# TARGETING MITOCHONDRIA
## DESIGN, SYNTHESIS AND APPLICATION OF GS-NITROXIDES

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Research Topic Seminar
16th May, 2015

# Mitochondria as a Target in Drug Discovery

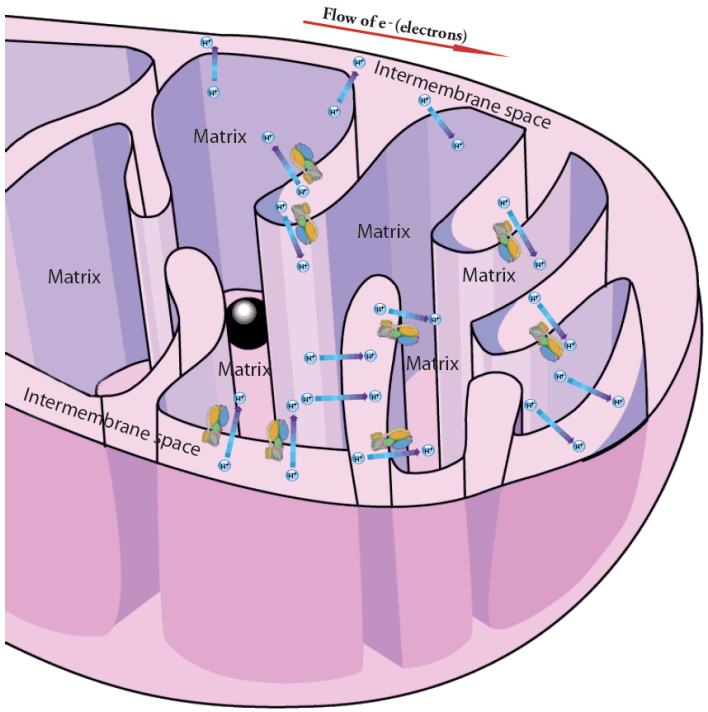
- Mitochondria play an essential role in cellular life and death
- Aging is the **greatest risk factor** for neurodegenerative disease due to accumulation of mtDNA mutations, **oxidative stress**
- Escaping ROS damage many intracellular targets in vicious cycle
- Radiation, Traumatic Brain Injury induce ROS generation

***"There is strong evidence to support the hypothesis that the source of pathogenesis in age-related neurodegeneration is oxidative stress caused by overproduction of ROS as a result of mitochondrial dysfunction"***

*Nature* **2006**, *443*, 787-795

# Mitochondria – The Energy Powerhouse

- Discreet organelles in most eukaryotic cells
- 80-90% of ATP is produced in mitochondria via oxidative phosphorylation (OXPHOS)




*Curr. Biol*. **2006,** *16*, R551
*Biomol. Therap*. **2010,** *18*, 235-245

## Other Roles of Mitochondria:

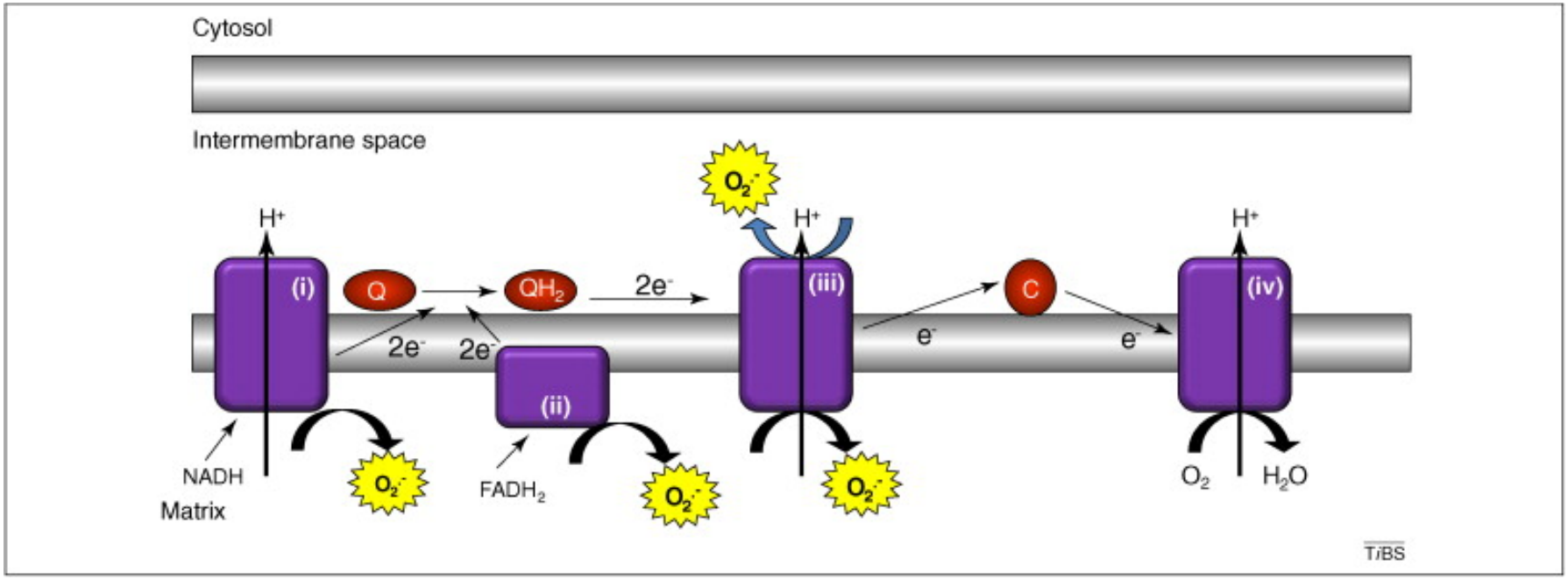
- Ion Homeostasis
- Regulation of Apoptotic Cell Death
- Biochemical pathways (Krebs Cycle, β-Oxidation, Lipid Cholesterol Synthesis)
- Calcium Regulation

## Mitochondria Morphology

- Porous outer membrane (< 5kDa)
- Intermembrane space
- Innermitochondrial membrane
- Mitochondrial Matrix

# ATP Production and Oxidative Stress

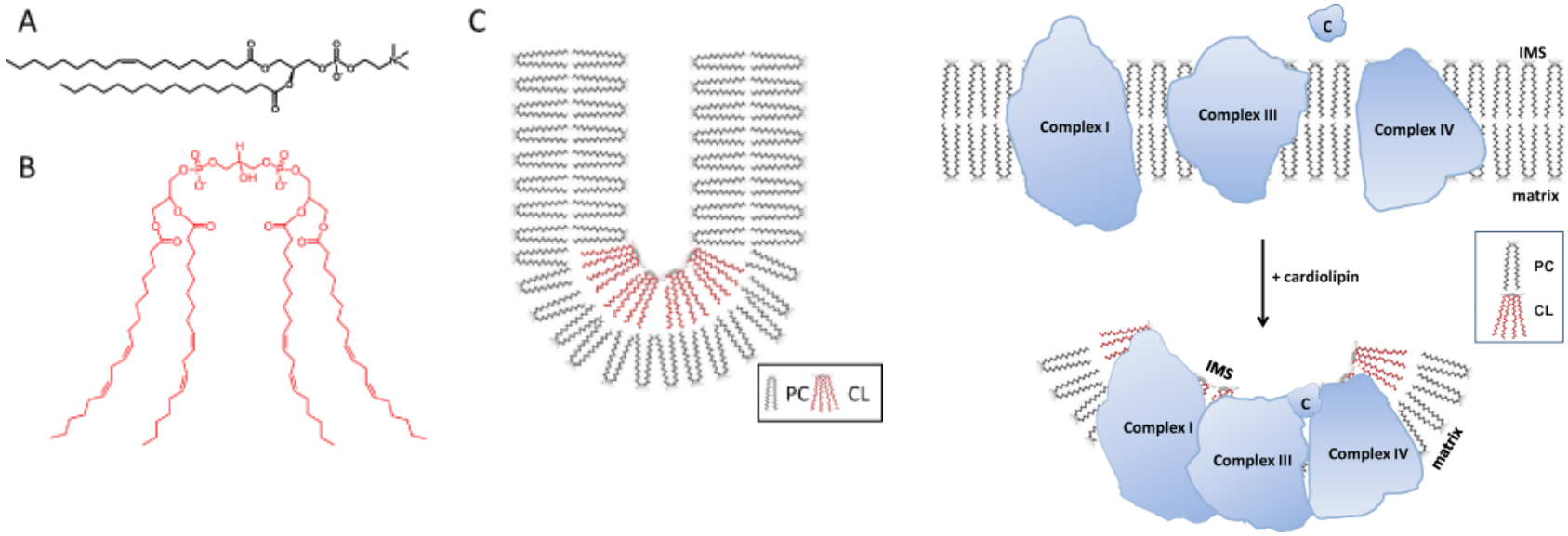
- In 1956 Harman proposed “free radical theory” of aging and related diseases
  *“the reaction of active free radicals, normally produced in the organism, with cellular constituents initiates the changes associated with aging”*
- Ca. 0.2% of cellular $O_2$ is converted to ROS




*Trends Biochem Sci*, **2010**, *35*, 505-513
*Biochem J.* **2009**, *417*, 1-13

# The Role of Cardiolipin

- **Cardiolipin (CL) is exclusively expressed in the IMM**

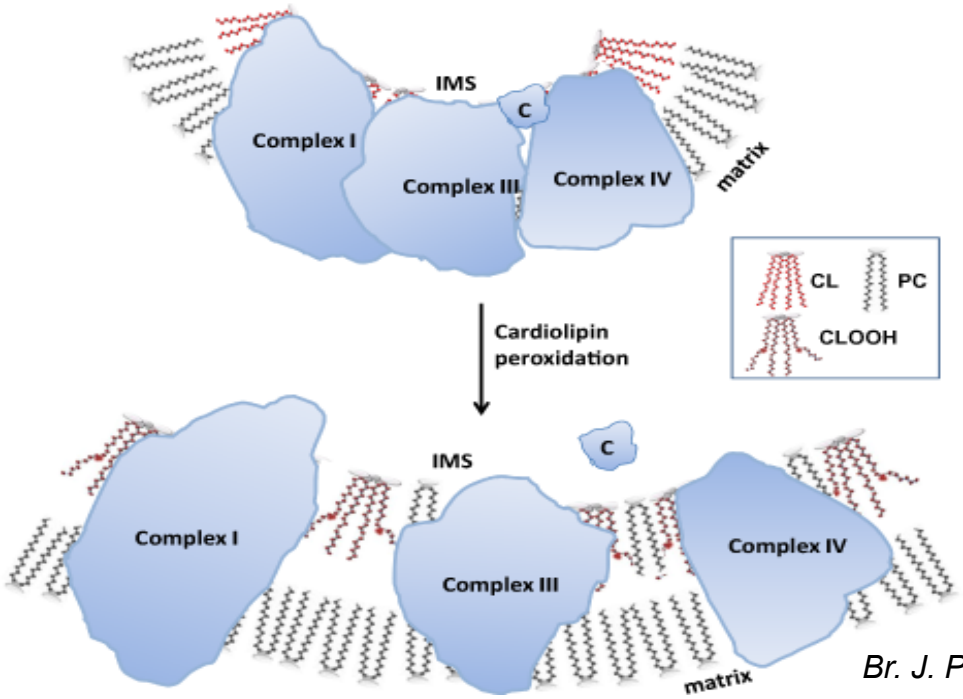



- **Roles:**
  - Cristae Formation
  - Organization of complexes into supercomplexes for optimal OXPHOS
  - Interaction with Cyt c for optimal electron transfer

*Br J Pharmacol*. **2014**, *171*, 2029-2050

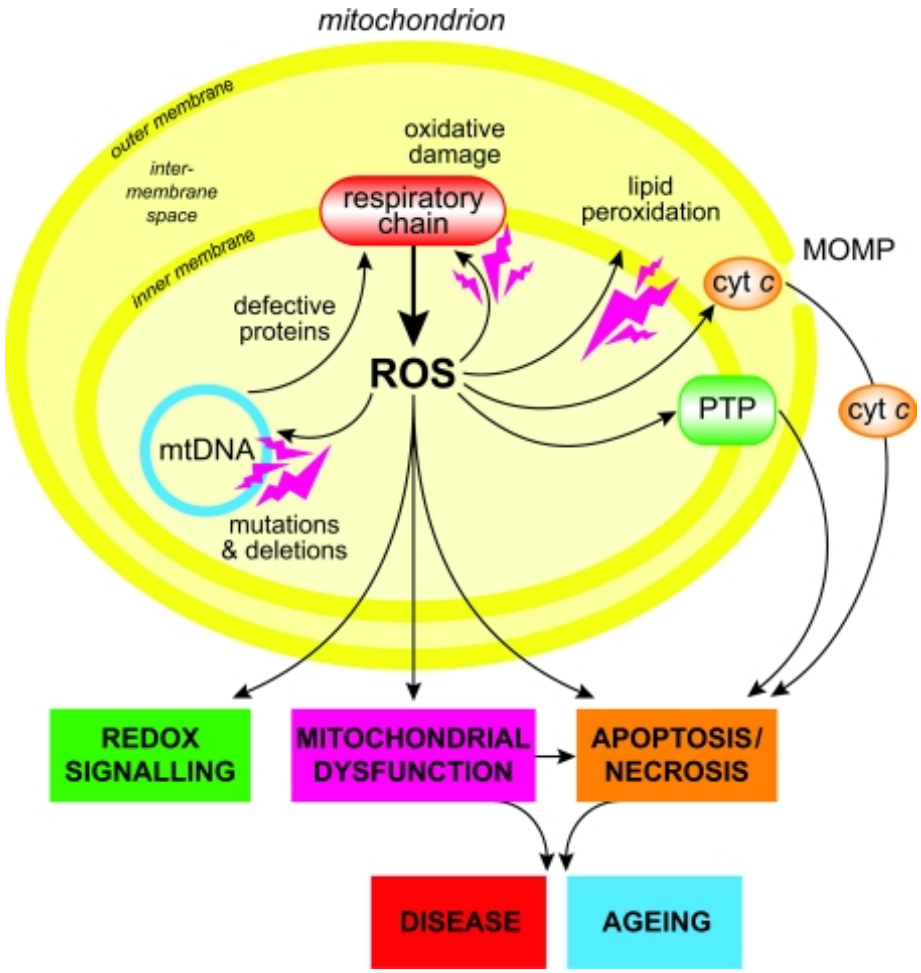
# Consequences of Cardiolipin (CL) Oxidation

- CL is particularly vulnerable to oxidation due to unsaturated fatty acid chains
- Located at ROS production site
- Reduction of CL affinity to cyt c leading to cyt c unfolding and enhances cyt c peroxidase activity
- Oxidized CL synergizes with $Ca^{2+}$ to induce opening of the mitochondrial permeability transition pore (MPT)




*Br. J. Pharmacol*. **2014**, *171*, 2029-2050

# Harmful Activities of ROS

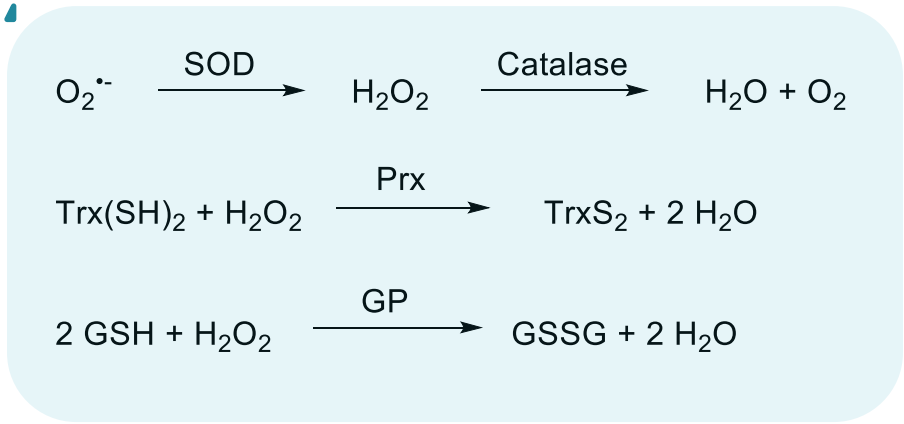


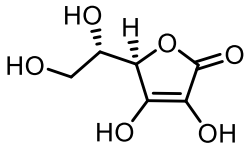

- ***Apoptotic and necrotic cell death***
- ***Mitochondrial diseases***
  - *Neurodegenerative disease (Huntington's, Alzheimer's, Parkinson's)*
  - *Neuromuscular*
  - *Obesity*
  - *Diabetes*
  - *Cardiovascular*
  - *Cancer*

*Biochem. J.* **2009**, *417*, 1-13

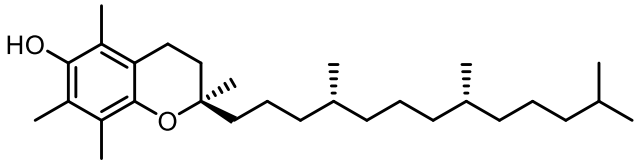
# Natural Antioxidants

- ***Superoxide dismutase*** (SOD)
- ***Catalase***
- ***Vitamin C***
- ***Vitamin E***
- ***Thioredoxin (Trx)***
- ***Glutathione peroxidase***

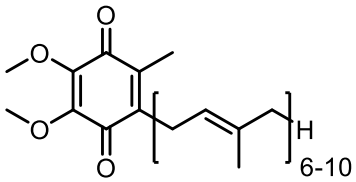


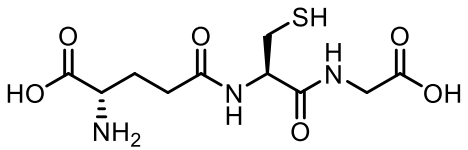

**Vitamin C**




**Vitamin E**



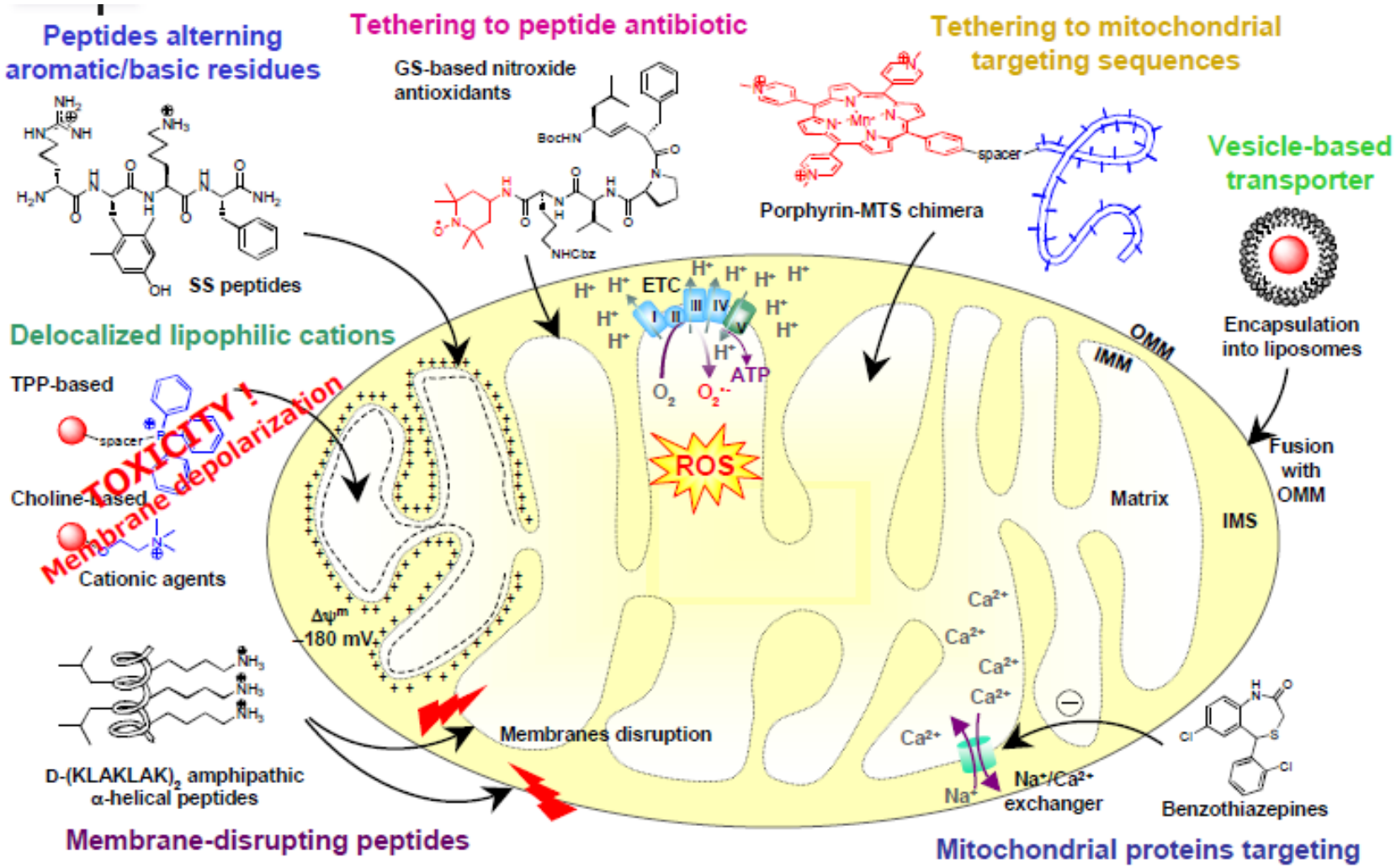

**$CoQ_{10}$**




**GSH (gluthatione)**

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

# Strategies in Mitochondrial Targeting

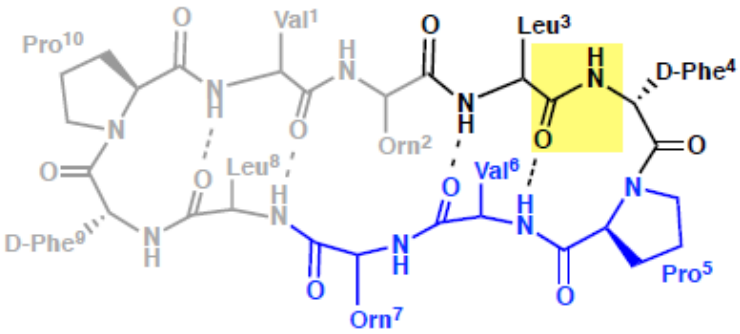



*Environ. Mol. Mutagen*. **2010**, *51*, 462

# Gramicidin S based Conjugates

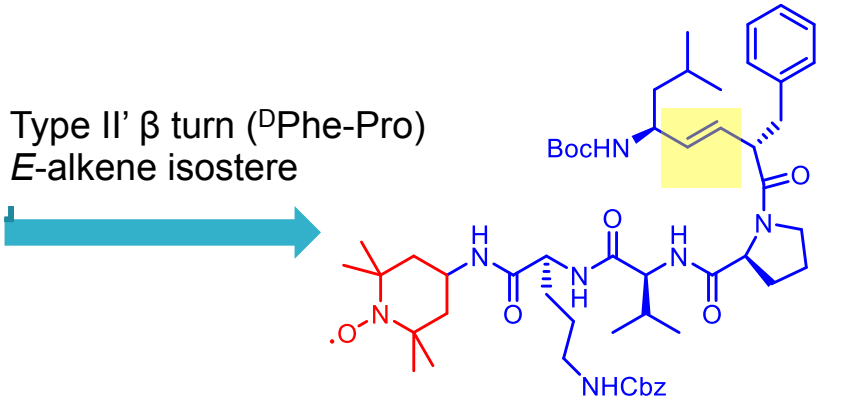
## ❑ Gramicidin S Scaffold for Subcellular Targeting Probes

Cyclic Peptide (*Bacillus brevis*)
Disrupts bacterial inner membrane




**Gramicidin S**

Type II' β turn ($^{D}$Phe-Pro)
*E*-alkene isostere




**Payload**
**TEMPO**

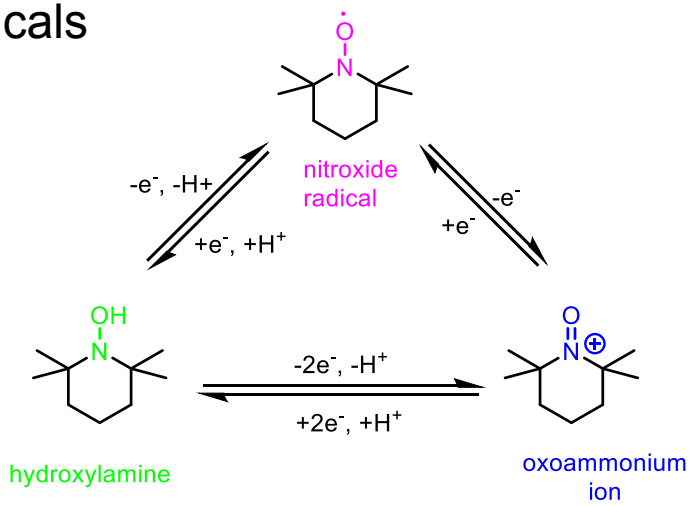
**Vehicle (hemi GS)**

Replacement of amide bond with alkene → extended bioavailability due to increased resistance against protease action

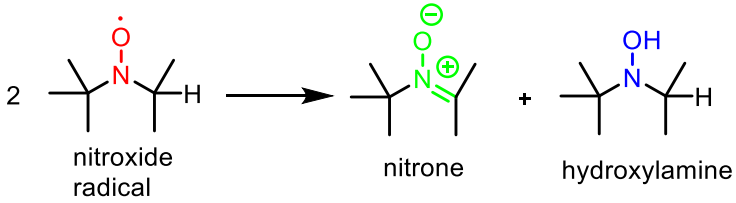
*Acc. Chem. Res.* **2008**, *41*, 87

# Cyclic Nitroxides

- **ROS scavengers and SOD mimetics**
  - Cyclic Nitroxides are stable free radicals



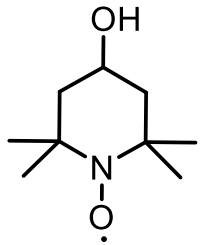

  - Nitroxides with α-Hs are unstable due to dismutation to nitrones




  - Very efficient radical scavengers
  - Inhibit lipid peroxidation

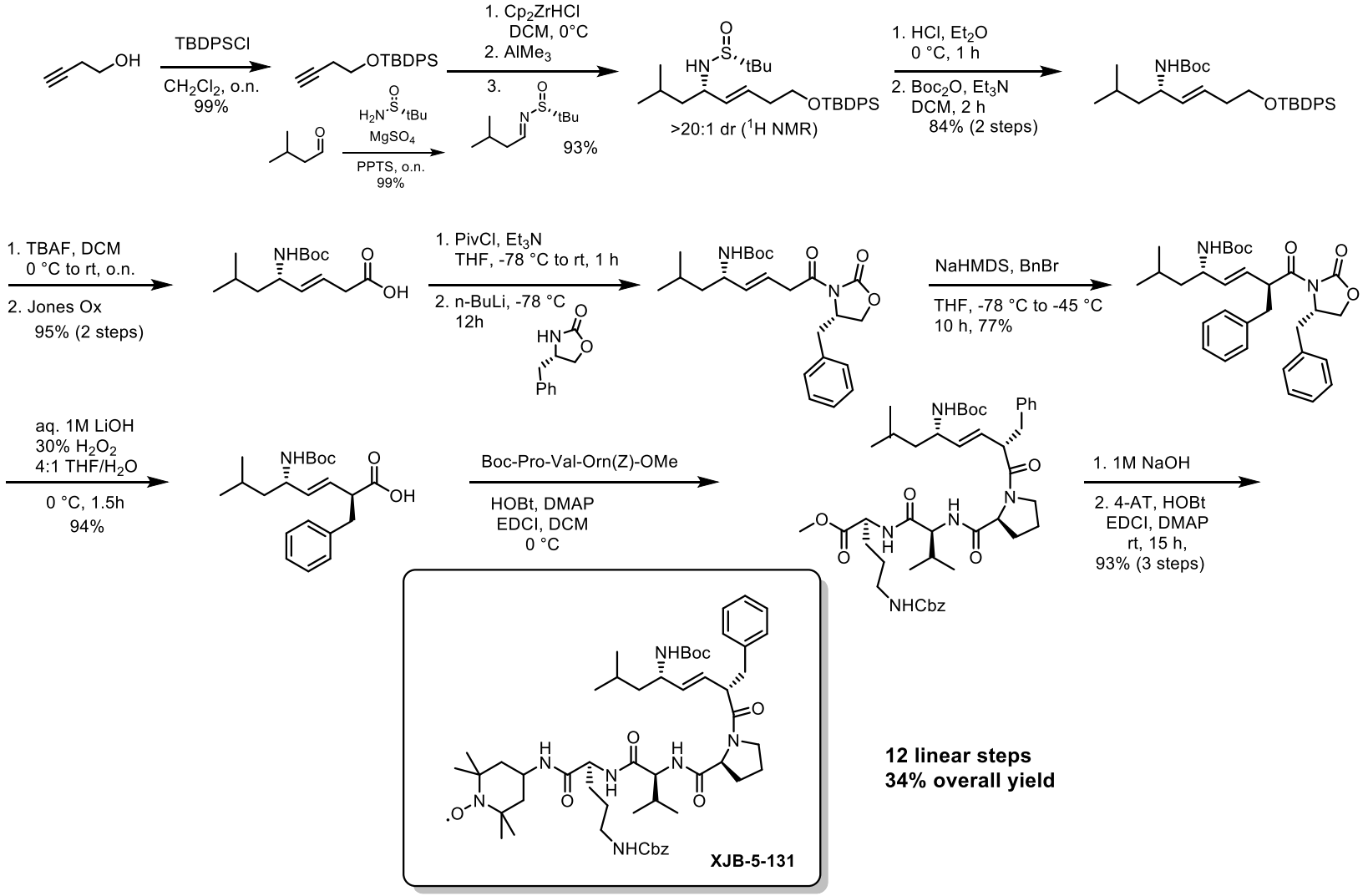
- **Cyclic Nitroxides in therapeutic applications:**

TEMPOL: completed phase I in radiation oncology
Challenges: High dosage necessary due to poor cellular partitioning



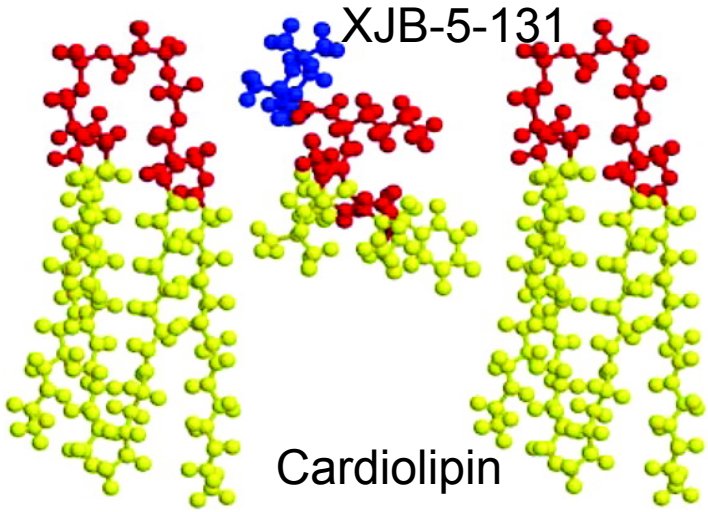
*J. Org. Chem*, **2008**, *73*, 6763

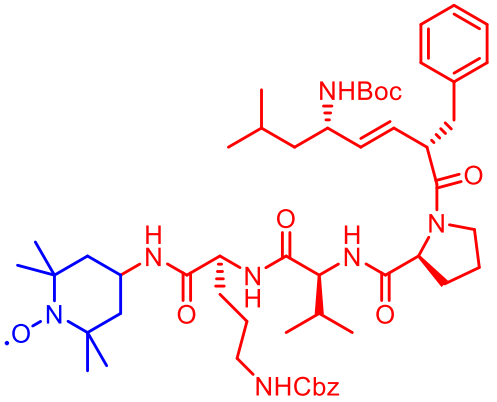
# Synthetic Route to XJB-5-131



# Monte Carlo Simulation

- Effective partitioning of nitroxide is **necessary but not sufficient** for the protection against oxidative stress



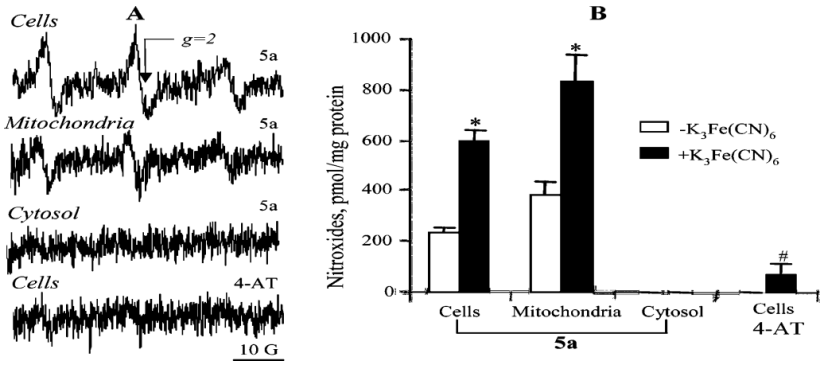



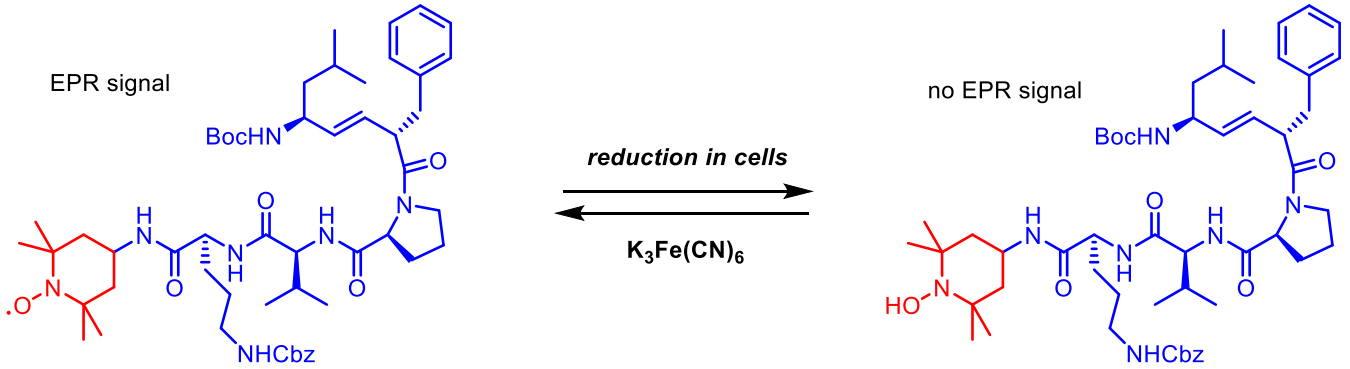

- Positioning of the nitroxide at the *polar/nonpolar interface* of the lipid membrane is essential for activity to allow successful competition with $O_2$ for electrons from ETC.
- Accomplished by the intact β-turn motif

*J. Pharmacol. Exp. Ther*. **2007**, *320*, 1050-1060

# XJB-5-131 Delivery into Mitochondria

- EPR monitoring of mitochondrial uptake of XJB-5-131 in Mouse Embryonic Cells (MECs) → incubation with 10 μM XJB-5-131



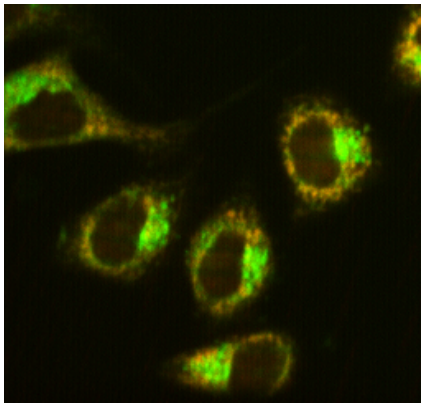




***Mitochondrial enrichment of XJB-5-131 and reduction of nitroxide to hydroxylamine***

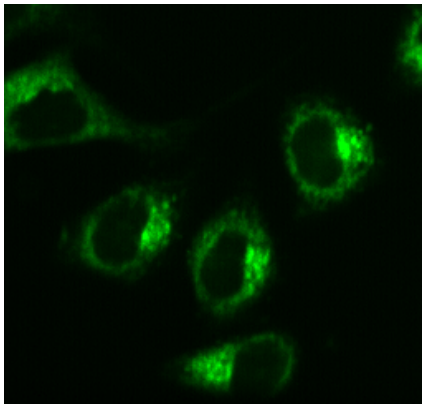
*J. Am. Chem. Soc.* **2005**, *127*, 12460

# Fluorescent Labelling of XJB-5-131

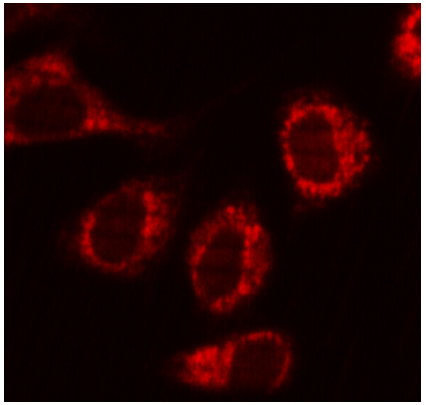
- Mitochondrial Localization of labelled XJB-5-131



**Overlay**



**XJB-BODIPY-FL**



**MitoTrackCMXROS**

# Independent studies with XJB-5-131

- Anti-inflammatory properties
- Radioprotection
- Lethal Haemorrhagic Shock
- Protection against gamma irradiation
- Huntington's disease
- Ischemia reperfusion
- Traumatic Brain Injury

# *In Vivo* Studies: Lethal Haemorrhagic Shock

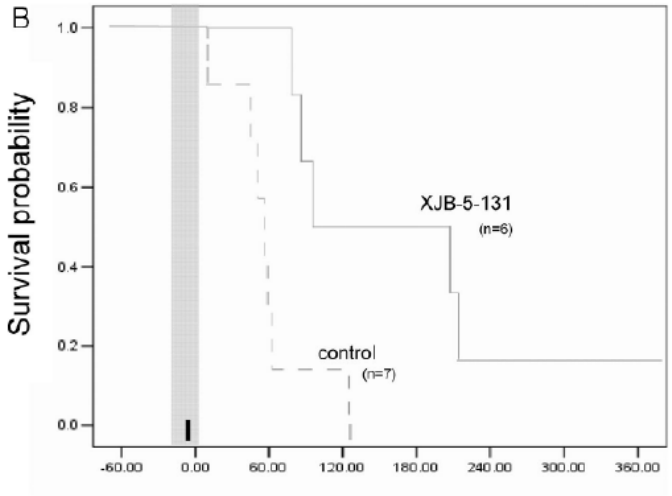
- Leading cause of death of people under 45 years of age.
  *Current Treatment*: controlling of bleeding and restoring intravascular volume

16 rats

**0h** — 55% blood removal — **1h** — Post bleeding observation — **6h**

3 rats died

*i.v.* injection of XJB-5-131 (total dose 2 µmol/kg)

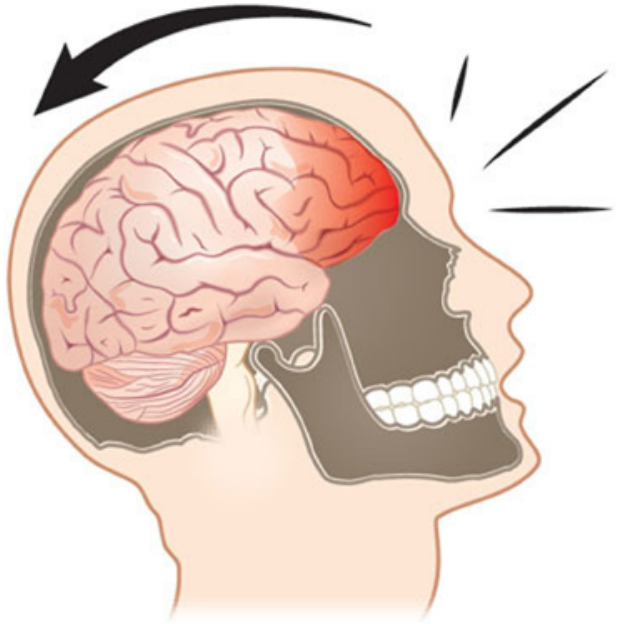



**XJB-5-131 prolongs survival of rats with lethal haemorrhagic shock**

*Ann. Surg.* **2007**, *245*, 305
*Biochem. Pharmacol.* **2007**, *74*, 801

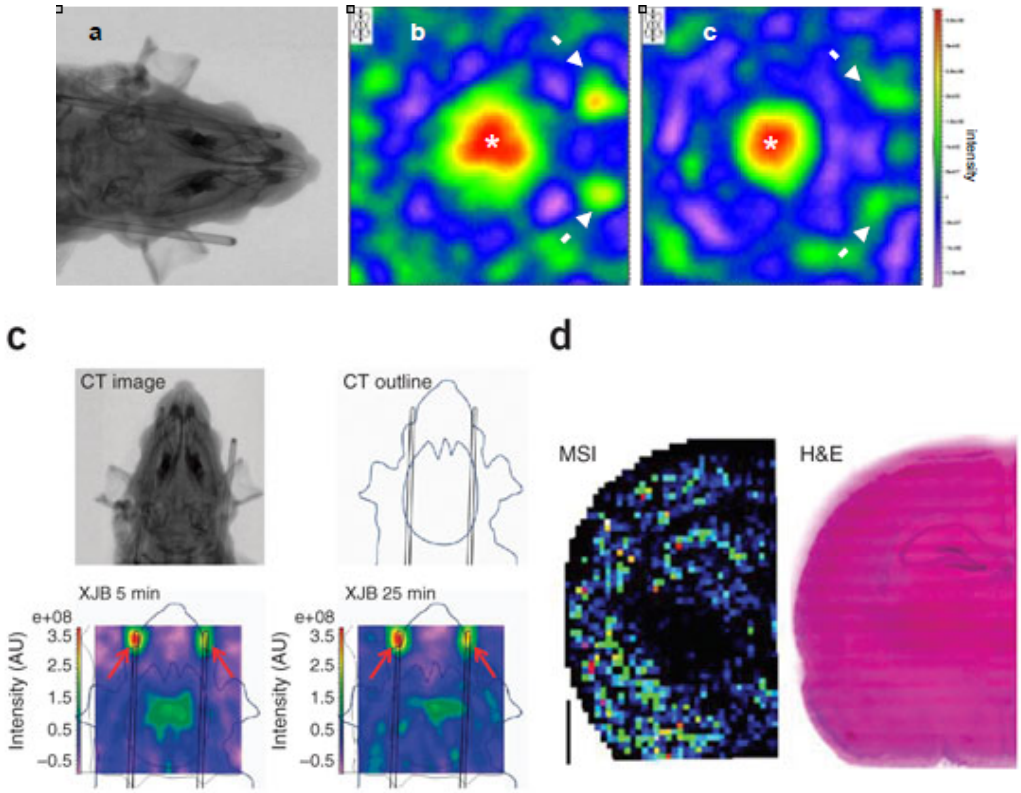
# Traumatic Brain Injury (TBI)

- Leading cause of death and disability in children and young adults
- Ca. 1.7 million people/year in US alone sustain acute brain injury
  - ~52,000 deaths
  - ~85,000 suffer from long term disabilities.
- No specific therapy– standard treatment remains supportive care



*Nat. Neuro*. **2012**, *15*, 1407-1415

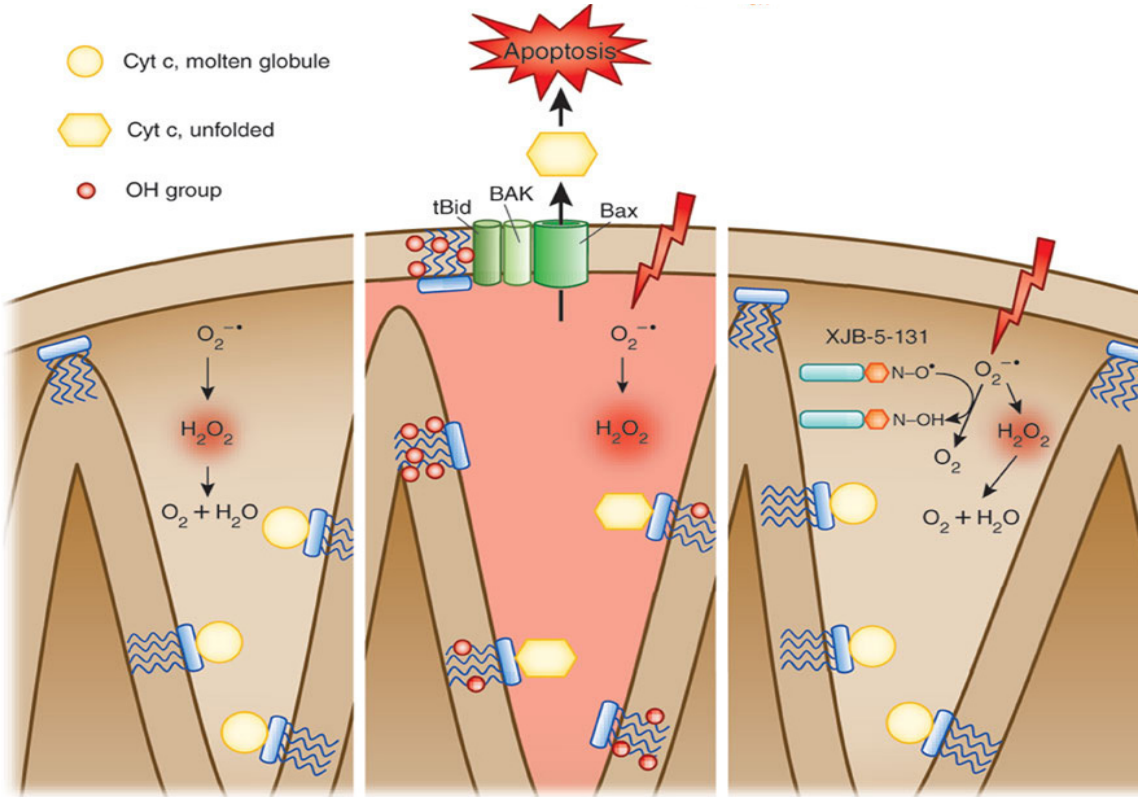
# XJB Partitioning in the Brain




- Imaging of XJB-5-131 in the brain by L-B and EPR spectroscopy
- Dose and time-dependent distribution of XJB-5-131 in naïve rat brain after interperitoneal injection

# Cytochrome C Release Triggers Apoptosis

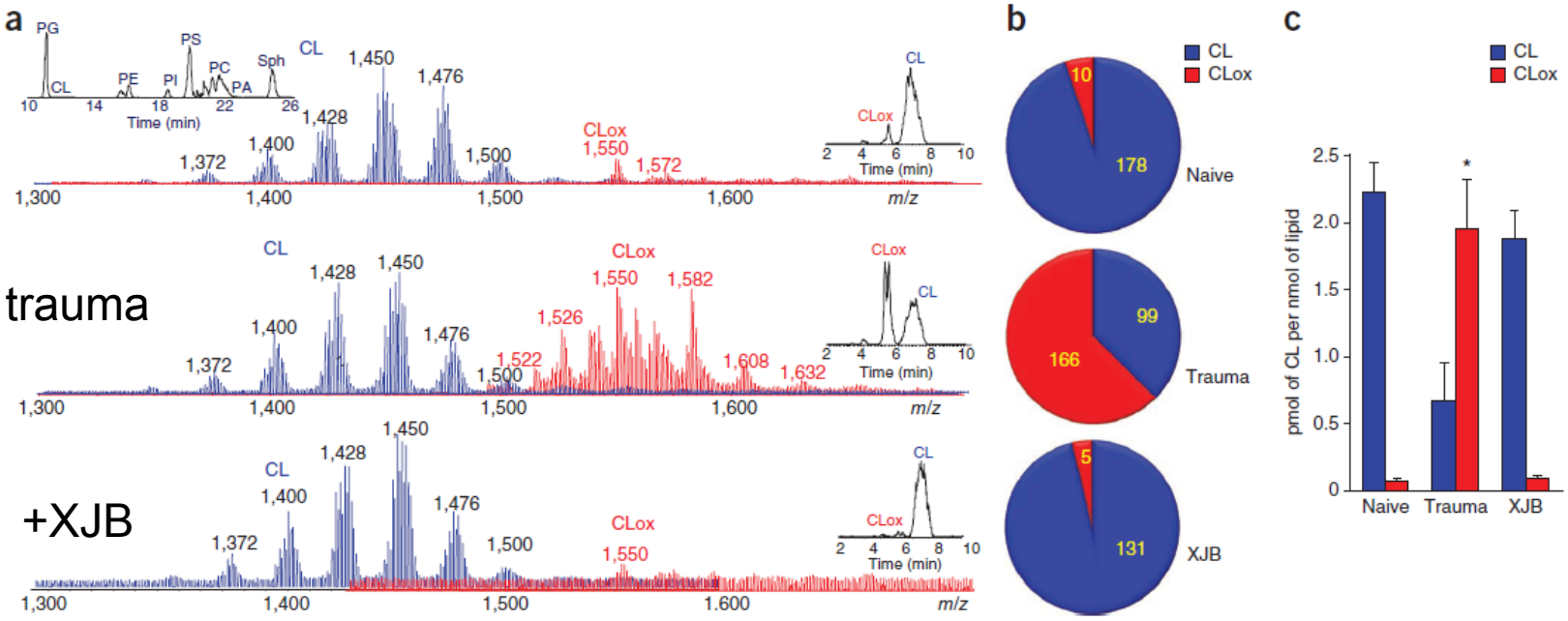
- Accumulation of $H_2O_2$ during TBI leads to **unfolding of Cyt c**
- Enhancement of Cyt c peroxidase activity **targeting side chains of CL**
- Oxidation of **CL weakens interaction with Cyt c**
- **Release of Cyt c** to cytosol via transition pore




*Nat. Neurosc.* **2012**, *15*, 1325-1327

# Traumatic Brain Injury

- **2D-LCMS assessment of molecular species of CL and its oxidation products**




- ***XJB-5-131 substantially reduces neuronal death in both in vitro and in vivo, and markedly reduced behavioural deficits and cortical lesion volume.***

# Huntington's Disease

- Huntington's disease is a **progressive brain disorder** that causes uncontrolled movements, cognitive difficulties and emotional disturbance
- Disease more prevalent in people of European ancestry
  - 3-7 out of 100,000 in Europe have HD
  - 30,000 cases in US
  - Only 1-3% of HD cases are not inherited

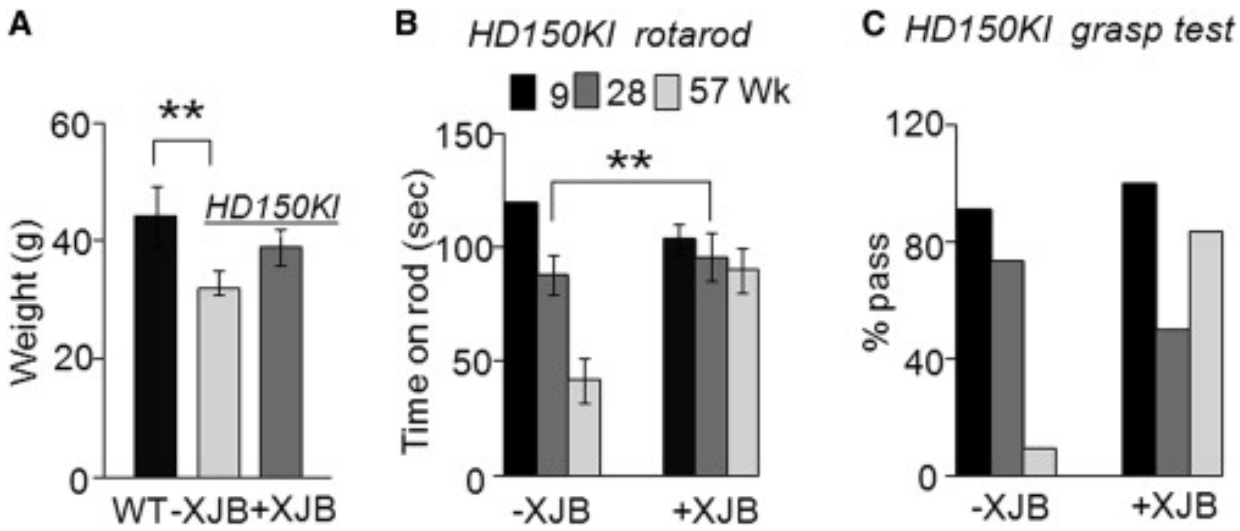
**This study:**

Mouse Model of HD harbouring disease length 150CAG tract "knocked into" both alleles of the mouse HD gene homologue.

HD150KI mice treated up to 57 weeks with XJB-5-131 (1 mg/kg up to 3 times/ week)

# *In Vivo* Studies: Huntington's Disease

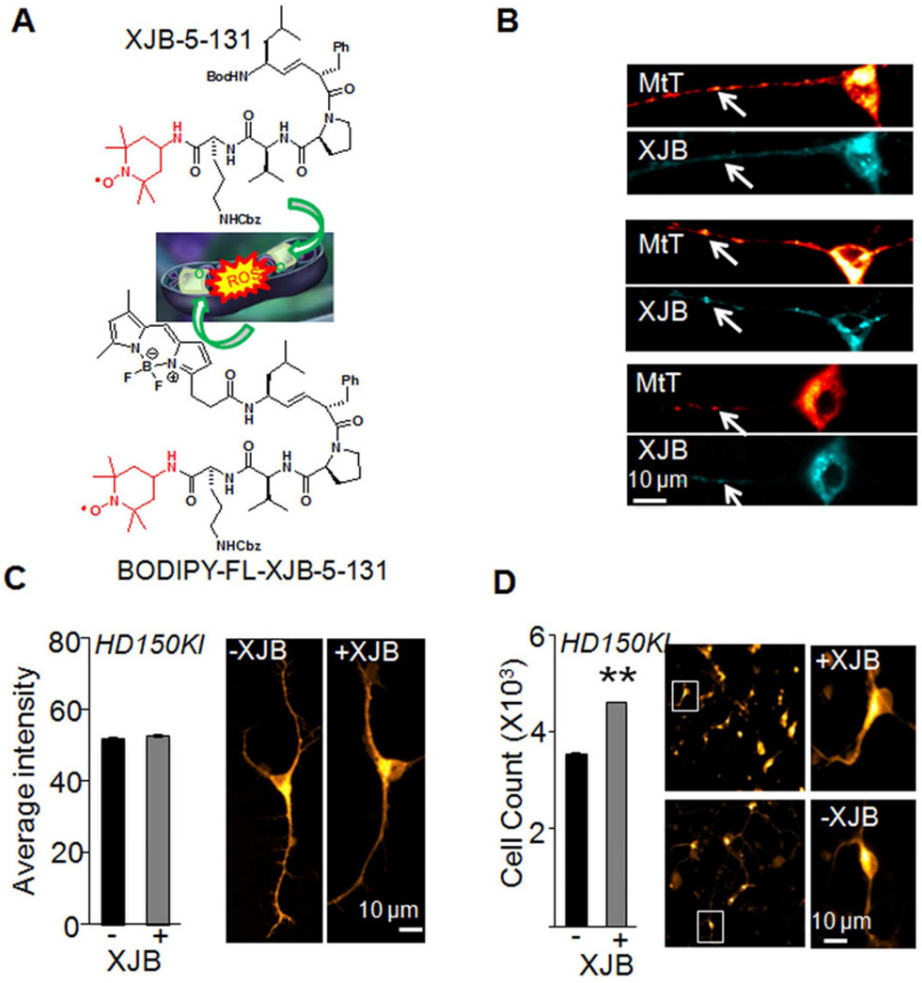
**Early signs of HD**: Weight loss
Motor Abnormalities




***XJB-5-131 supresses weight loss in HD mice and significantly improves motor performance***

*Cell Rep*. **2012**, *2*,1137

# Huntington's Disease: XJB Enhances Neuronal Survival




**B**. MtT=Mitotracker co-labelled in primary striatal neurons from embryonic day 17 HD150KI mice.

**C.** XJB treatment (1 µM) for 1 week does not induce measurable changes in the number of MT as quantified after Mitotracker intensity.

**D**. XJB treatment protects survival of primary striatal neurons after 7 days in culture

# Conclusion and Future Outlook

**Demonstrated effectiveness of XJB-5-131 in:**

- Traumatic Brain Injury
- Lethal Haemorrhagic Shock
- Huntington's Disease

- BODIPY - analogues for visualization and co-localization in mitochondria

**Challenges:**

- More concise synthesis of XJB-5-131
- Metabolic stability
- Solubility

# Acknowledgements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- Prof Louis Falo