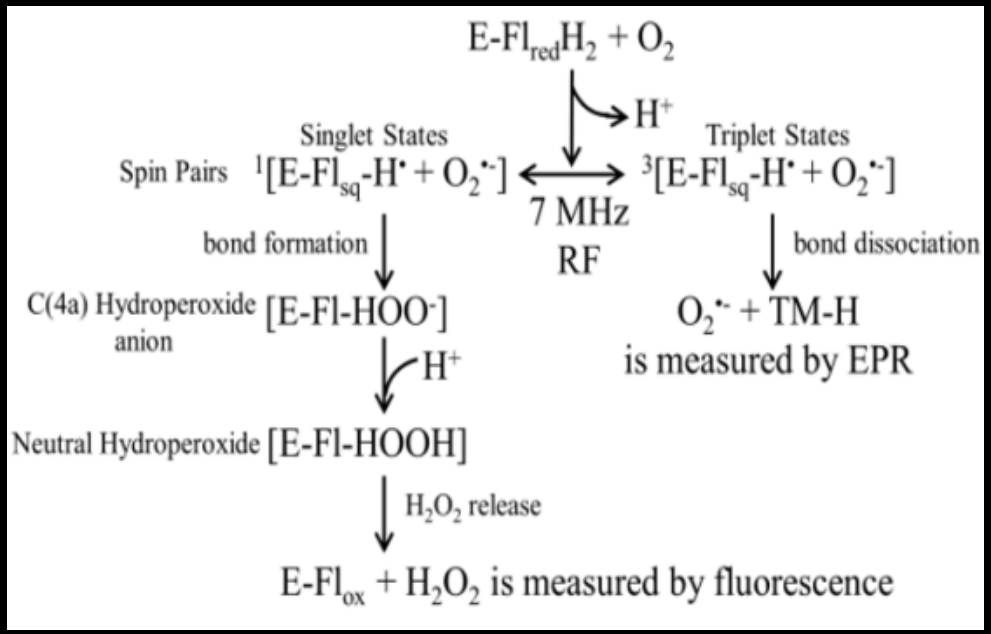
Hypothesis: The RP spin dyamics is influenced by magnetic fields via Zeeman energies and hyperfine couplings.

[1]J.A. Imlay *Nature Reviews Microbiology* 11 443-454 (2013)

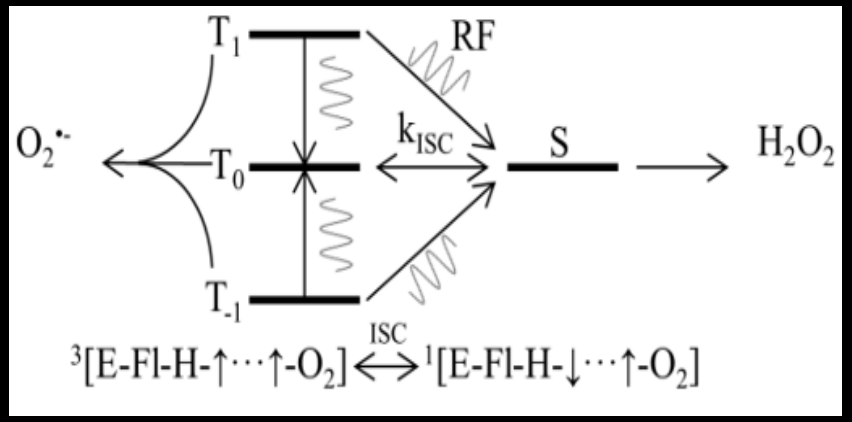
V. Massey *J. Biologcial Chemistry 269* (36) (1994)

Product Yield Detected Magnetic Resonance (PYDMR)

Optical and Spin Probe Assays



Singlet-Triplet Interconversion



Separately measure H_2O_2 and $O_2^{-\bullet}$ by ROS cellular assays.

M. Okazaki, S. Sakata, R. Konaka, T. Shiga, *J. Chem. Phys.* 86: 6792-6800 (1987)

Distinguish between Magnetic Resonance Signals and Product Yields

Magnetic Resonance:

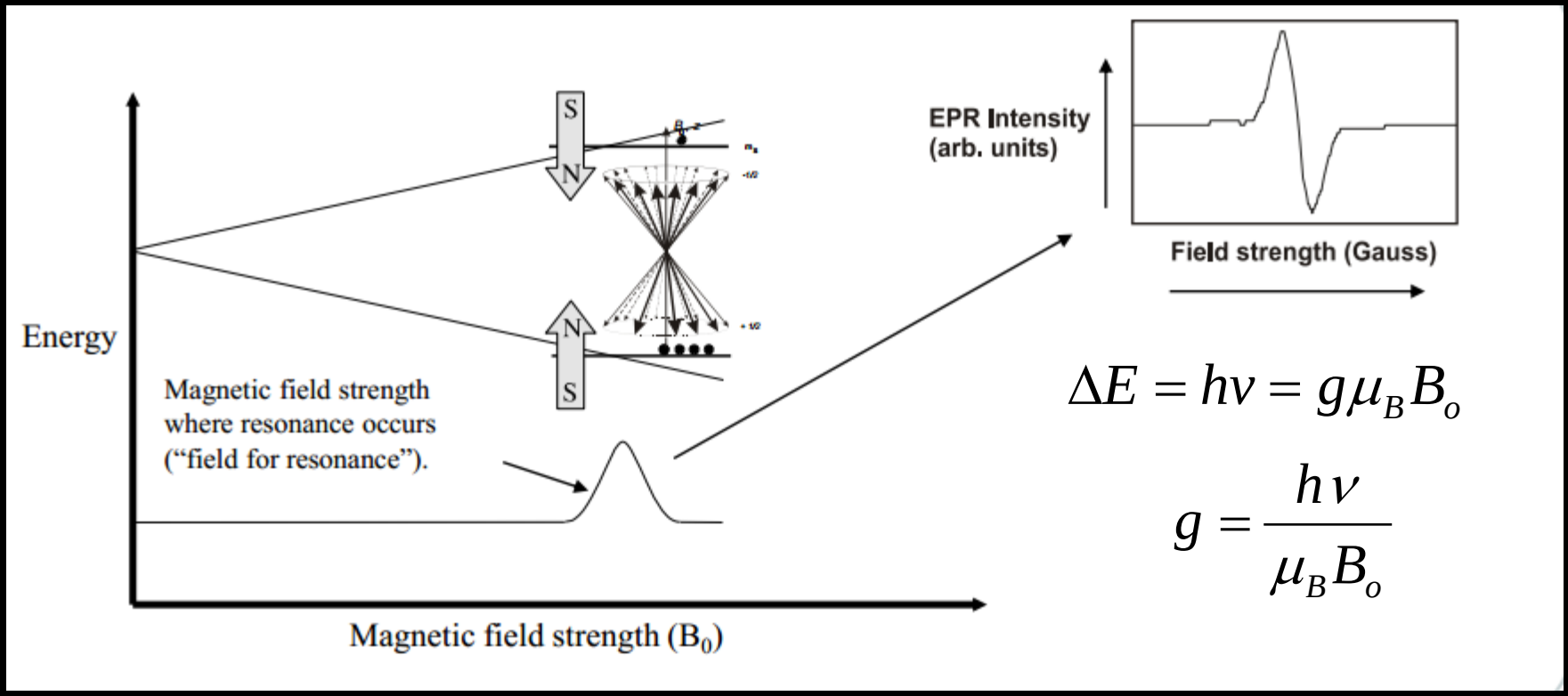
Larmor precession of spins and applied RF frequency

–Spin Hamiltonian

Singlet-Triplet Product Yields:

Stochastic Liouville Equation, time-dependent quantum mechanics.

Basic Continuous Wave (CW) EPR Experiment



$$\Delta E = h\nu = g\mu_B B_o$$

$$g = \frac{h\nu}{\mu_B B_o}$$

The EPR spectrometer provides a linear magnetic field sweep, while exposing the sample to a fixed frequency of microwave irradiation.

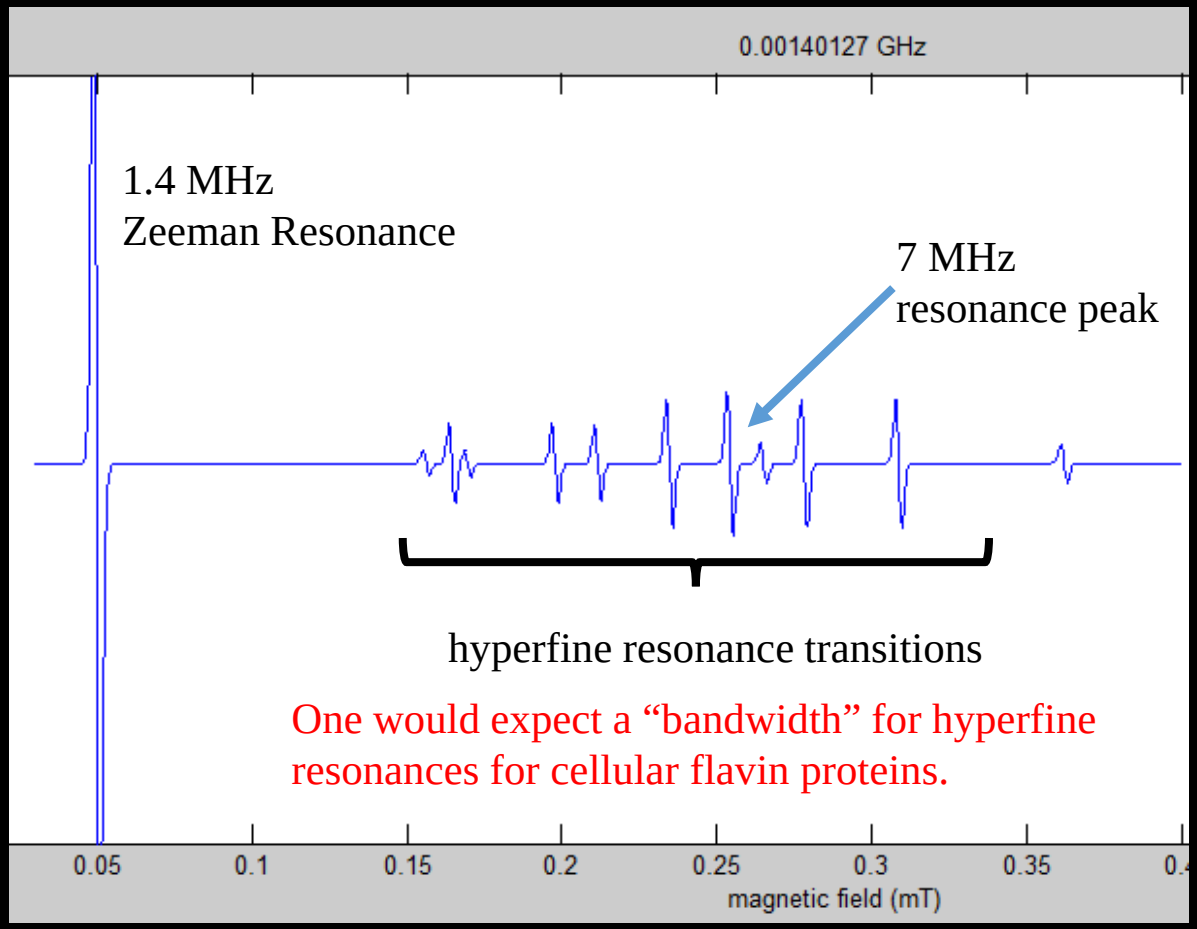
Spin Hamiltonian $H = \mu_B \mathbf{S} \mathbf{g} \mathbf{B} + \mathbf{SAI} + \mathbf{SDI} + \ldots$

$\mu_B \mathbf{S} \mathbf{g} \mathbf{B}$: Zeeman energy

$\mathbf{SAI}$: Hyperfine Coupling

$\mathbf{SDI}$: Dipolar Interaction

EasySpin[1] Resonance Simulation of Two Coupled Spin 1/2s



Two Coupled Spin 1/2s

Superoxide – no hyperfine coupling

Flavin Semiquinone – hyperfine couplings

[2]Hyperfine couplings energies for flavoproteins range from 1 to 35 MHz

Spin Hamiltonian $H = \mu_B \mathbf{S}_1 \mathbf{g} \mathbf{B} + \mathbf{S}\mathbf{A}\mathbf{I} + \mu_B \mathbf{S}_2 \mathbf{g} \mathbf{B}$

Zeeman energy | Hyperfine Coupling | Zeeman energy

[1]S. Stoll and A. Schweiger, *J. Mag. Res.* 178 (1) 42-55 (2006)

[2]E. Schleicher, R. Wenzel, M. Ahmad, A. Batschauer, and S. Weber, *Appl. Mag. Res.* 37 339-352 (2010)

Electron Paramagnetic Resonance (EPR) Spectroscopy

X-band (9 GHz) EPR

MSU Chemistry Department EPR Spectrometer

Calculation of Singlet-Triplet Yields

General formalism: Haberkorn Approach

$$\dot{\rho}(t) = -\frac{i}{\eta}[H, \rho(t)]_- - \frac{k_S}{2}[Q^S, \rho(t)]_+ - \frac{k_T}{2}[Q^T, \rho(t)]_+$$

Stochastic Liouville Equation (Haberkorn approach)

$$\rho(0) = \frac{Q^T}{Tr[Q^T]}$$

Initial condition: Triplet Born radical-pair

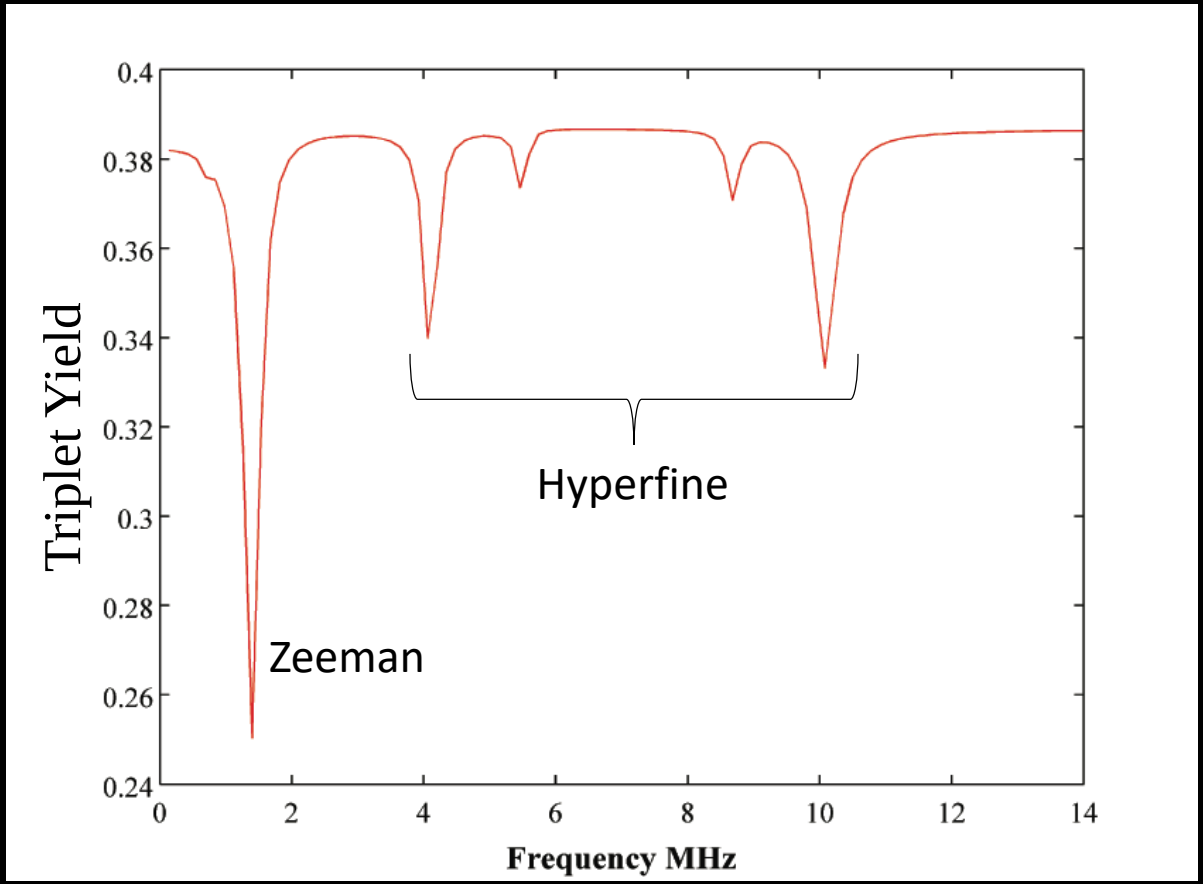
$$T(t) = Tr[Q^T \rho(t)]$$

Triplet probability:
probability that a radical-pair is in a triplet states.
Projection Operator into the Triplet States

$$\Phi^T(t) = \int_0^\infty k_T T(t) dt$$

Triplet Yield is the amount of products decaying via triplet channel -> biochemical signal

Triplet Yield for Zeeman and Hyperfine Resonances

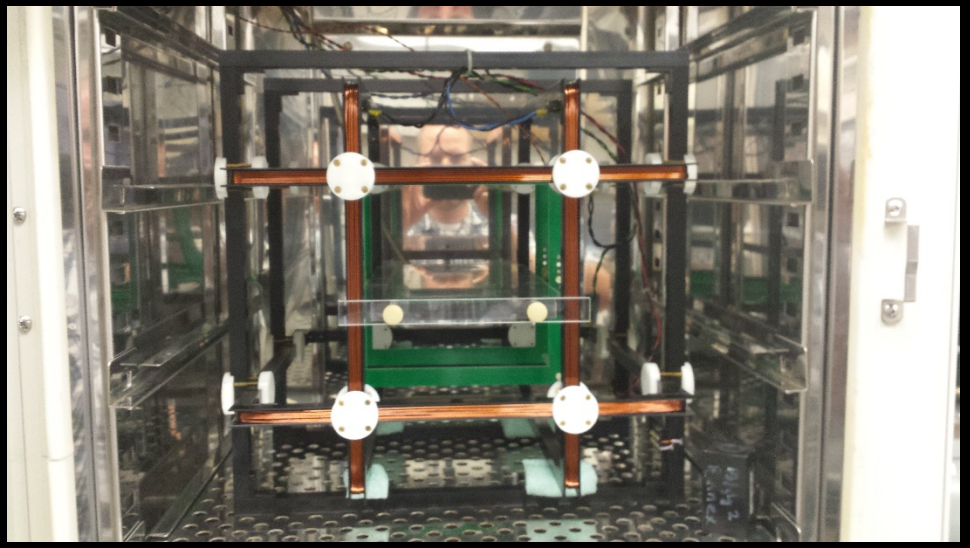


"The intention behind these simulations is to illustrate the general behavior of a model radical pair rather than to mimic faithfully the test conditions of the experiments, a task that would be computationally intractable."

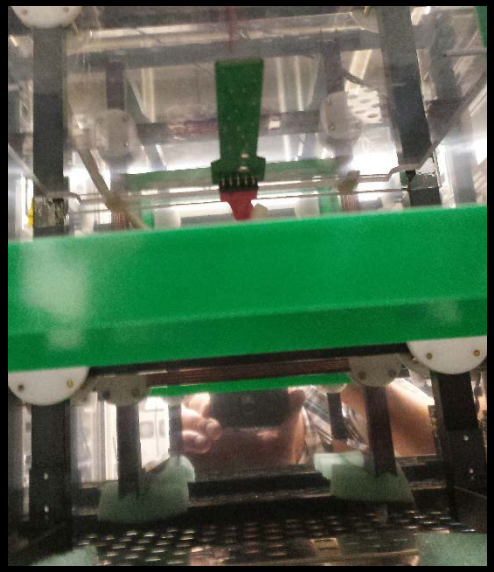
Ritz T, Wiltschko R, Hore PJ, Rodgers CT, Stapput K, et al. Biophysical Journal 96: 3451-3457 (2009).

Quantum Biology Spectrometer

Dual Incubators - Cell Cultures

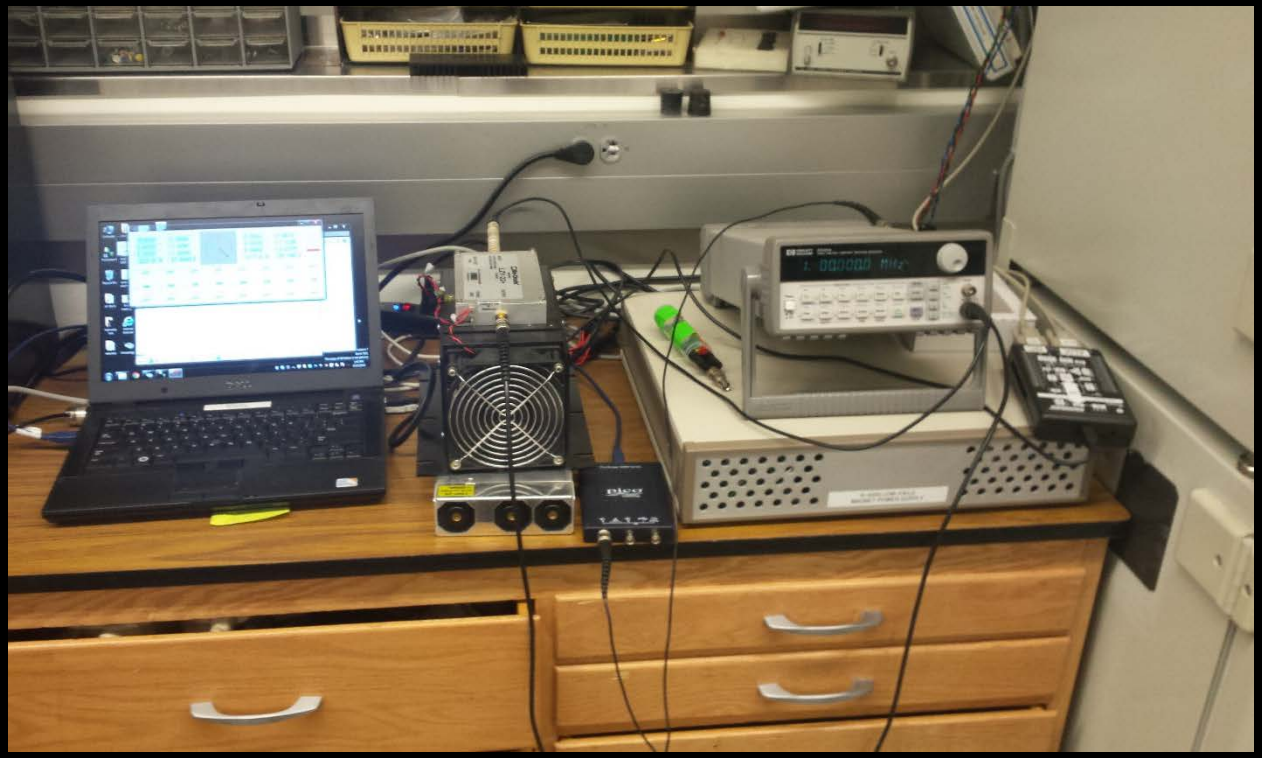


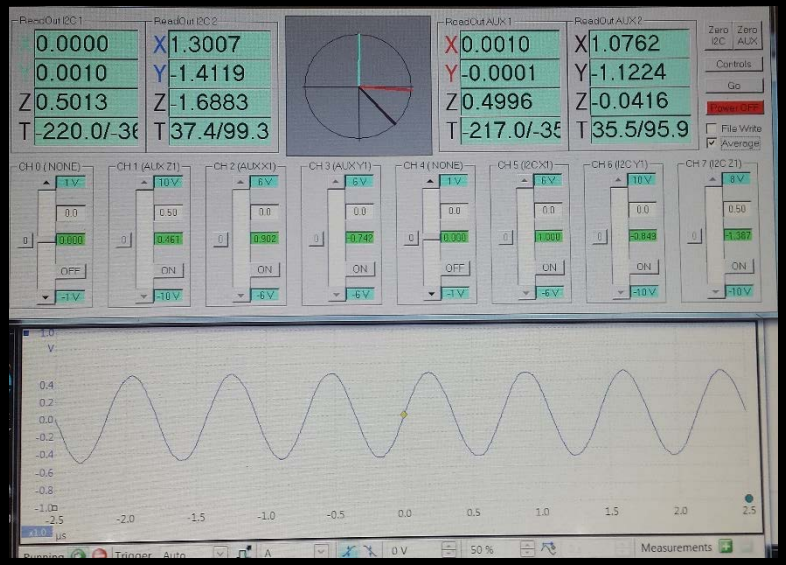
Tri-axial Square Helmholtz Coils, RF Coil (green)



FreeScale 3-axis Magnetometer

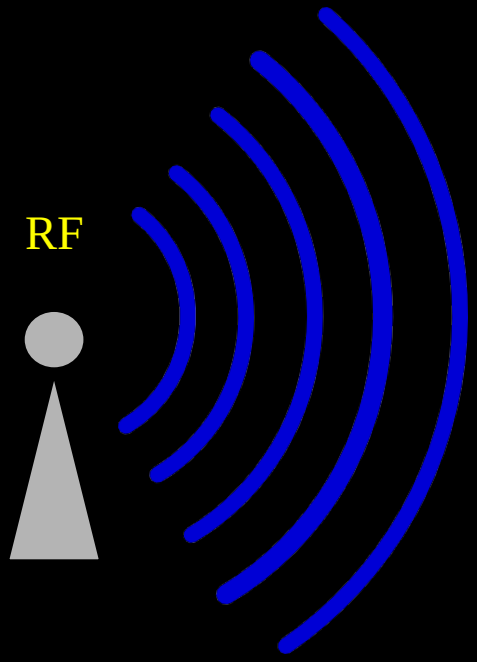
Quantum Biology Spectrometer

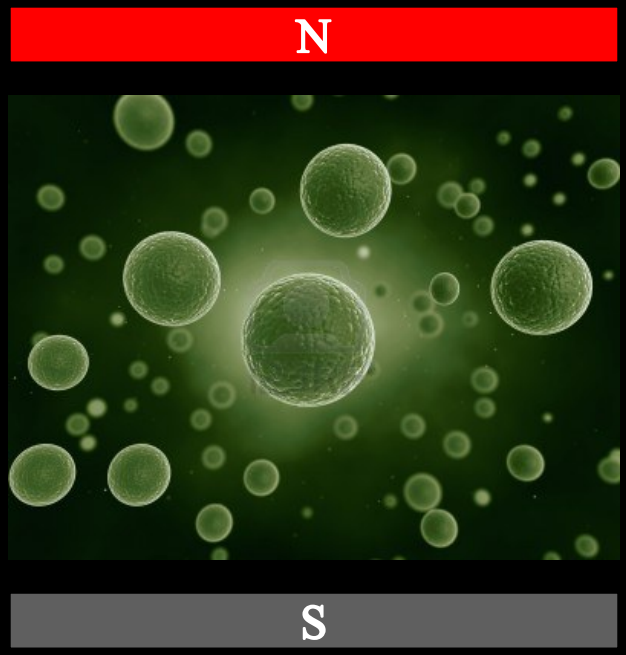


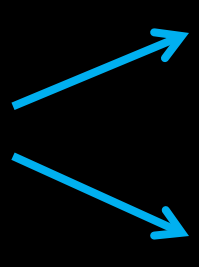


Computer Controlled

Spin Biochemistry and the Biological Production of Reactive Oxygen Species (ROS)







Assay ROS Products (PYDMR)

45 μT Magnetic Field

rat pulmonary arterial smooth muscle cells (rPASMC)

R.J. Usselman, et. al. *PLoS ONE* 9(3) e93065 (2014)

R.J. Usselman, et. al. *Scientific Reports (6) 38543 (2016)*

Spin Biochemistry of ROS - Hyperfine Resonance

Cell Type rat pulmonary arterial smooth muscle cells (rPASMCs)

Experiment Static Magnetic Field – 50 μT

Radio Frequency - 7 MHz

RF perpendicular with respect to SMF

Assays Hydrogen peroxide detection by horse radish peroxidase assay

EPR detection of superoxide with cyclic hydroxylamine spin probes

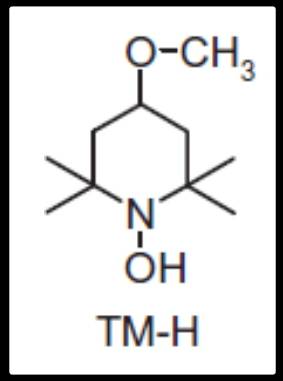
Inhibitors such as paraquat, DPI

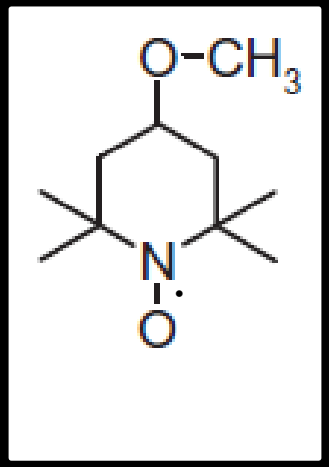
Cellular growth rates: direct cell counts

Intra-cellular Superoxide Detection

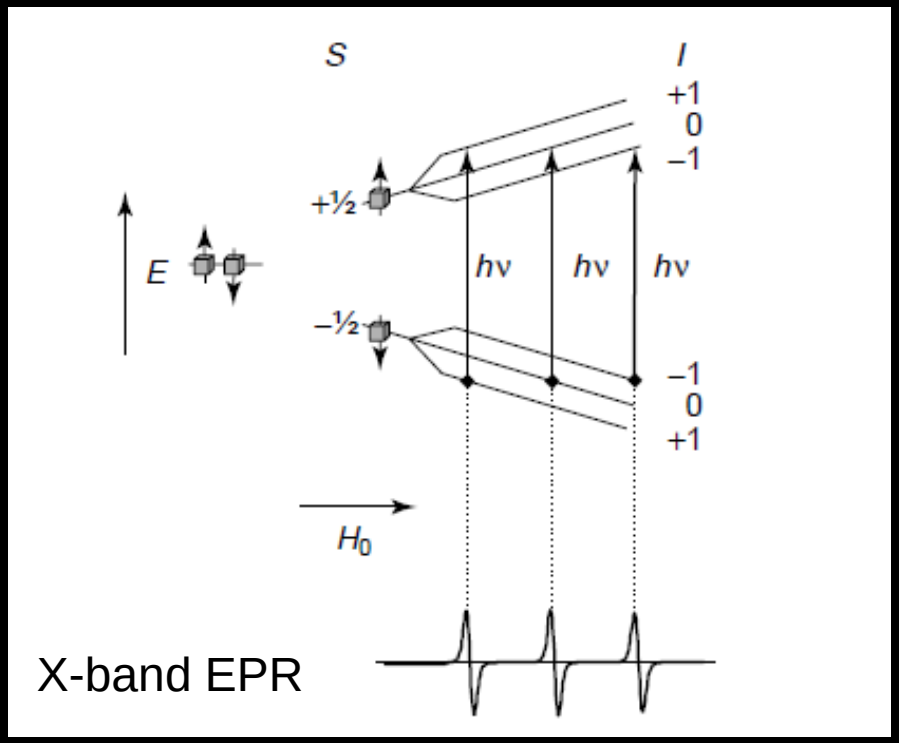
Cyclic Hydroxylamines

$O_2^{-\bullet}$ ⟶





Nitroxyl
Free Radical



Spin ½ electron is coupled to spin 1 nitrogen, gives rise to hyperfine coupling.

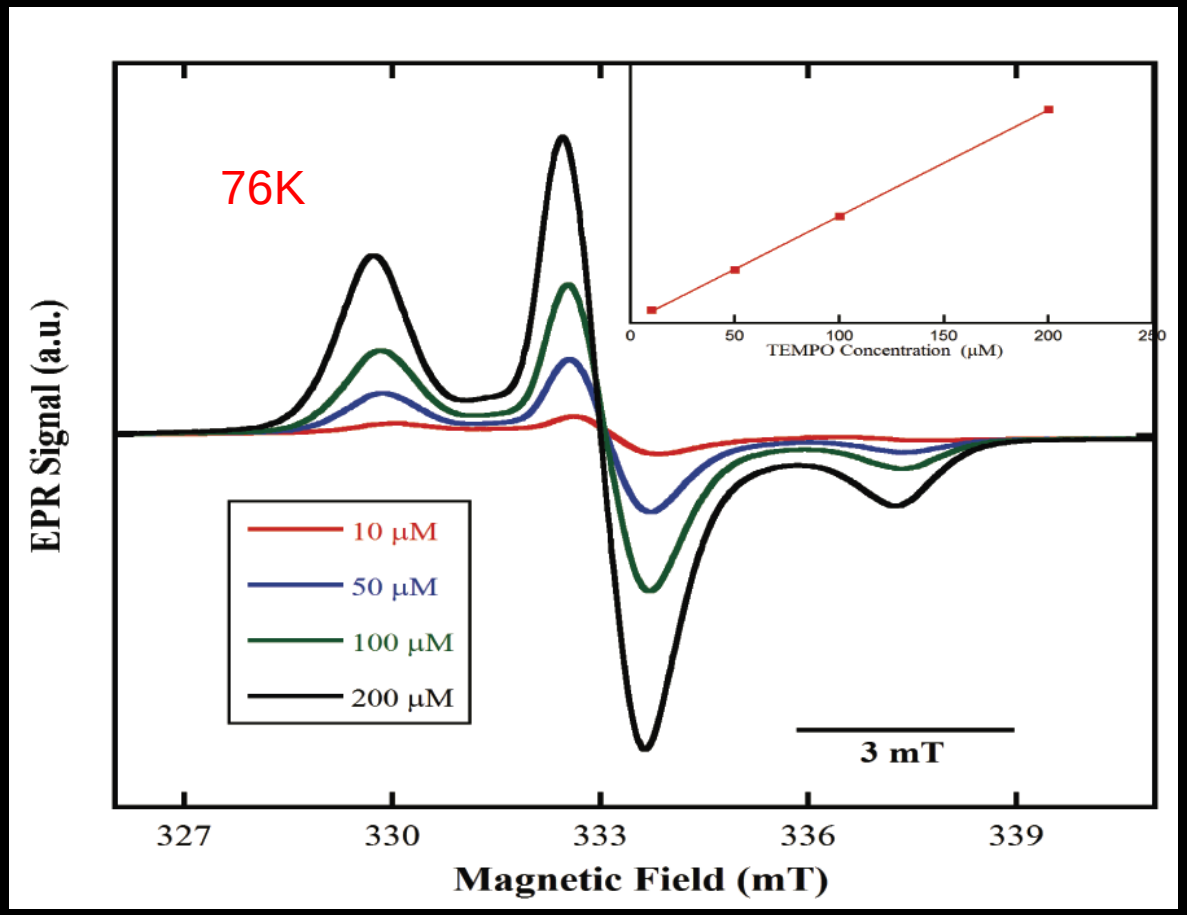
Spin Hamiltonian $H = \beta_e \mathbf{S} g \mathbf{B} + \mathbf{S} A \mathbf{I}$

$\beta_e \mathbf{S} g \mathbf{B}$: Zeeman energy

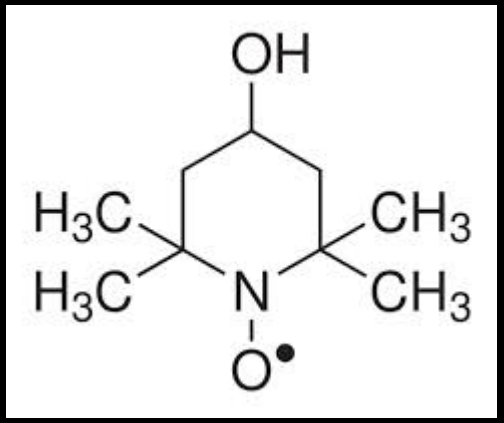
$\mathbf{S} A \mathbf{I}$: Hyperfine Coupling

S.I. Dikalov, I.A. Kirilyuk, M. Voinov, I.A. Grigor'ev, *Free Radical Research* 45(4): 417-430 (2011)

X-band EPR Nitroxide Standard Curve

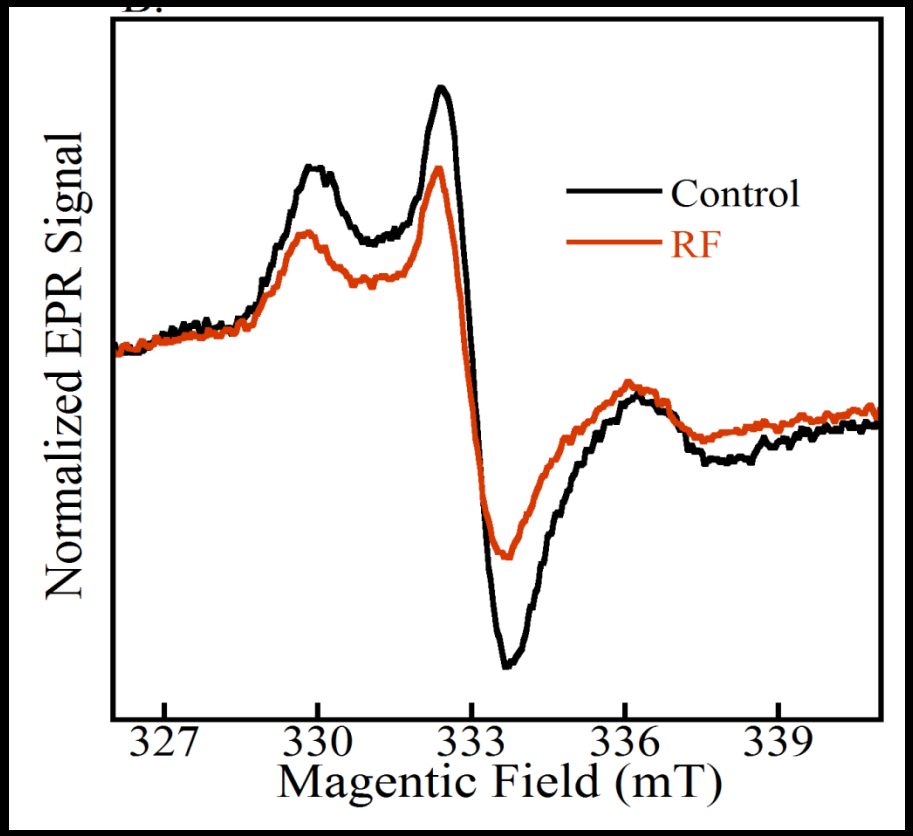


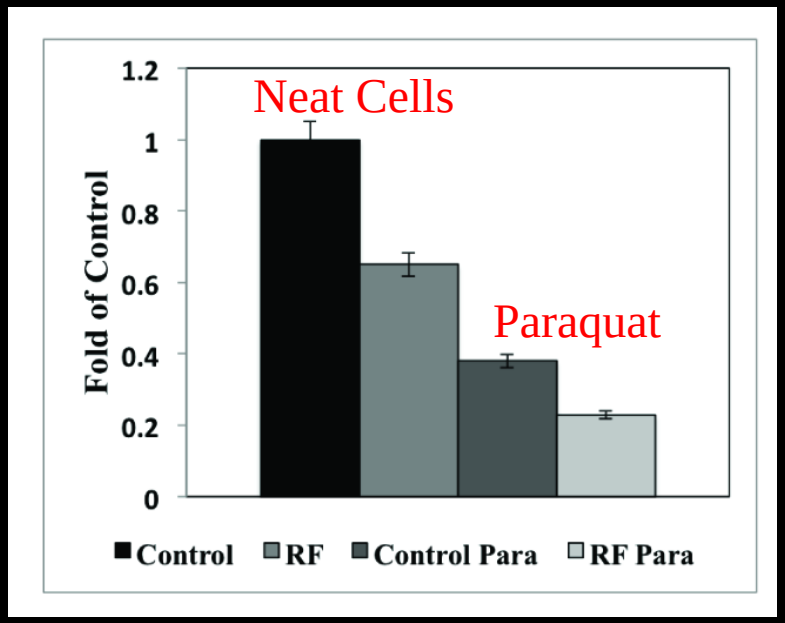
TEMPO

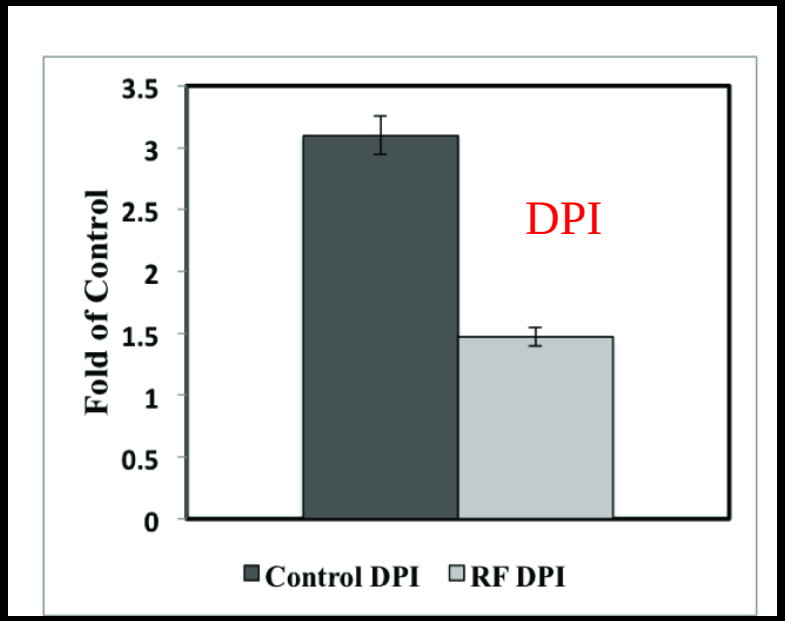


Standard curves for TEMPO spin probe.
EPR signal intensity is proportional to the number of spins.

7 MHz (10 μT_{rms}) RF vs. Control EPR Data

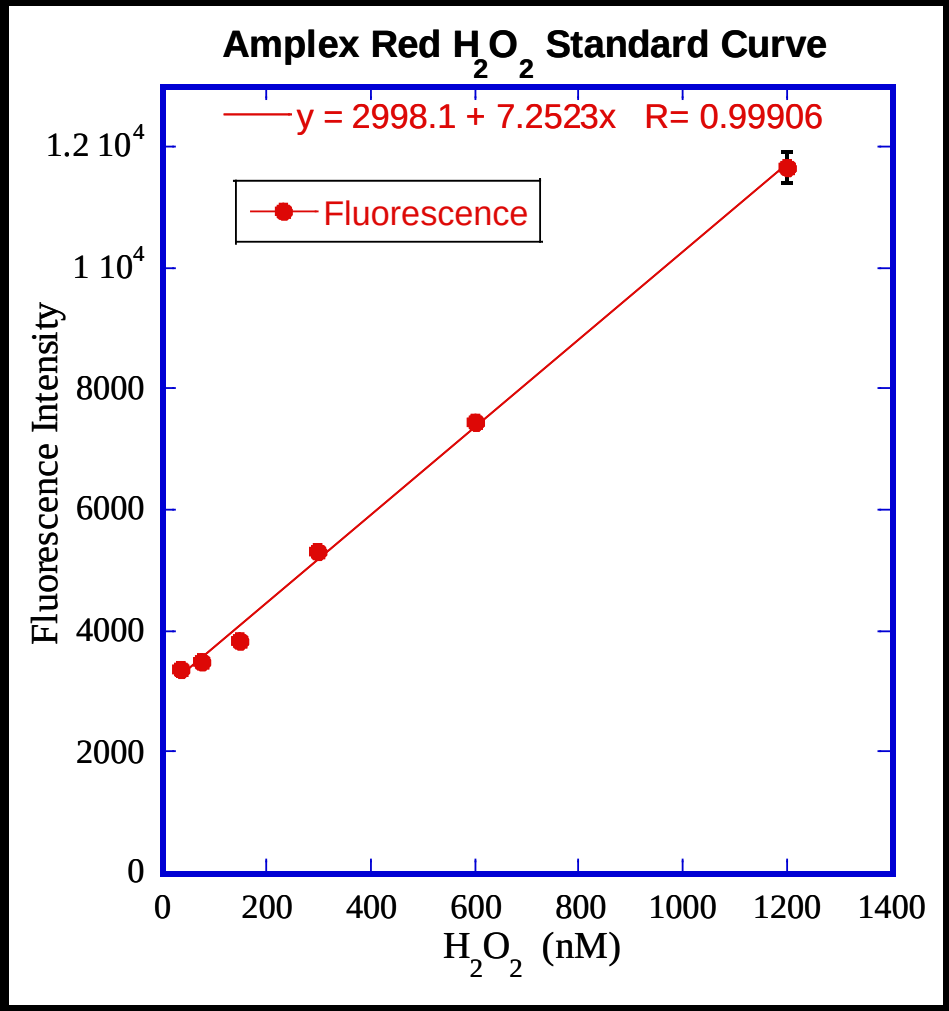


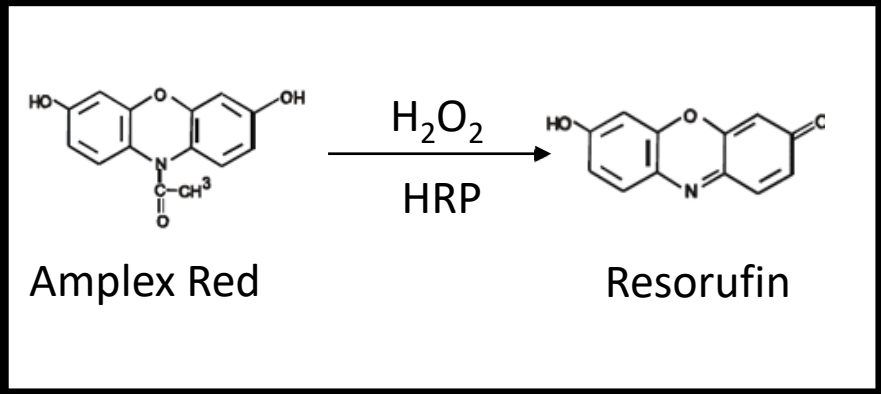




Herbicides were used to study Nox signaling.

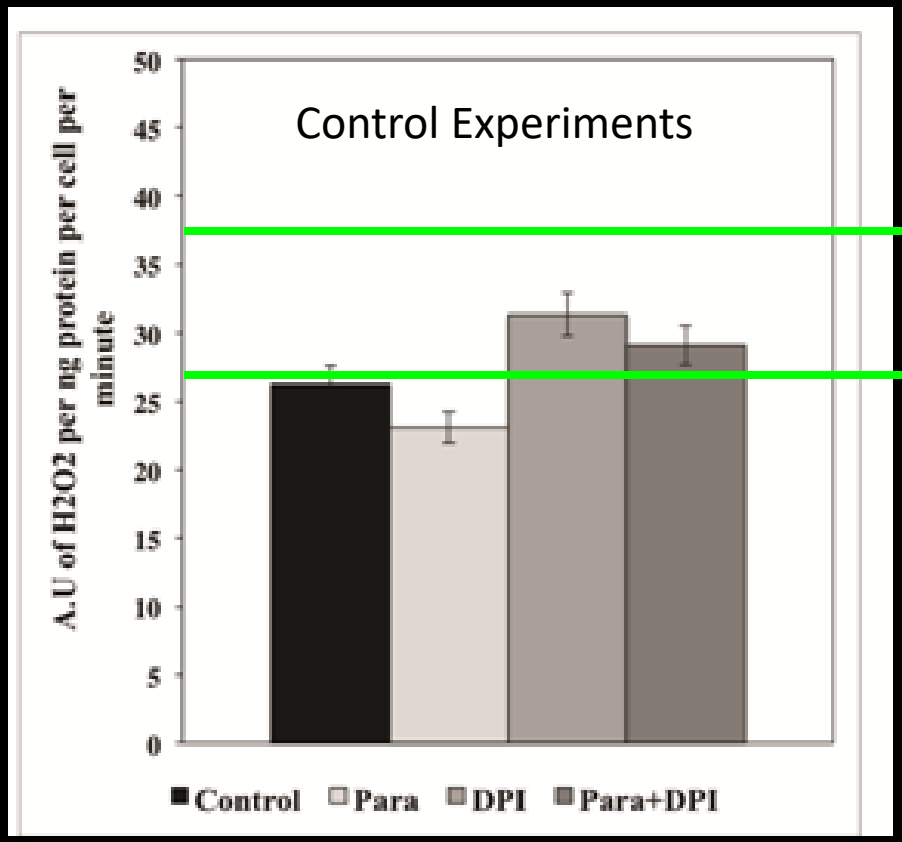
Hydrogen Peroxide Standard Curve

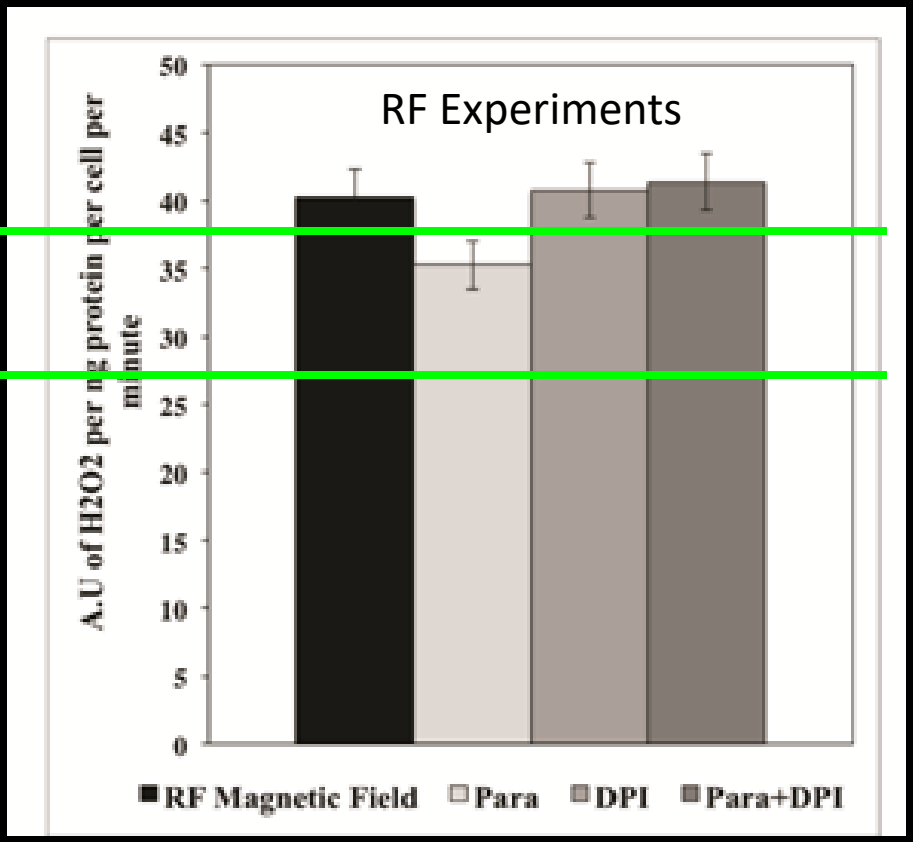




Biotek Plate Reader

RF and Herbicide H_2O_2 Production



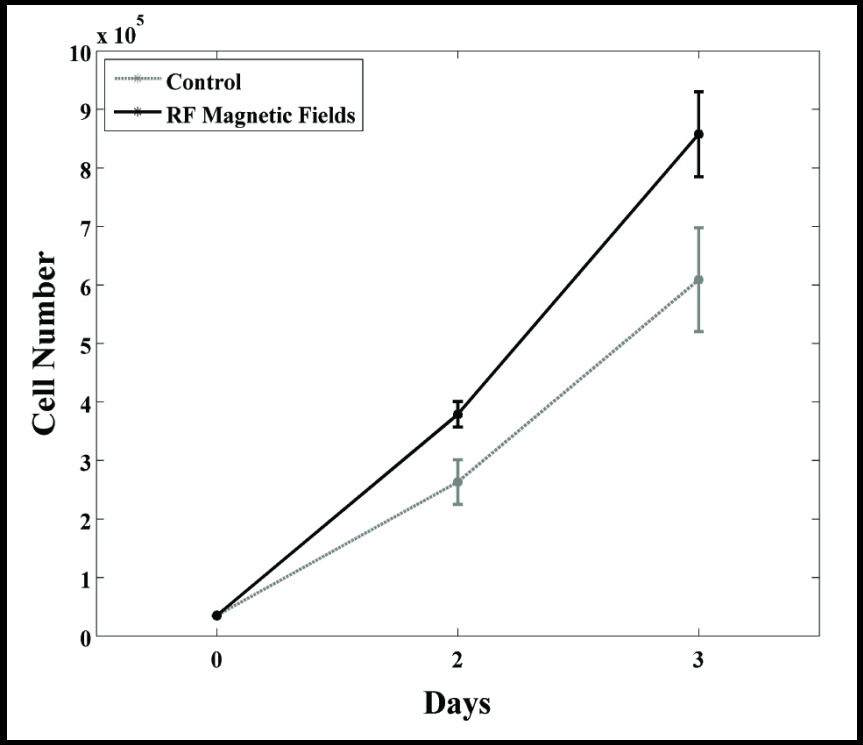


<u>Salient Feature:</u>
Collective RF baseline is larger for all measured samples.

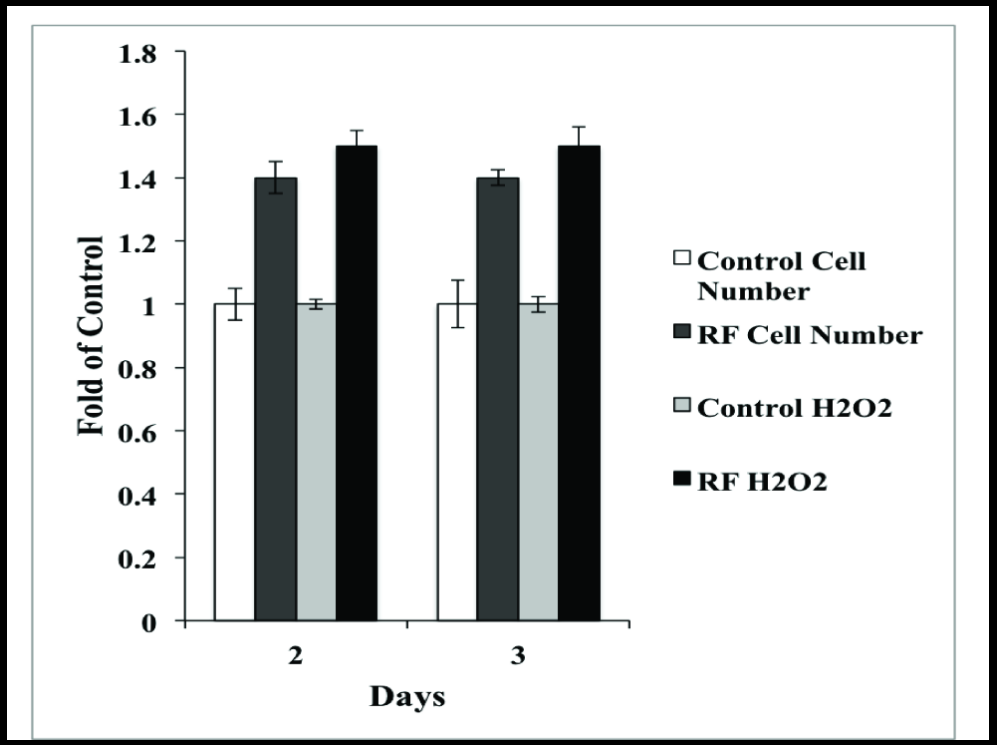
Increased H_2O_2 Production
- 30-40%

Correlation between Increased Cell Proliferation and H_2O_2 Production

Cellular Growth Curves



Amplex Red H_2O_2 Assays



Increased Cellular Proliferation

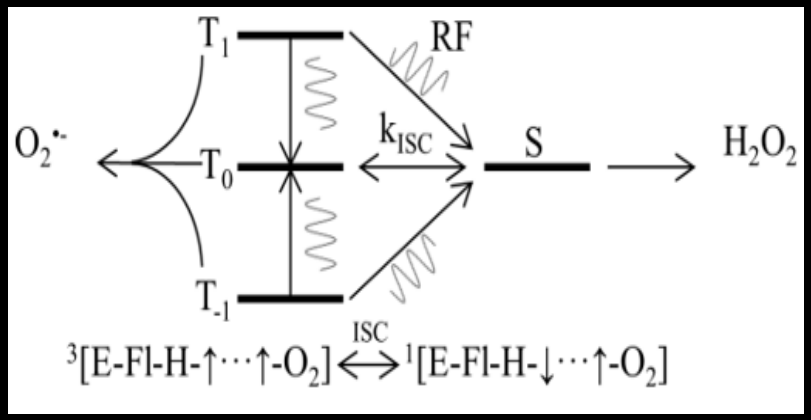
- Day 2 ~ 40%
- Day 3 ~ 45%

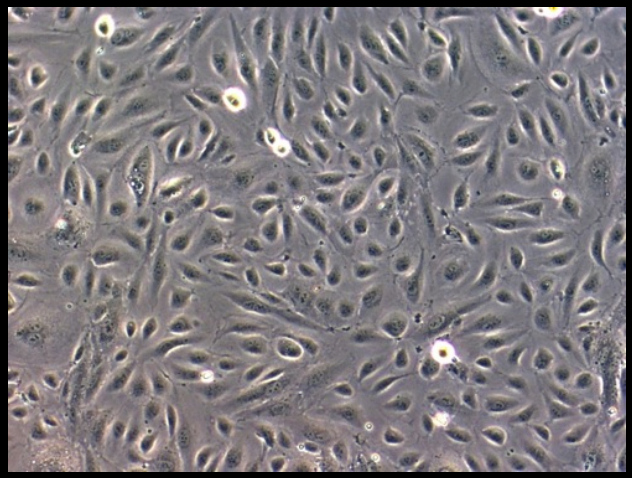
Increased H_2O_2 Production

- Day 2 ~ 50%
- Day 3 ~ 50%

Summary – Hyperfine Resonance

Singlet-Triplet Interconversion





Increased cell proliferation

- **ROS singlet-triplet yields are changed by magnetic fields, which elicit a biological response – quantum biology**

Spin Biochemistry of ROS - Zeeman Resonance

Cell Type Human umbilical vein endothelial cells (HUVECs)

Experiment

Static Magnetic Field – 50 μT

Radio Frequency – 1.4 MHz

RF perpendicular or parallel with respect to SMF

Assays

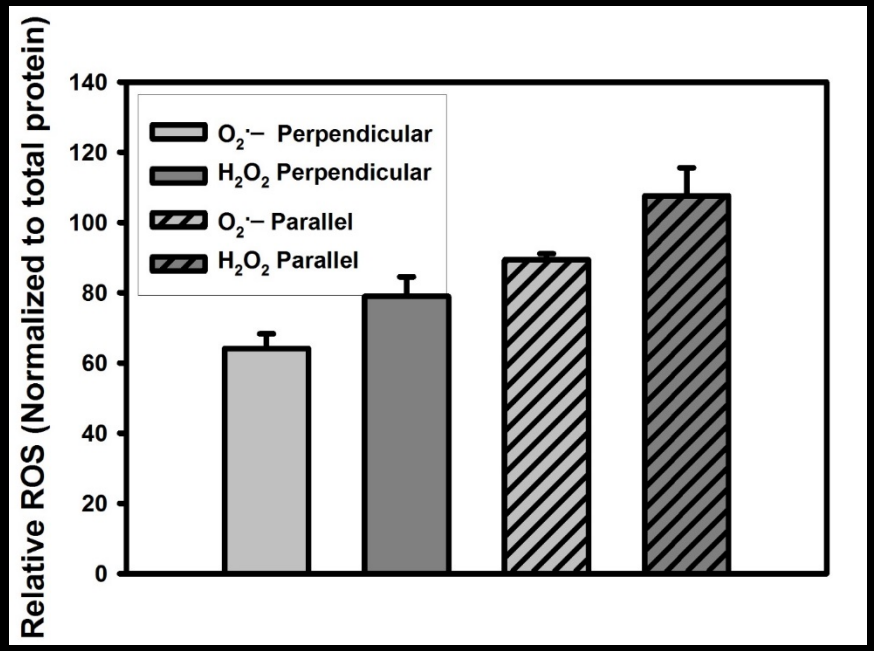
HPLC detection of superoxide by dihydroethidium

Hydrogen peroxide detection by horse radish peroxidase assay

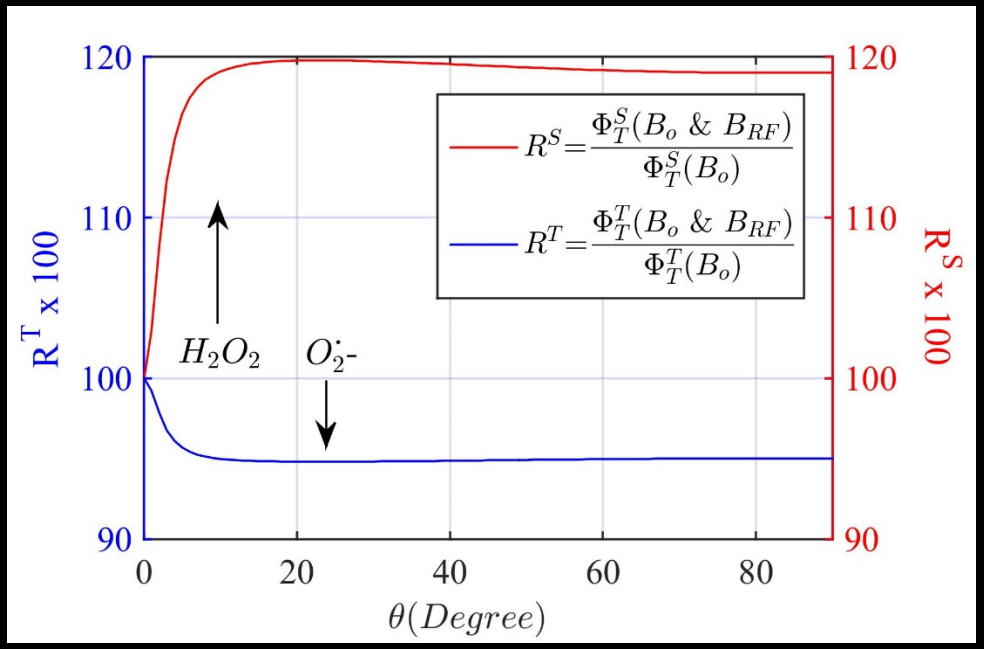
Bioenergetics: oxygen consumption rate and extracellular acidification rates

ROS Partitioning at Zeeman Resonance: Orientation Dependence

Experimental



Simulations



Bioenergetics

Seahorse XFp Extracellular Flux Analyzer

- Mitochondrial Respiration: measured by Oxygen Consumption Rate (OCR)
- Glycolysis: measured by Extracellular Acidification Rate (ECAR)

Seahorse Metabolic Stress Test

Cell Mito Stress Test

Measure of mitochondrial functioning through measure of oxygen consumption

Oligomycin

Inhibits ATP synthase

Decrease linked to mitochondrial respiration linked to ATP production

FCCP

Collapse of proton gradient

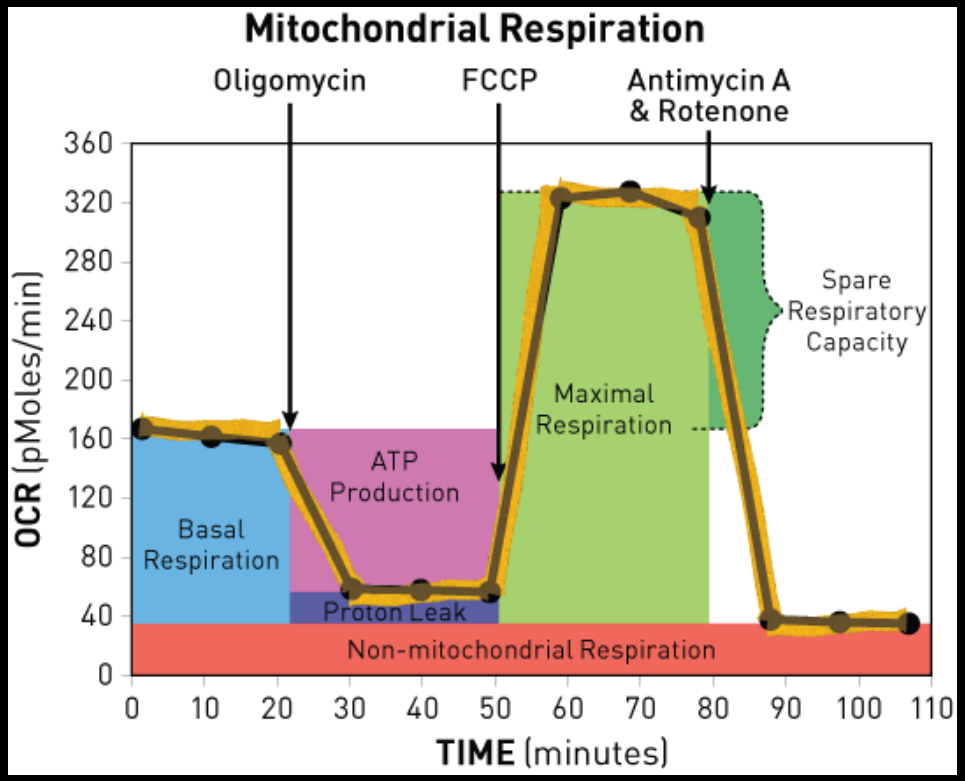
Disrupts mitochondrial membrane potential

Spare Capacity

Antimycin A/ Rotenone

Complex I/Complex III inhibitor

Shuts down mitochondrial respiration

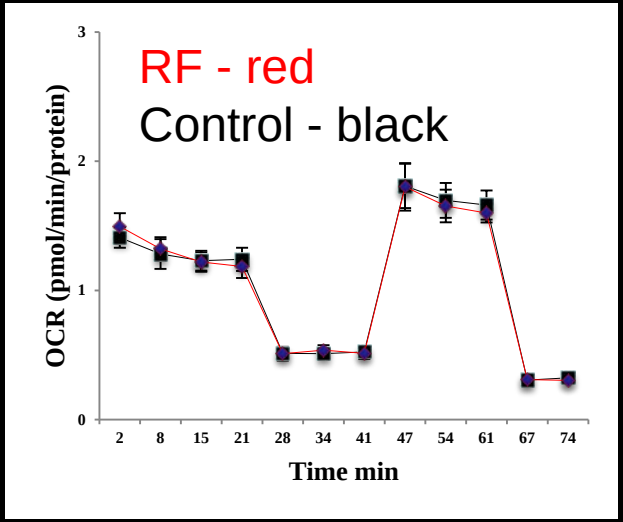


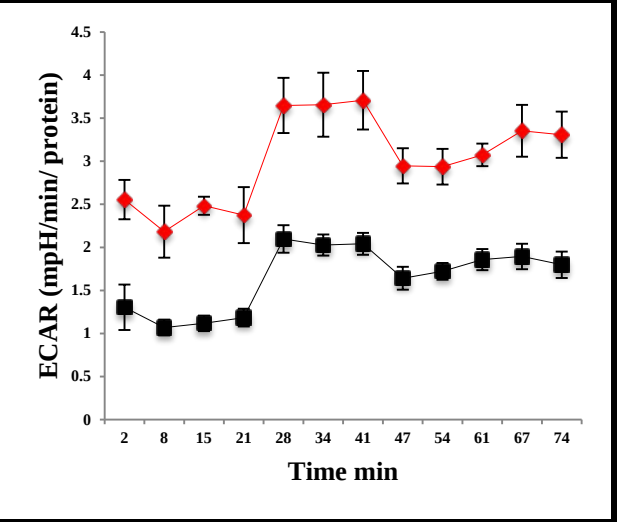
The Quantum Biology of ROS Impacts Cellular Bioenergetics

Respiration

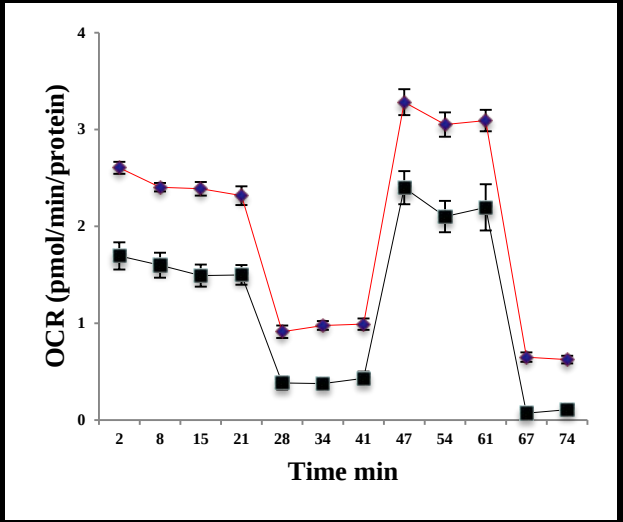
Glycolysis

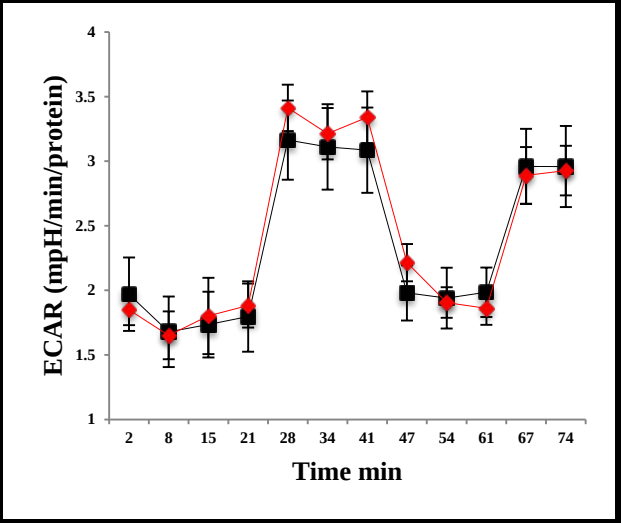
Zeeman Perpendicular





Zeeman Parallel

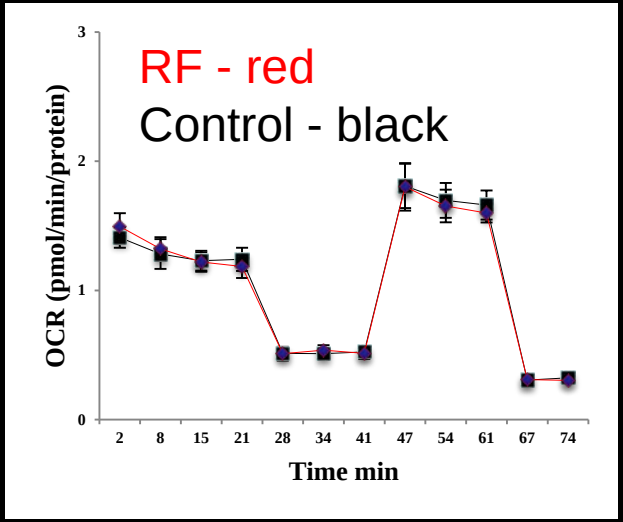




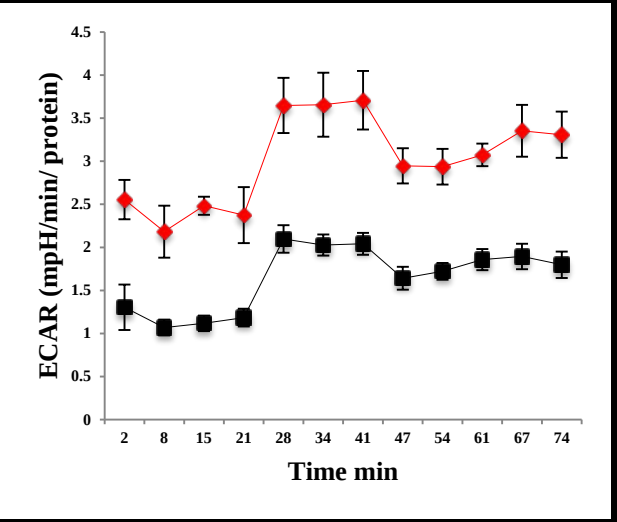
Bioenergetics – Zeeman Perpendicular

Zeeman Perpendicular

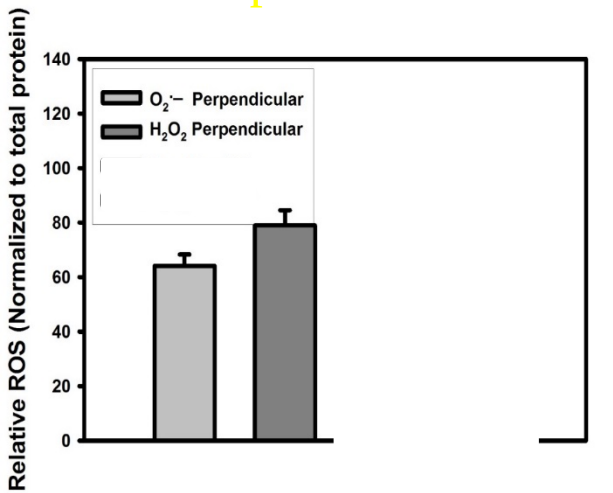
Respiration



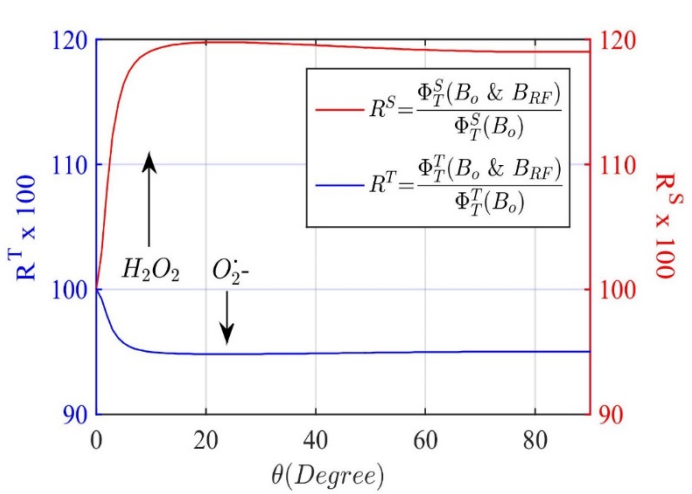
Glycolysis



Experimental



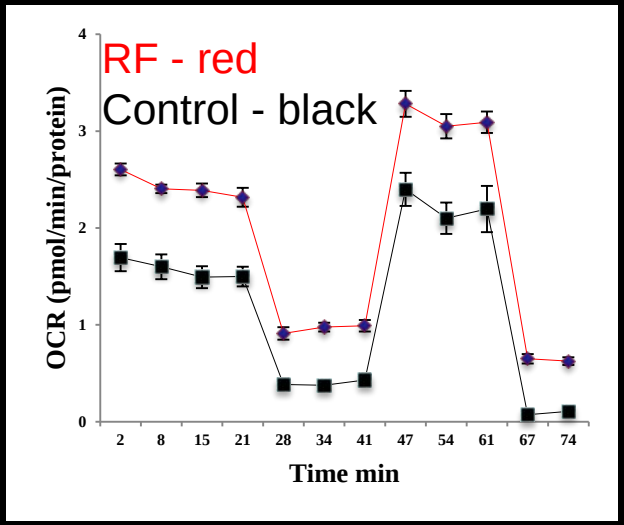
Simulations



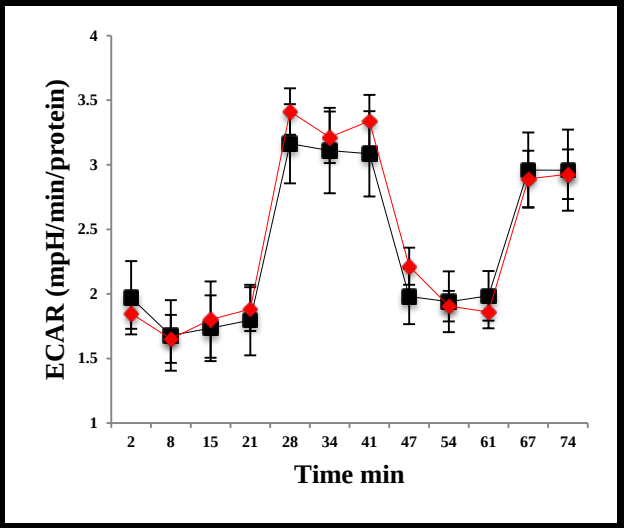
Bioenergetics – Zeeman Parallel

Zeeman Parallel

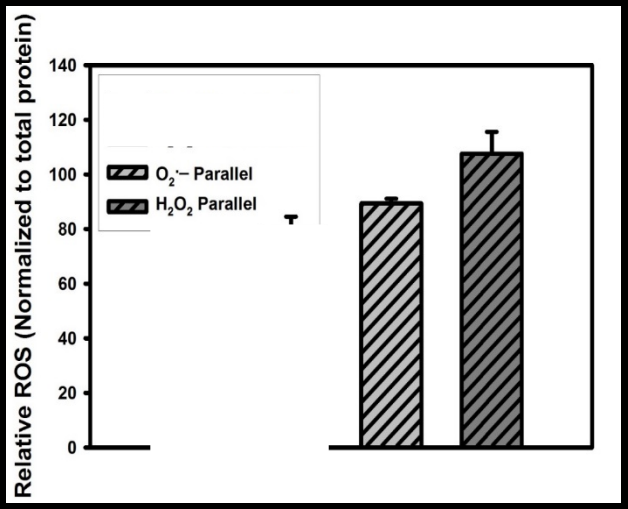
Respiration



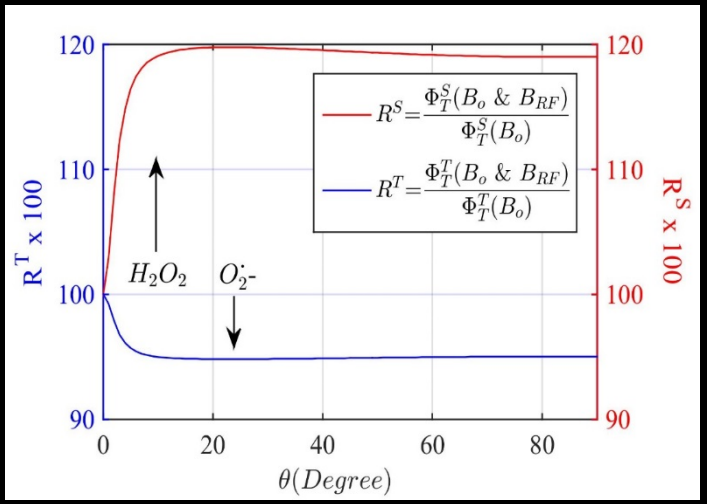
Glycolysis



Experimental



Simulations

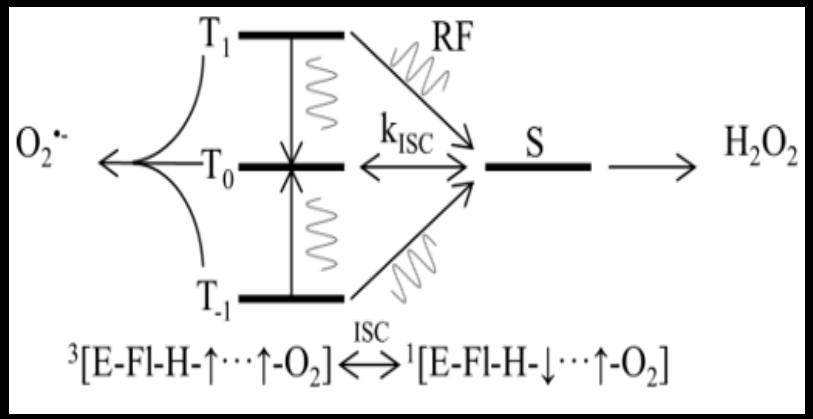


A New Quantum (Coherence) Biology Domain in Redox Cell Biology

- **Indirect evidence of quantum coherence in the biological production of ROS.**
- **Electron spin dynamics, altered by magnetic fields, determine relative yields of cellular H_2O_2 and $O_2^{\bullet -}$ products.**
- **Connect persistent quantum effects in ROS production to cell proliferation and bioenergetics, bridging the atomic and cellular levels.**
- **Results provide fundamental insights into the role of the RPM in ROS redox cell biology.**

Future Direction

Singlet-Triplet Interconversion



Real-time spatio-temporal evolution of ROS production

ROS signaling in cellular bioenergetics

Theoretical optimization of ROS partitioning

Thank You!

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