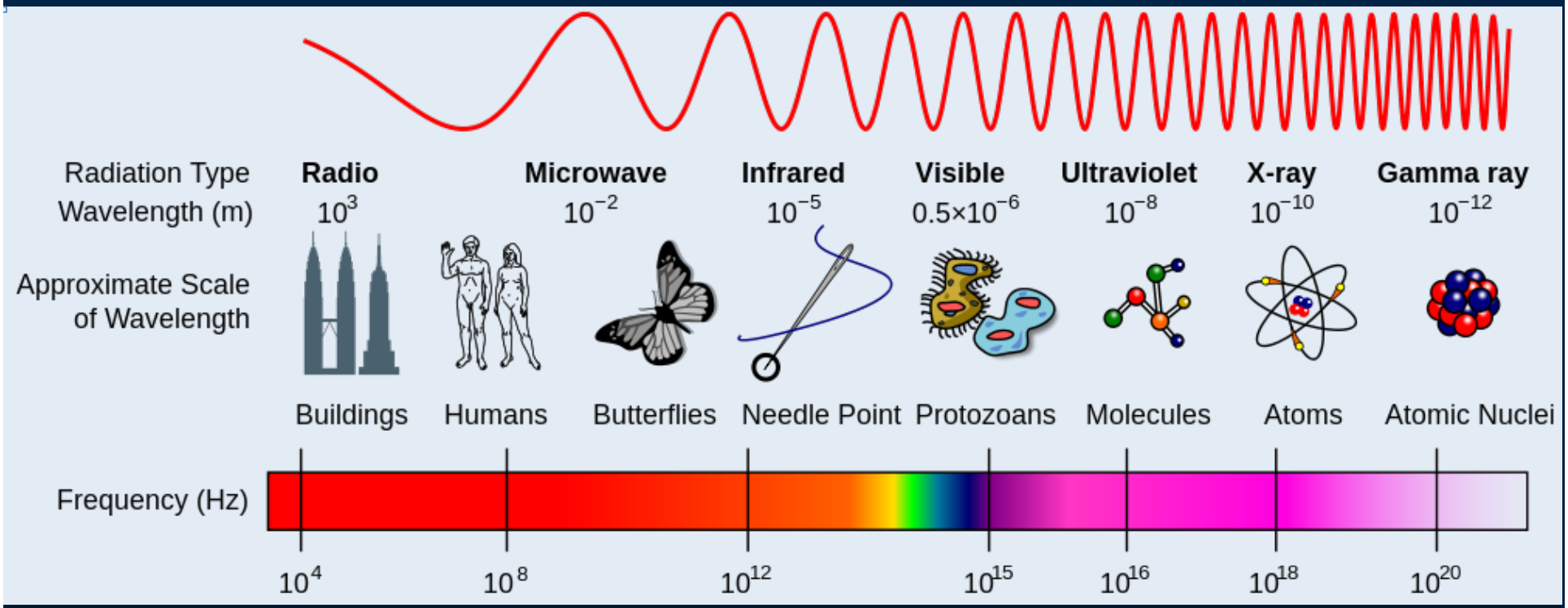
GS-Nitroxides for Radiation and ROS-Related Disease

Joshua Sacher

Topic Seminar

15 Dec 2012

Electromagnetic Radiation



http://commons.wikimedia.org/wiki/File:EM_Spectrum_Properties_edit.svg

Ionizing Radiation

Units of Radiation

- Gray (Gy)
  - 1 J/kg
  - Independent of IR type, location
- Sievert (Sv)
  - Equivalent dose
  - Factors in IR "quality," area exposed

Exposure to IR

- Unintended exposure
  - Industrial accidents (Fukushima)
  - Nuclear weapons
- Intentional exposure
  - X-rays
  - Radiation therapy (cancer)

Dose Reference Scale

Yearly Background	~ 2-4 mSv
Full Body CT	< 30 mSv
Highest Dose at Fukushima	~ 670 mSv
Predicted Dirty Bomb Dose	< 10 mSv

http://www.bioterrorism.slu.edu/dirty/dirty.pdf

Whole-Body Irradiation Effects

Phase	Symptom	Whole-body absorbed dose (Gy)				
		1–2Gy	2–6Gy	6–8Gy	8–30Gy	Greater Than 30Gy
Immediate	Nausea and vomiting	5–50%	50–100%	75–100%	90–100%	100%
	Time of onset	2–6h	1–2h	10–60 min	< 10 min	Minutes
	Duration	< 24h	24–48h	< 48h	< 48h	N/A (patients die in < 48h)
	Diarrhea	None	None to mild (<10%)	Heavy (>10%)	Heavy (>95%)	Heavy (100%)
	Time of onset	—	3–8h	1–3h	< 1h	< 1h
	Headache	Slight	Mild to moderate (50%)	Moderate (80%)	Severe (80–90%)	Severe (100%)
	Time of onset	—	4–24h	3–4h	1–2h	< 1h
	Fever	None	Moderate increase (10-100%)	Moderate to severe (100%)	Severe (100%)	Severe (100%)
	Time of onset	—	1–3h	< 1h	< 1h	< 1h
	CNS function	No impairment	Cognitive impairment 6–20 h	Cognitive impairment > 24h	Rapid incapacitation	Seizures, Tremor, Ataxia, Lethargy
Latent period		28–31 days	7–28 days	< 7 days	none	none
Illness		Mild to moderate Leukopenia Fatigue Weakness	Moderate to severe Leukopenia Purpura Hemorrhage Infections Epilation after 3 Gy	Severe leukopenia High fever Diarrhea Vomiting Dizziness and disorientation Hypotension Electrolyte disturbance	Nausea Vomiting Severe diarrhea High fever Electrolyte disturbance Shock	N/A (patients die in < 48h)
Mortality	Without care	0–5%	5–100%	95–100%	100%	100%
	With care	0–5%	5–50%	50–100%	100%	100%
	Death	6–8 wks	4–6 wks	2–4 wks	2 days–2 wks	1–2 days

Manhattan Project (1945, 1946):

Harry Daghlian: ~3.3 Gy (2.2 n + 1.1 γ), died 25 d

Louis Slotin: ~11.1 Gy (10 n + 1.1 γ), died 9 d

Wood River Junction (1964): ~100 Gy dose, died 49 h

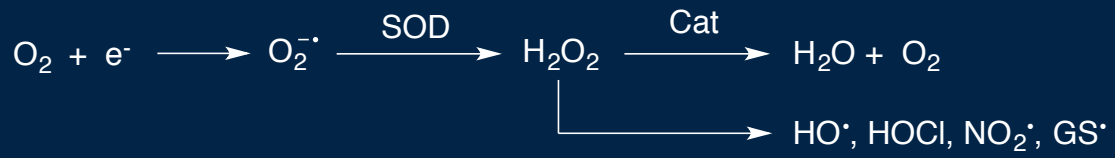
Current treatment: Supportive

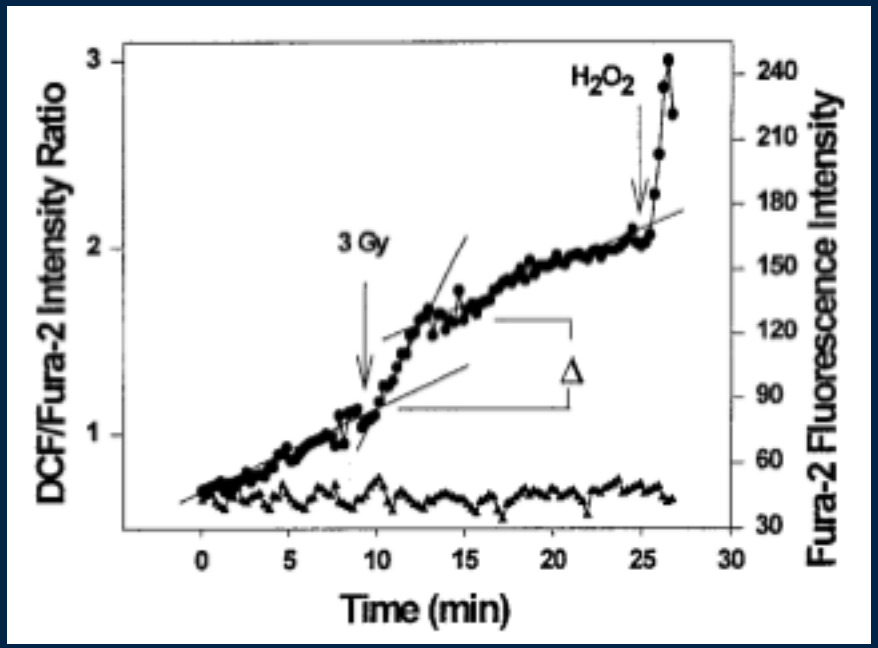
Merck Manuals

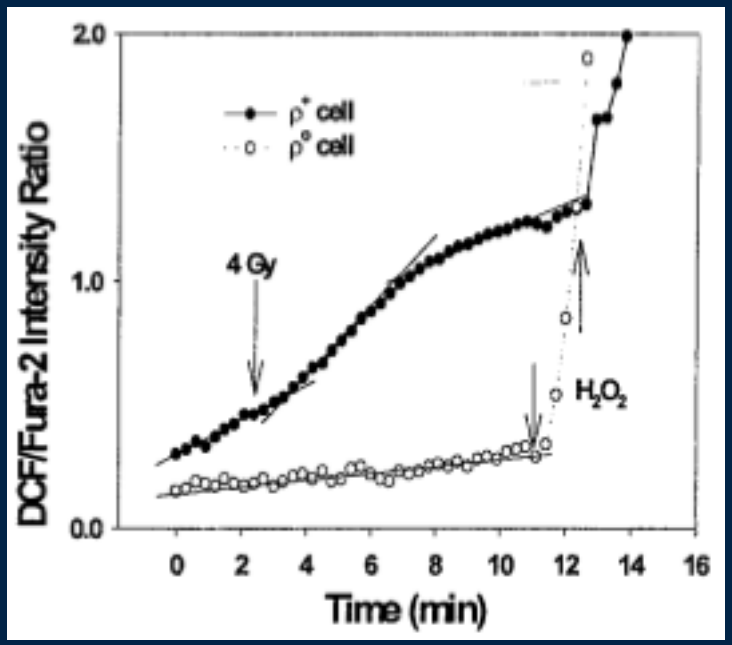
A Review of Criticality Accidents, Los Alamos Labs

Cellular Effects of Radiation

- Protein damage
- DNA damage (ss and ds breaks, base damage, crosslinking)
- ROS generation
  - Occurs in mitochondria



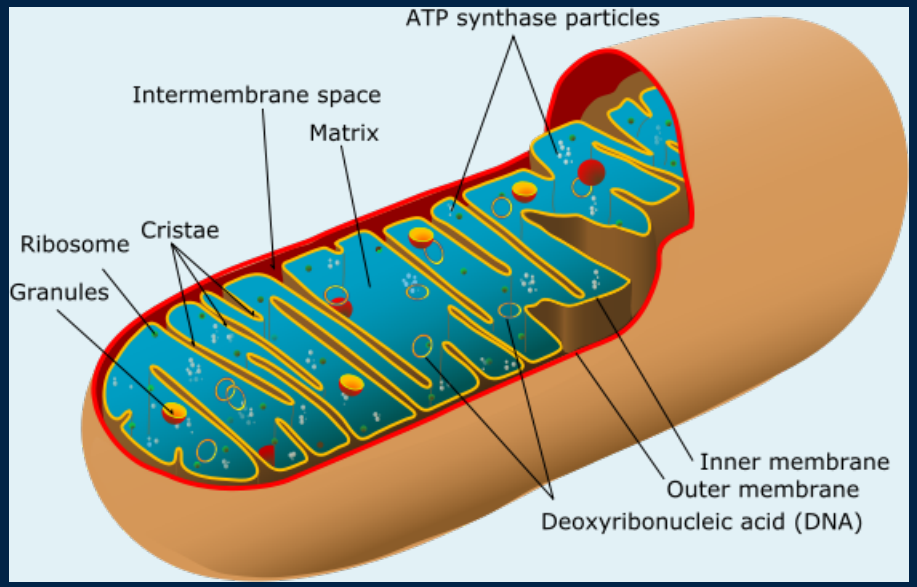




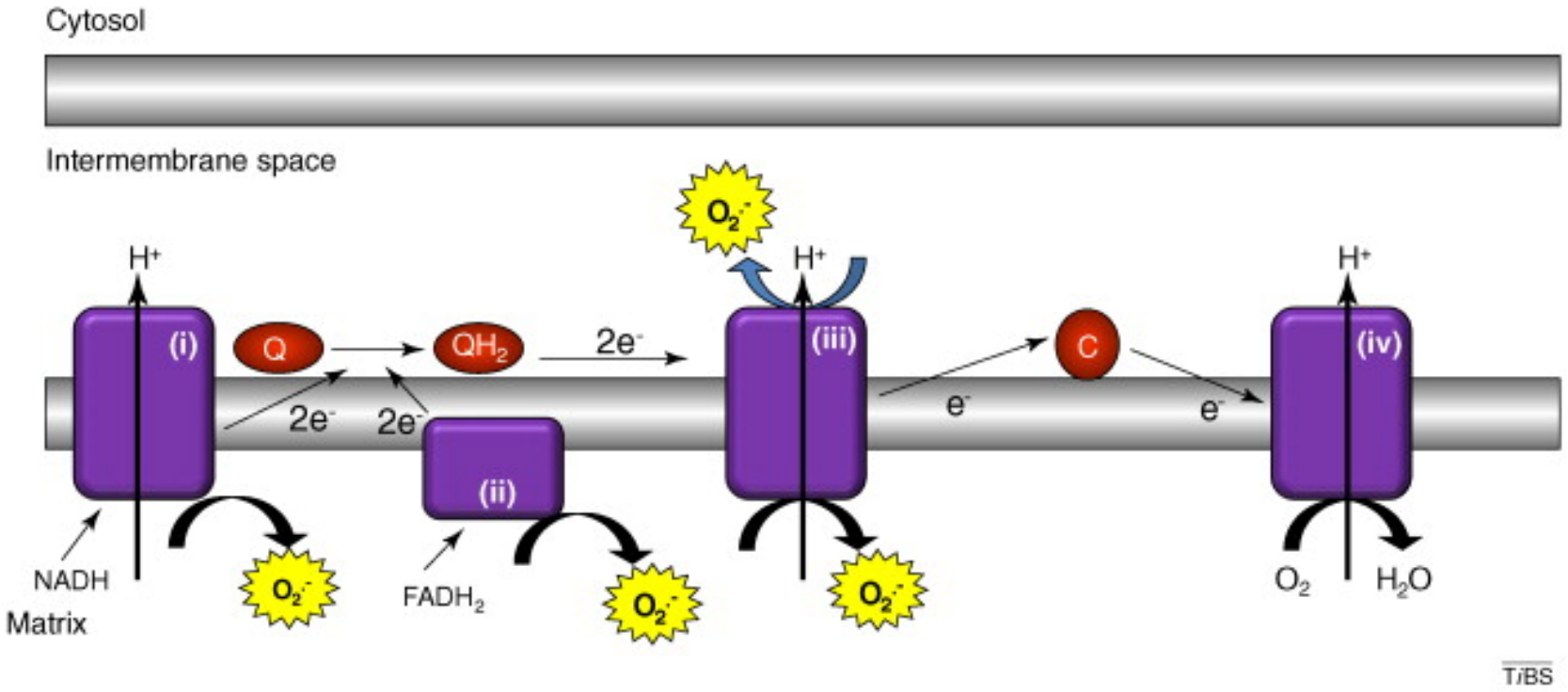
Rad. Prot. Dosim. **2006**, *122*, 124
Cancer Res. **2001**, *61*, 3894

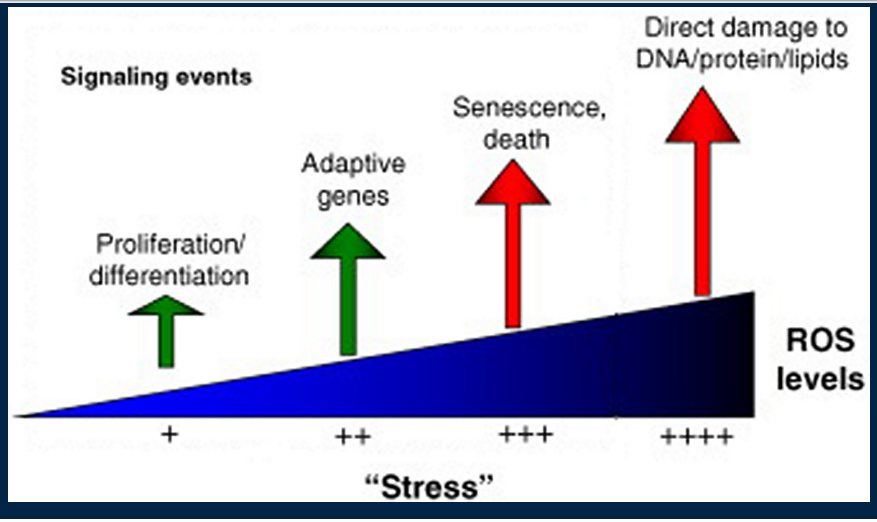
Mitochondria

- Origin likely from endosymbiosis
- Large number per cell
- Energy production through OxPhos



ROS Production by ETC



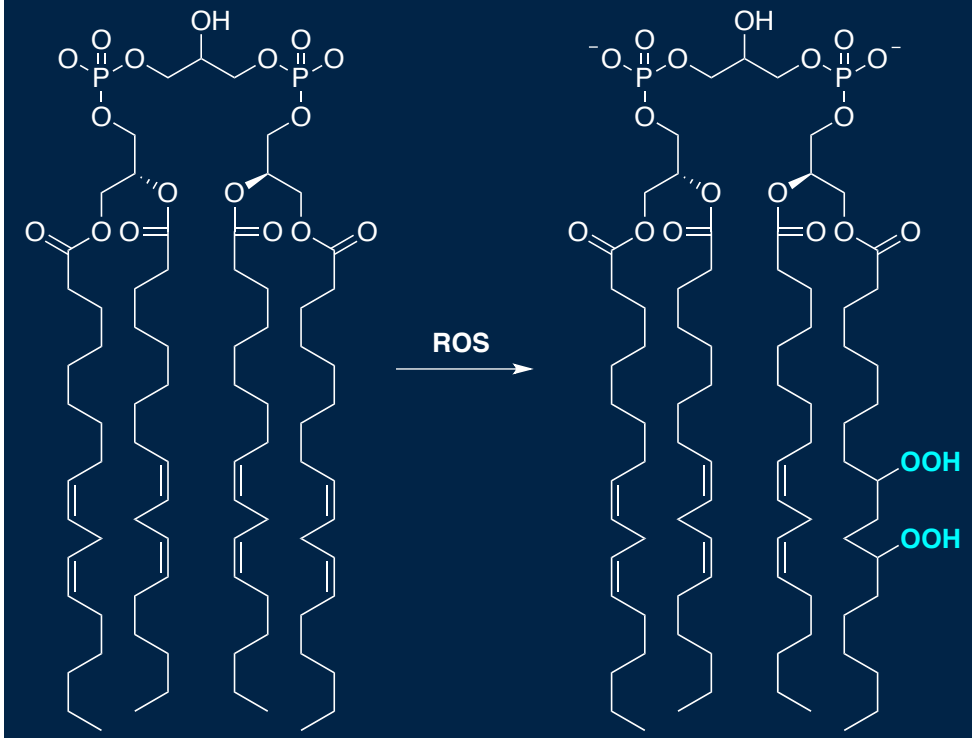


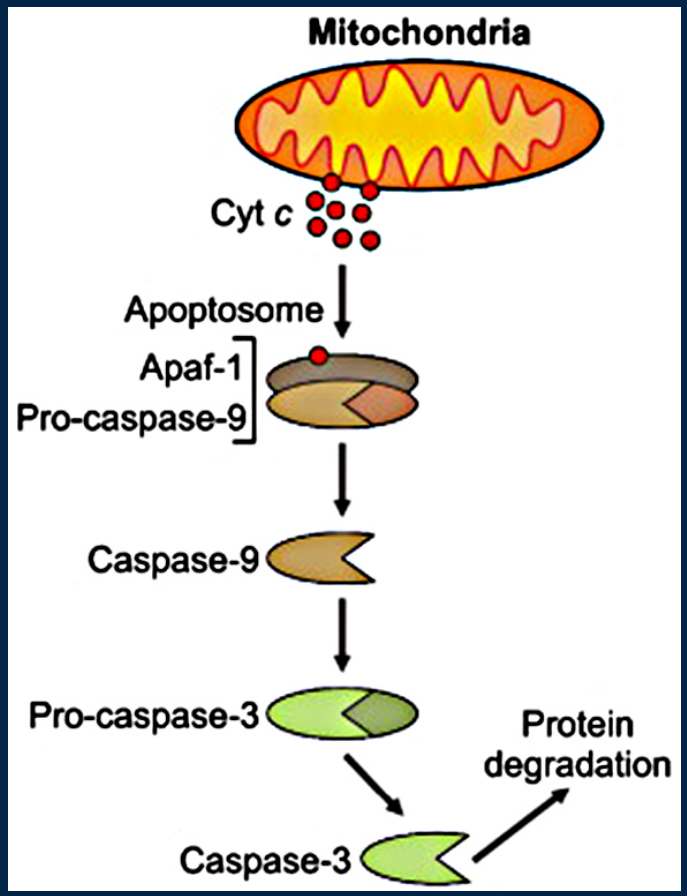
Trends Biochem. Sci. **2010**, *35*, 505

ROS and Apoptosis

Cardiolipin:

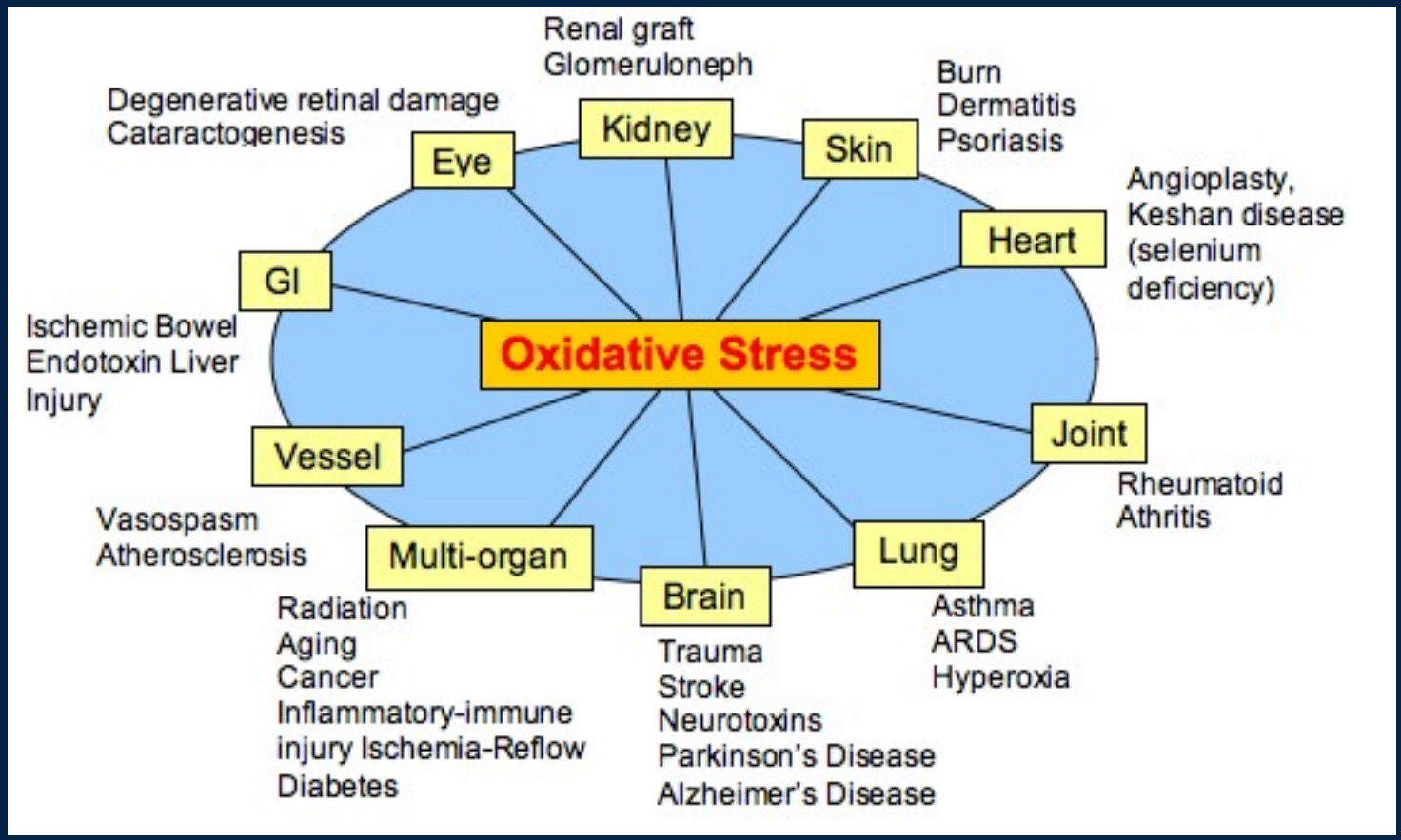
- Mitochondrial inner membrane
- Oxidized by ROS
- Translocates to outer membrane
- Allows release of Cyt C; triggers apoptosis





Trends Cell Biol. **2000**, *10*, 369
Biochem. Biophys. Res. Commun. **2008**, *368*, *145*
J. Biol. Chem. **2011**, *286*, 26334
Phys. Behav., **2007**, *92*, 87

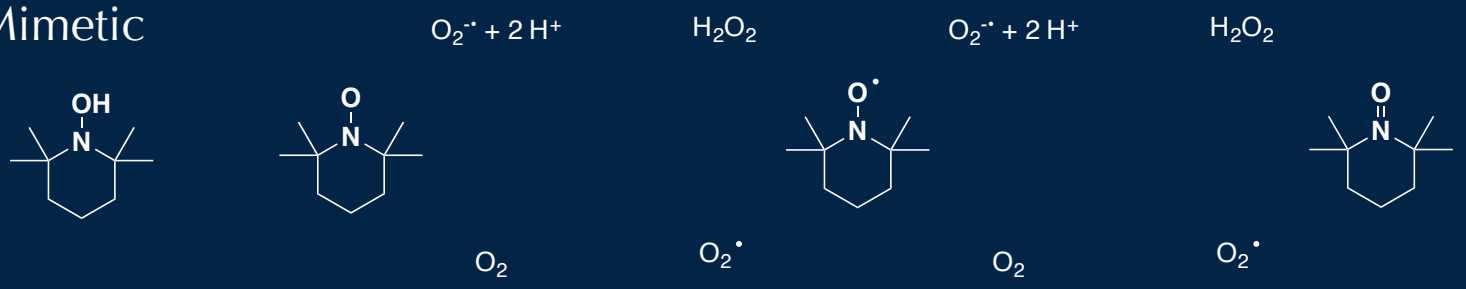
Mitochondrial ROS and Disease



J. Biomed. Biotech. **2012**, Article ID 936486
NIST *via* http://www.oxidativestressresource.org

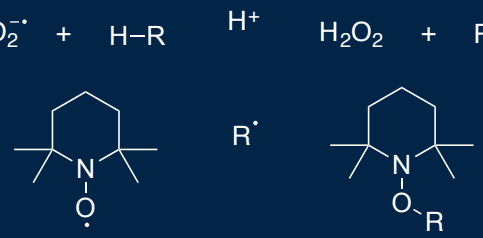
Nitroxides and ROS

SOD Mimetic



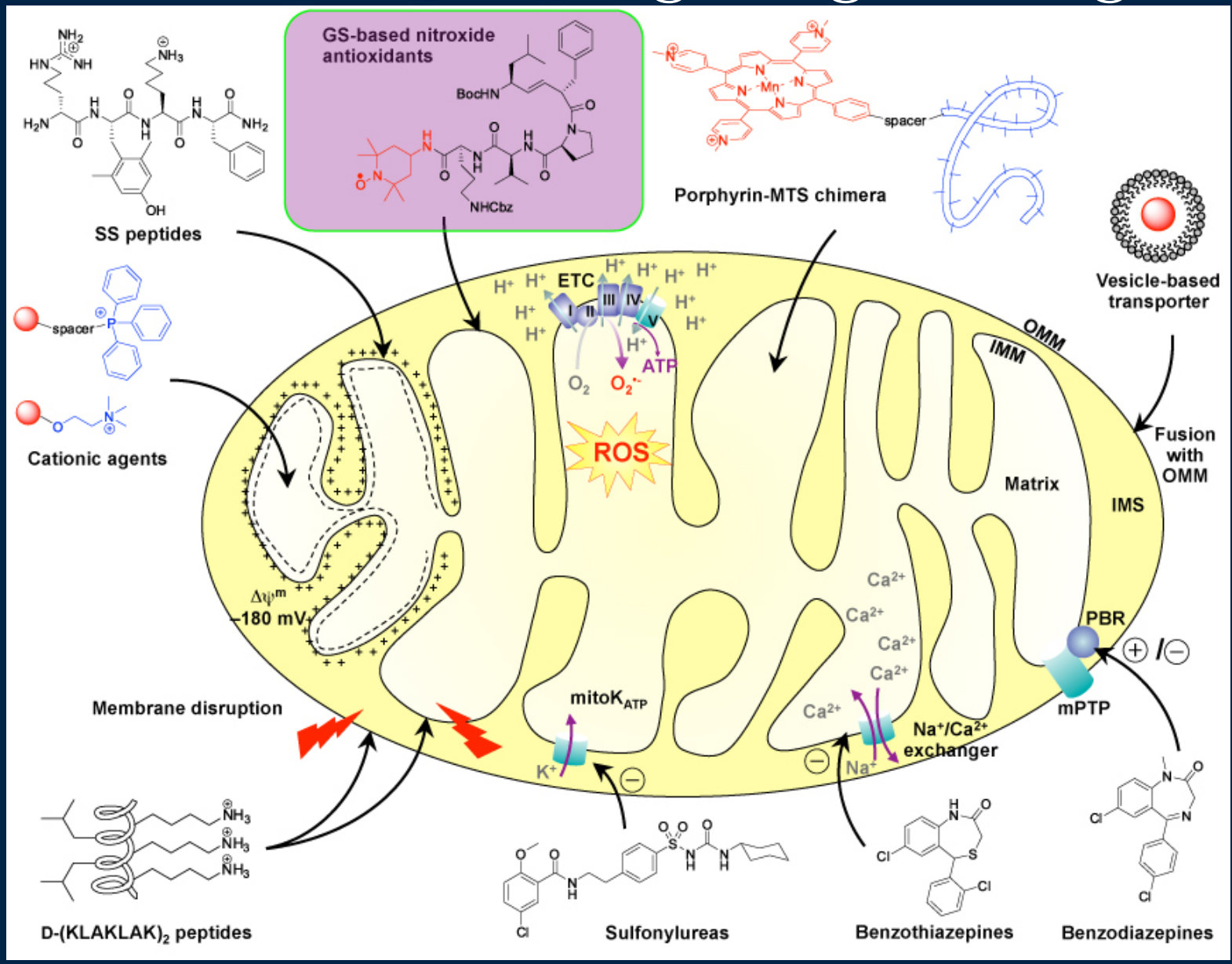
Electron Scavenger

Radical Trap



J. Phys. Chem. A, **2006,** *110*, 3679
Free Rad. Biol. Med. **2007**, *42*, 1632
Nat. Neuro. **2012**, *15*, 1407

Mitochondrial Targeting Strategies

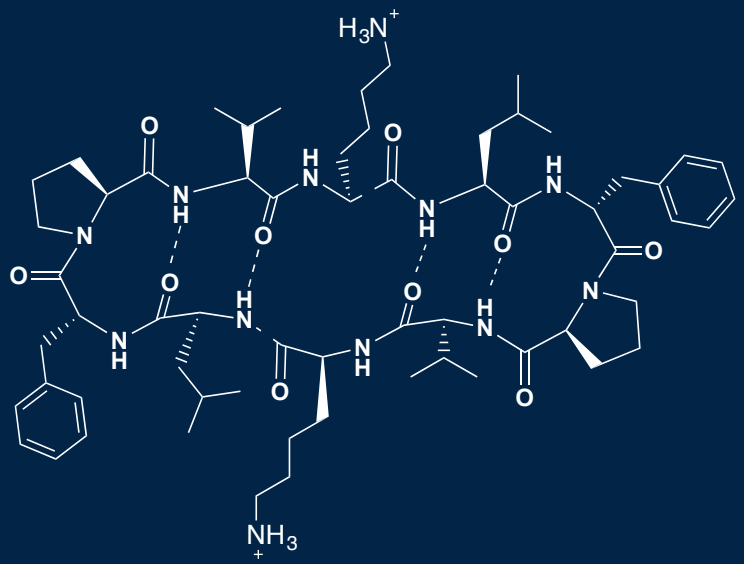


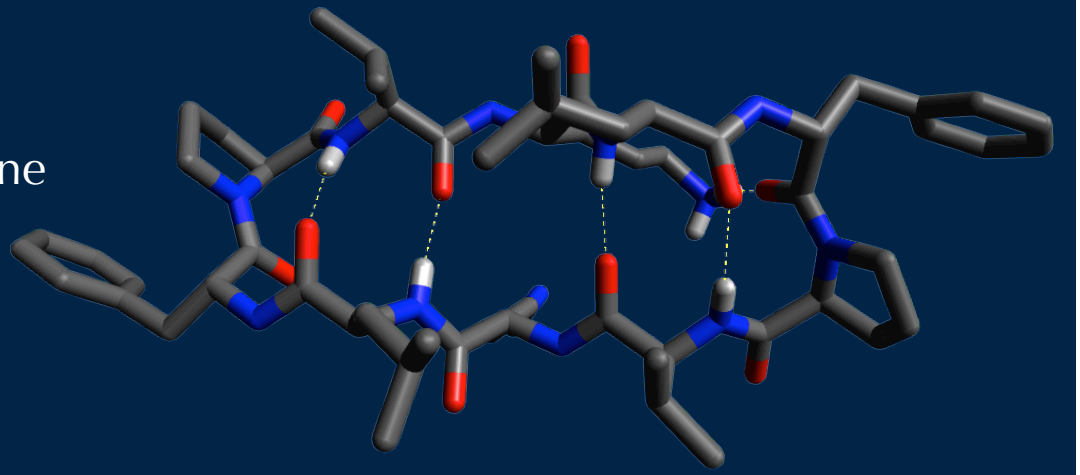
Environ. Mol. Mutagenesis, **2010**, *51*, 462

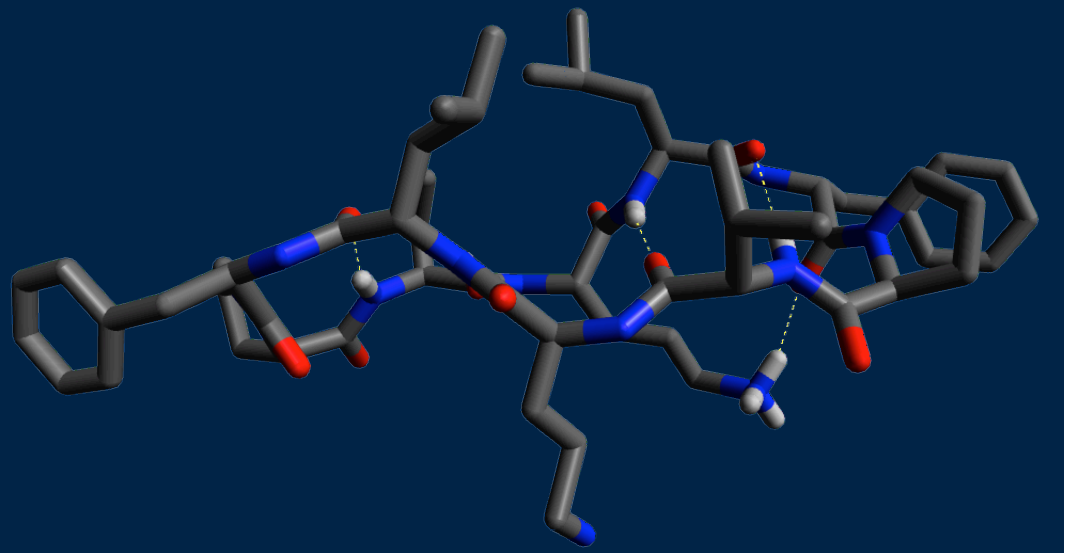
Gramicidin S

- Cyclic Peptide (*B. brevis*)
- Used as topical antibiotic
- Disrupts bacterial inner membrane
- Destabilizes lipid packing

- Shape
  - Antiparallel β-sheet
  - Type II′ β-turns
- Amphiphilicity

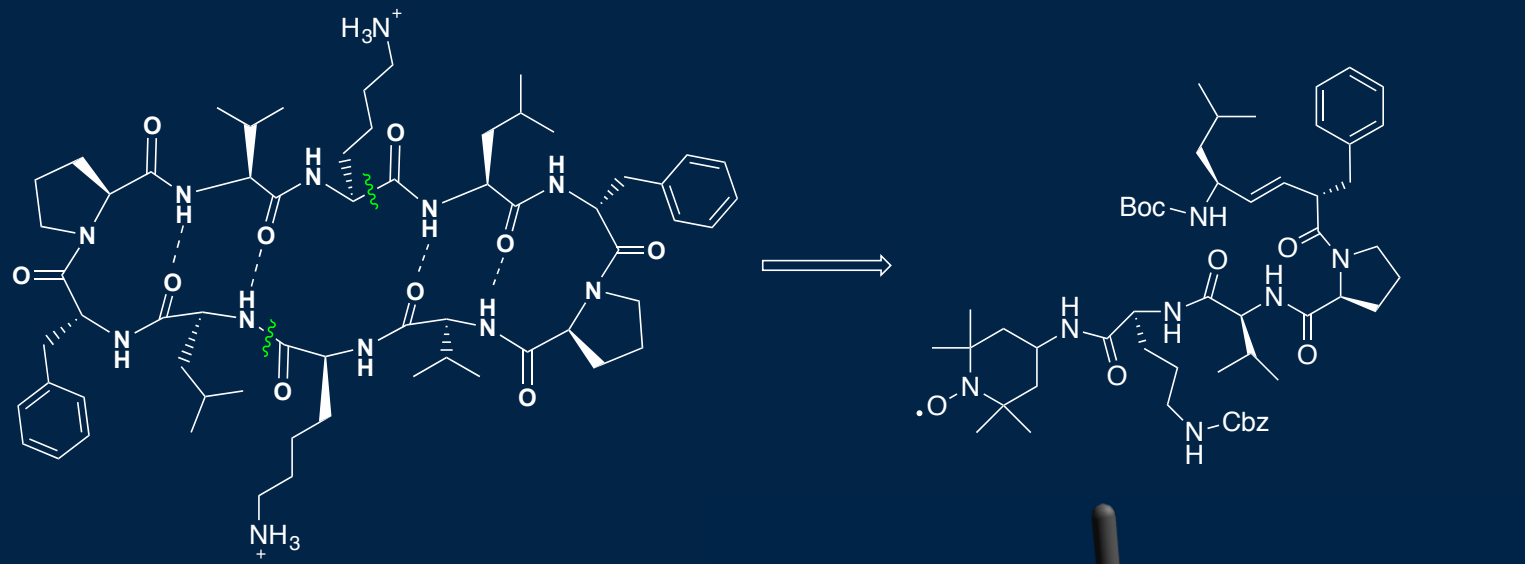




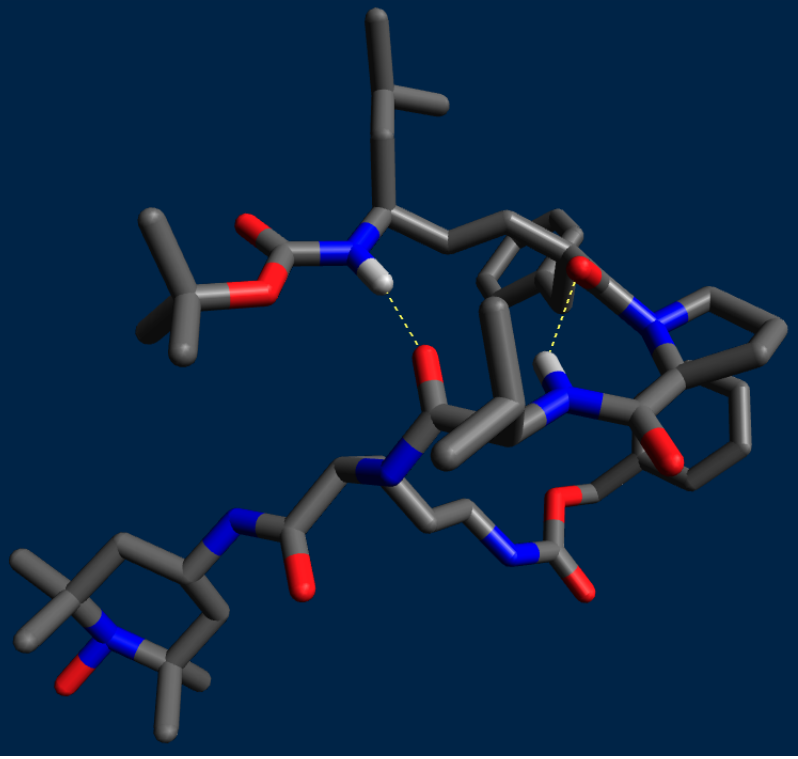


Acta Cryst. **1997**, *D53*, 151
Biochim. Biophys. Acta, **1999**, *1462*, 201

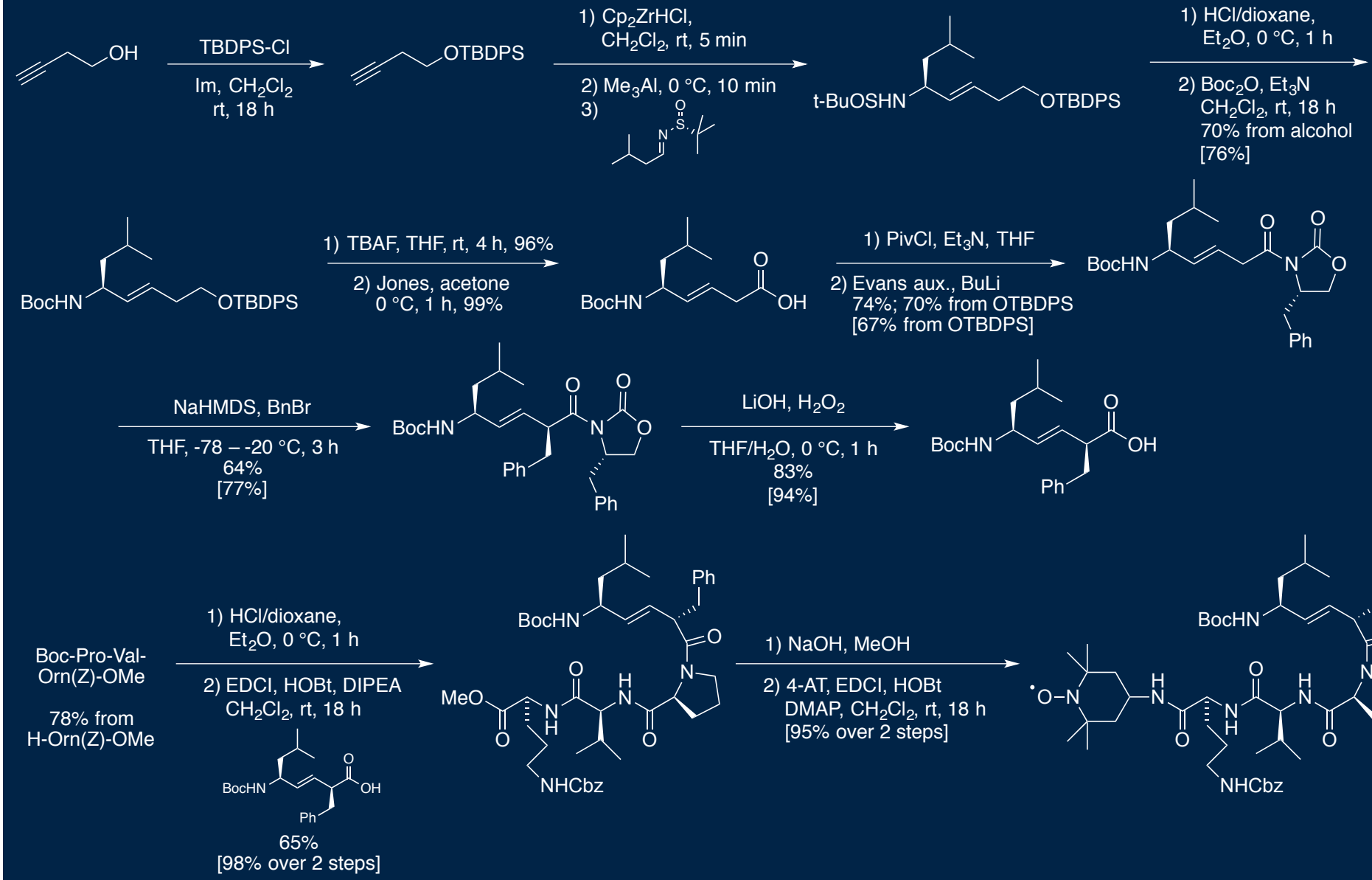
Modified Gramicidin S: XJB-5-131



- Shape
  - Type II′ β-turn
  - New H-bond arrangement
- Site to attach “payload”



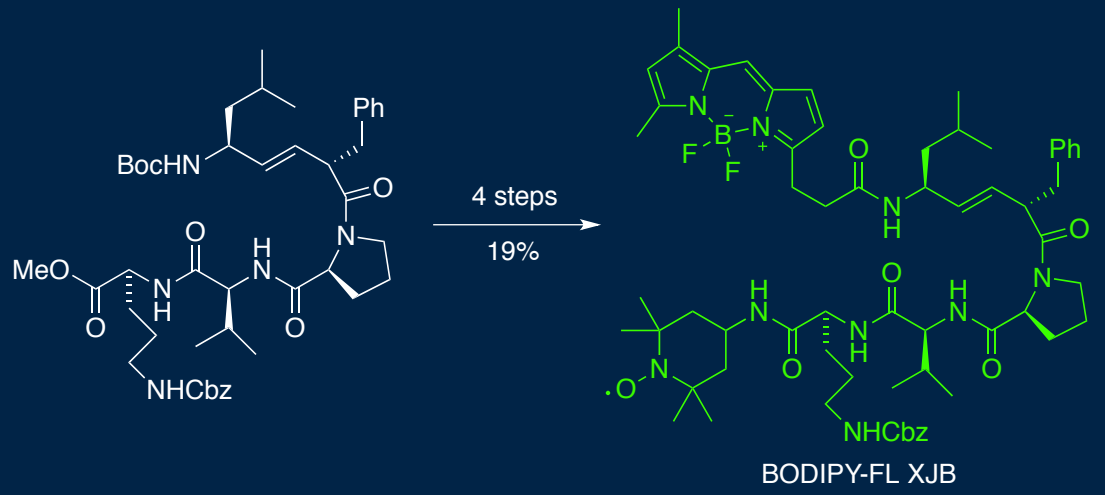
Synthesis of XJB-5-131



34% from commercial, 12 steps LLS, 15 steps total

J. Am. Chem. Soc., submitted

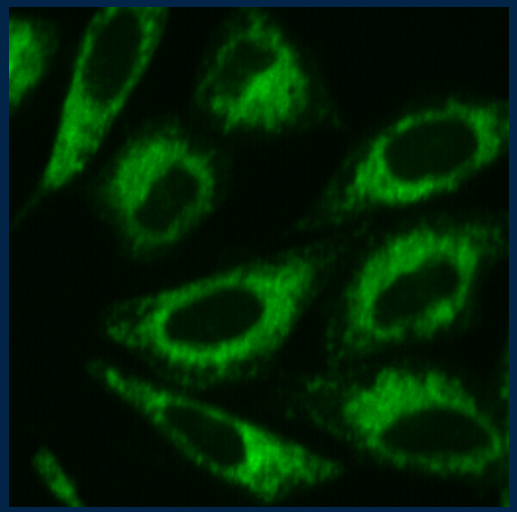
Mitochondrial Localization of XJB



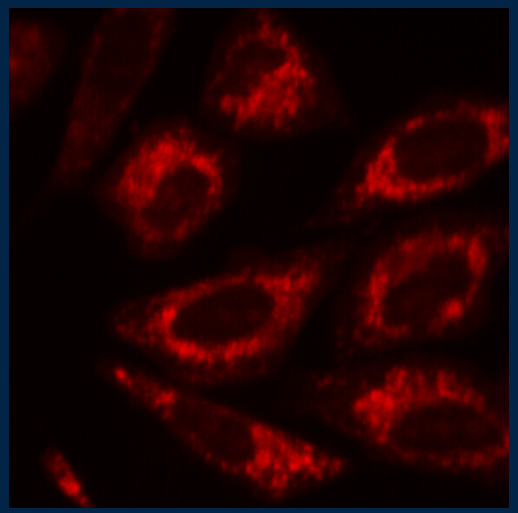
BODIPY-FL XJB

XJB-5-131

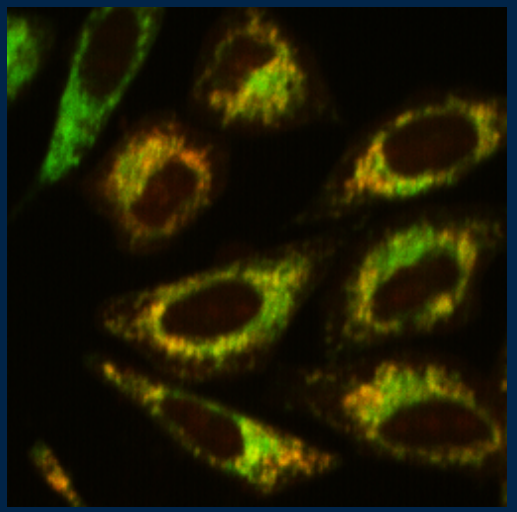
BODIPY



MitoTracker CMXRos



Overlay

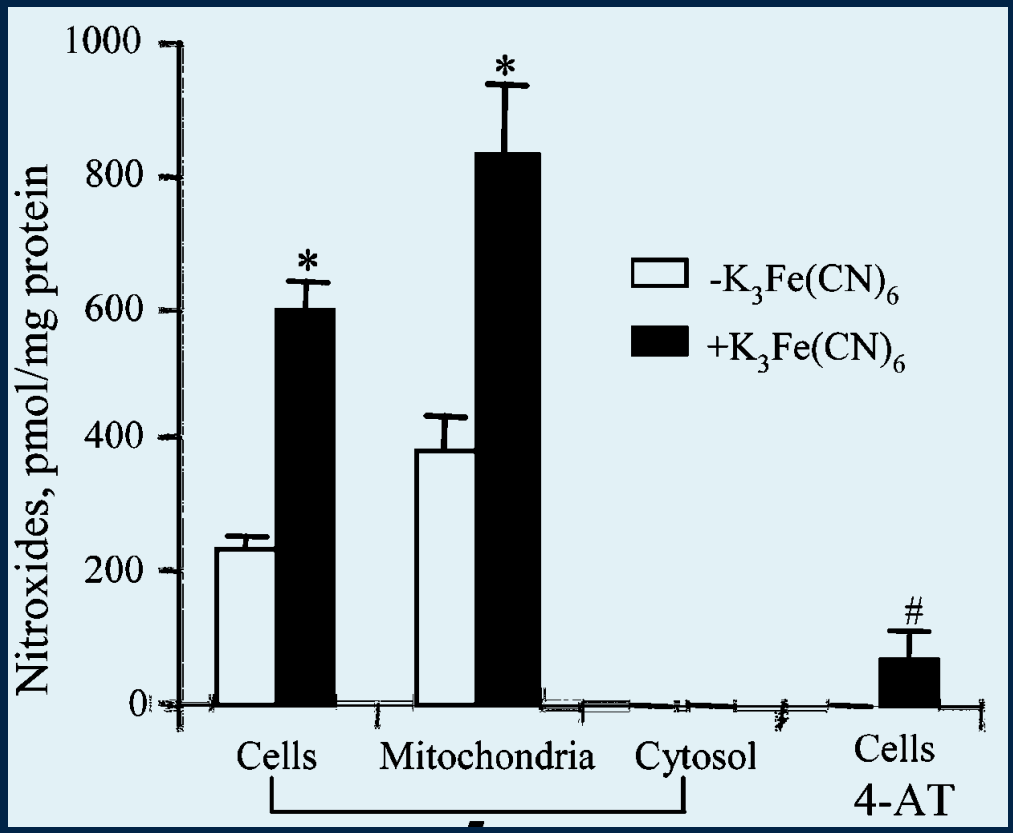


Erin Skoda; Julie Goff
J. Am. Chem. Soc., submitted

Mitochondrial Localization

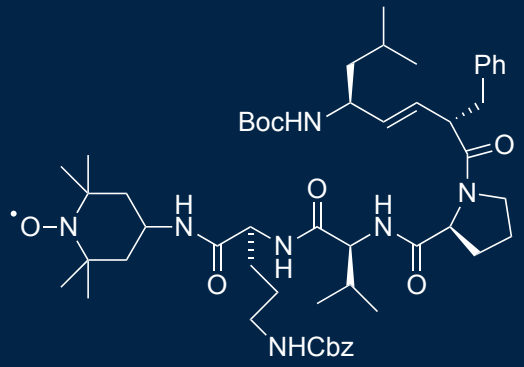
Quantified by EPR, MS:

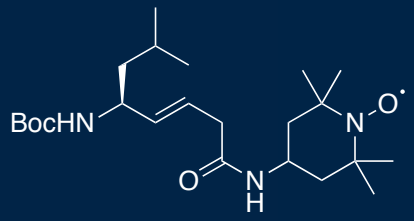
Mitochondria has ~600x concentration!

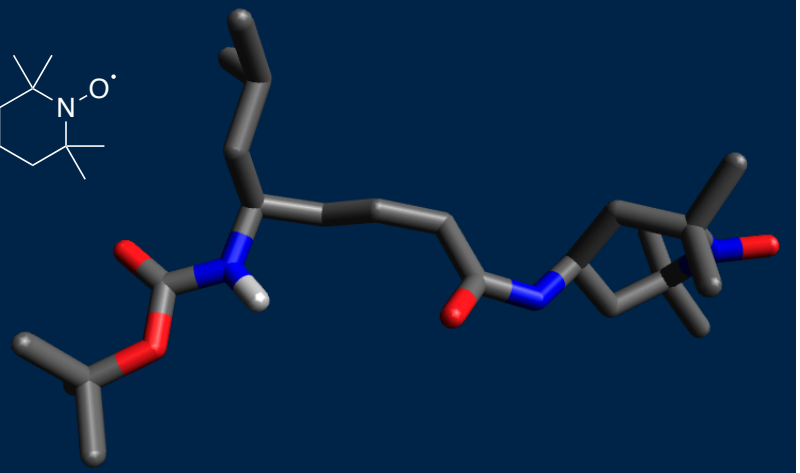


J. Am. Chem. Soc. **2005**, *127*, 12460

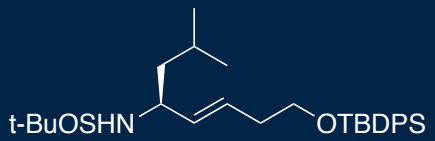
Simplification of XJB: JP4-039

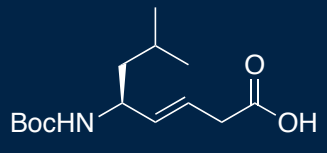






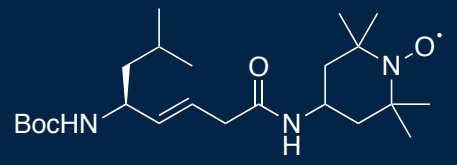
MW	960.3 g/mol	425.6 g/mol
cLogP	4.8	3.6
PSA	280 Å^2	97 Å^2
Solubility	0.05 mg/mL	0.50 mg/mL





4-AT

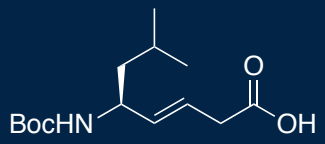
EDCI HOBt,
DMAP CH_2Cl_2



6 steps, 58%

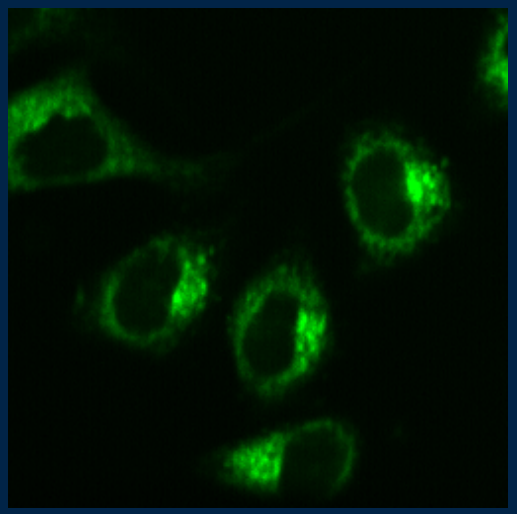
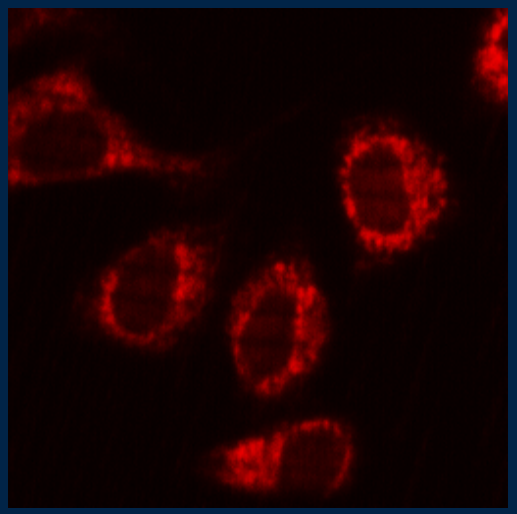
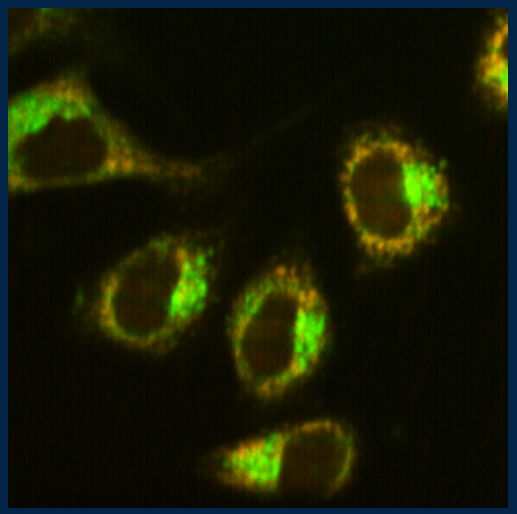
Calcs: QikProp
Synthesis: Marie-Céline Frantz
Solubility: Kayla Lloyd
Org. Lett. **2011**, *13*, 2318

Localization of JP4



4 Steps, 39%

10 days

	BODIPY	MitoTracker CMXRos	Overlay
JP4-039			

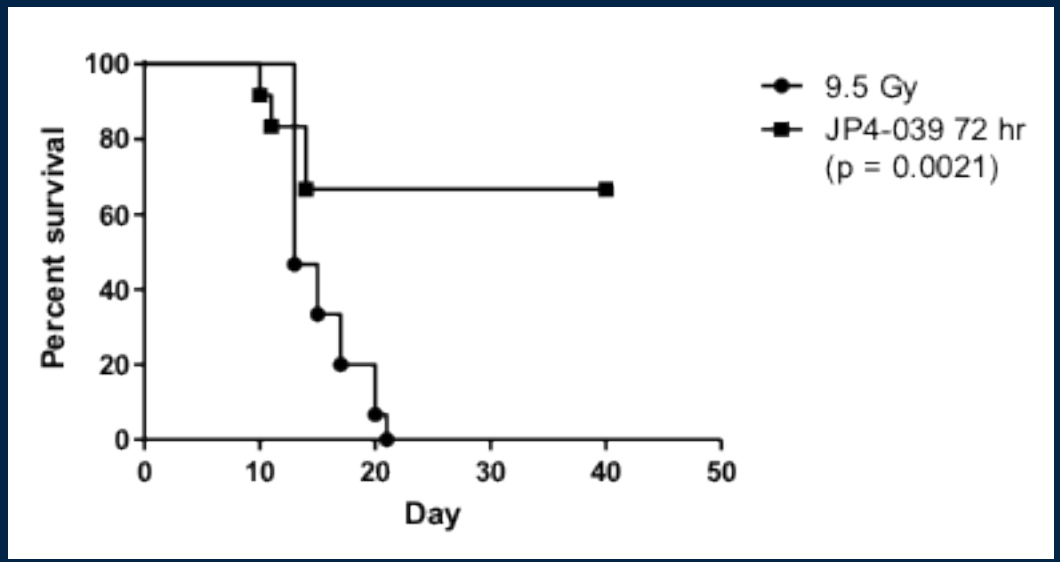
EPR & MS Measurements: ~30x enrichment

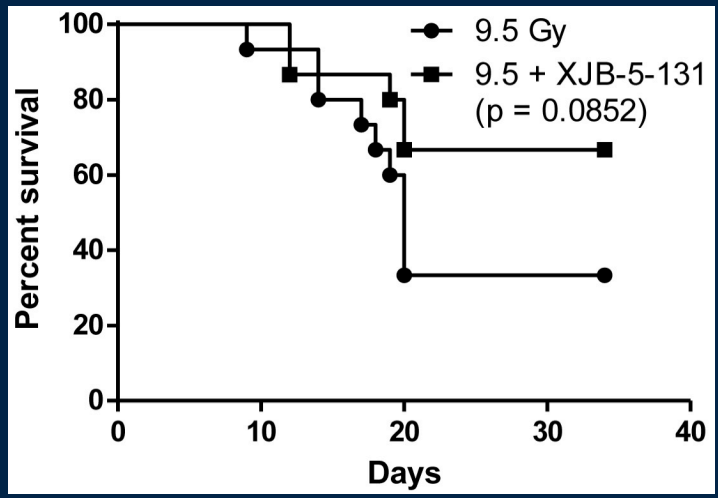
Erin Skoda, Julie Goff
J. Am. Chem. Soc., submitted

Radioprotection of GS-Nitroxides

JP4-039 is More Effective than Tempol in Radioprotective Capacity

Cell line	Concentration (μM)	$\tilde{n}$
FancG	0	2.5 ± 0.3
Tempol + FancG	0.1	4.5 ± 1.3
	1	4.9 ± 0.9
	10	7.2 ± 0.7 ($P = 0.001$)
JP4-039 + FancG	0.1	4.0 ± 0.1 ($P = 0.04$)
	1	5.3 ± 0.1 ($P = 0.01$)
	10	20.5 ± 4.5 ($P = 0.002$)

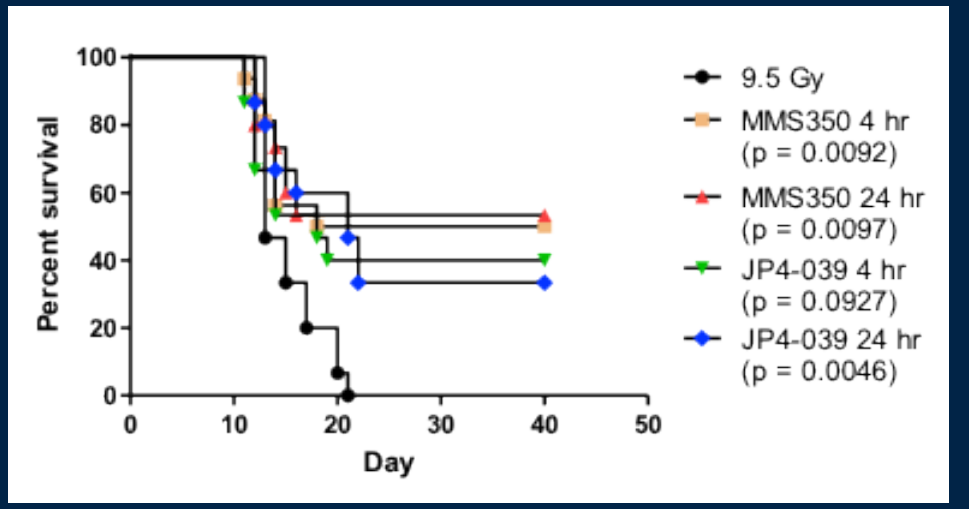


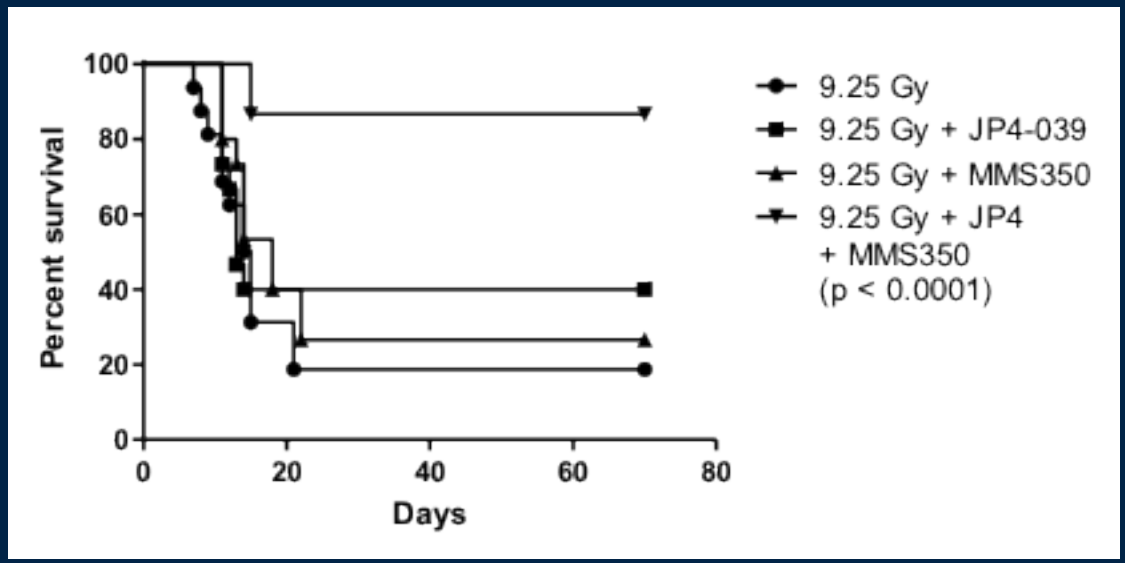


Mike Epperly
Rad. Res. **2011**, *126*, 603

MMS-350

Condition	D_0 (Gy)	ñ
C57BL/6	1.9 ± 0.1	5.8 ± 1.1
C57BL/6 + MMS350 Pre	1.7 ± 0.2 (p = 0.0039)	15.8 ± 2.9
C57BL/6 + MMS350 Post	2.4 ± 0.3 (p = 0.0444)	3.5 ± 0.3





Melissa Sprachman; Mike Epperly
Rad. Res. **2012**, accepted

Radiation Oncology

- Used in >50% of all cancer treatments
- Doses
- Side effects
  - Acute
    - Skin damage
    - Swelling
    - Area-specific
  - Late (months to years after)
    - Fibrosis
    - Epilation
    - Area-specific

Current project: Reduce late side effects of upper body irradiation

Nat. Rev. Cancer, **2006**, *6*, 702
Curr. Mol. Pharmacol. **2009**, *2*, 122

Other Applications of XJB-5-131

- Hemorrhagic Shock (*Ann. Surg.* **2007**, *245*, 305)
- Anti-inflammatory (*Crit. Care Med.* **2007**, *35*, S461)
- Huntington's Disease (*Cell Rep.*, **2012**, *2*, 1137)
- Traumatic Brain Injury (*Nat. Neuro.* **2012**, *15*, 1407)

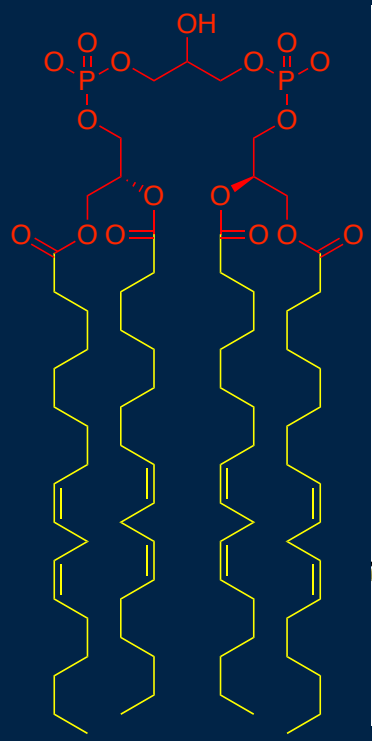
Focus has been on JP4-039 for radiation:

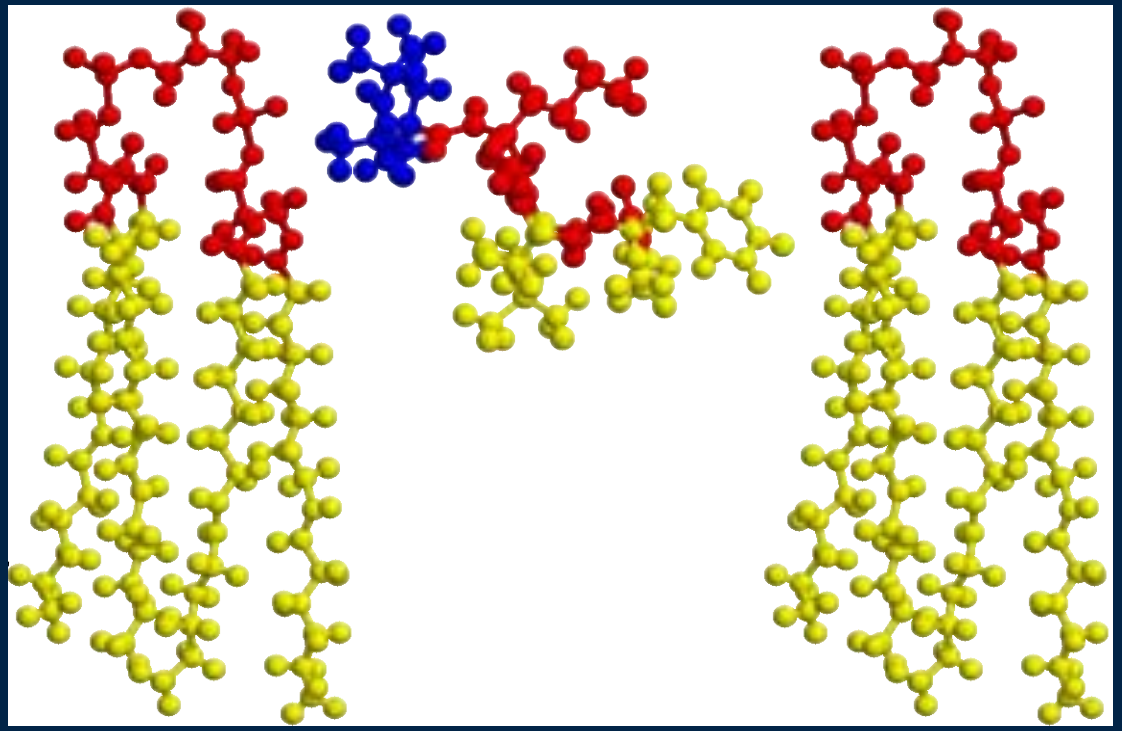
- Physical properties
- Ease of synthesis
- Good initial results

But XJB-5-131 may work BETTER

Modifications of XJB

Localization in the IMM





J. Pharmacol. Exp. Theor. **2007**, *320*, 1050

Other Challenges and Opportunities

- Streamline synthesis of alkene isostere portion
  - Chiral sulfinamide
  - Evans auxiliary
- New applications
  - Kidney disease
  - Diabetes
- New uses for hemi-GS
  - Targeted chemotherapeutics

Acknowledgements

- Dr. Peter Wipf
- CMCR
  - Joel Greenberger, Valerian Kagan
  - Mike Epperly
  - Julie Goff
- Pitt NMR Facilities
- Wipf Group