



**Using Telomere-  
Based Biological  
Chemistry to Develop  
Superior  
Anti-Oxidant  
Therapeutics.**

**Kelly Lamb**

# Overview

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- **Biological ageing**
- **Telomeres**
- **MTR**
- **Anti-oxidants**
- **Applications**

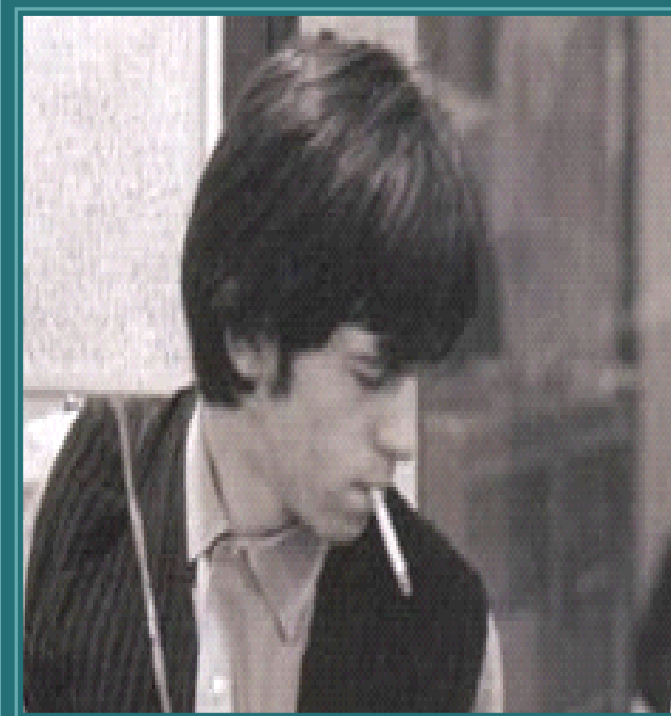
# How do we age?

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- **Chronological ageing**
- **Biological ageing**
  - **Level of the cell  
(cellular senescence)**
  - **Organ senescence**

# Stress accelerates biological ageing

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**1963**



**1993**

# Chronological and Biological ageing do not always equate

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57



43

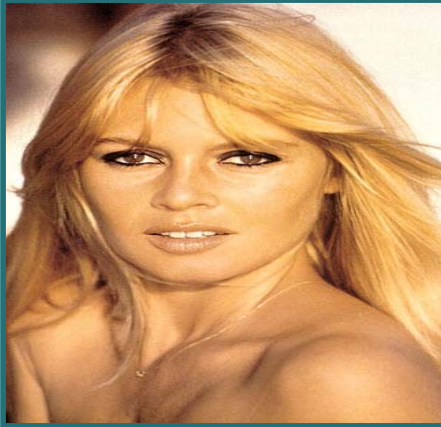


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52

# Face reflects health



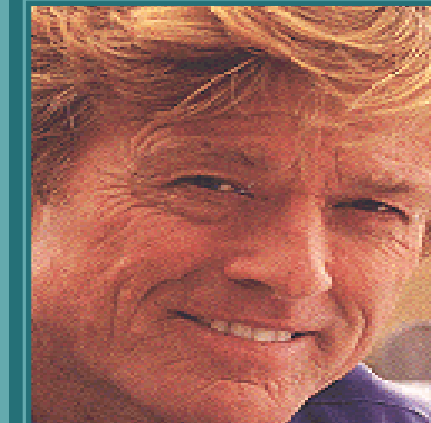
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52



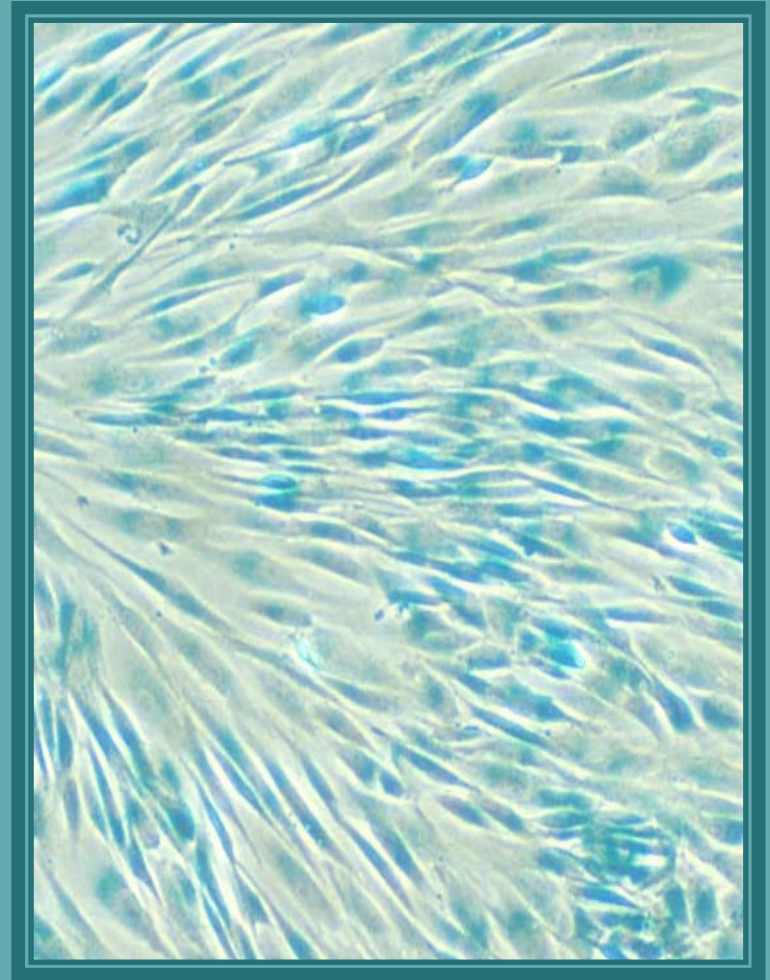
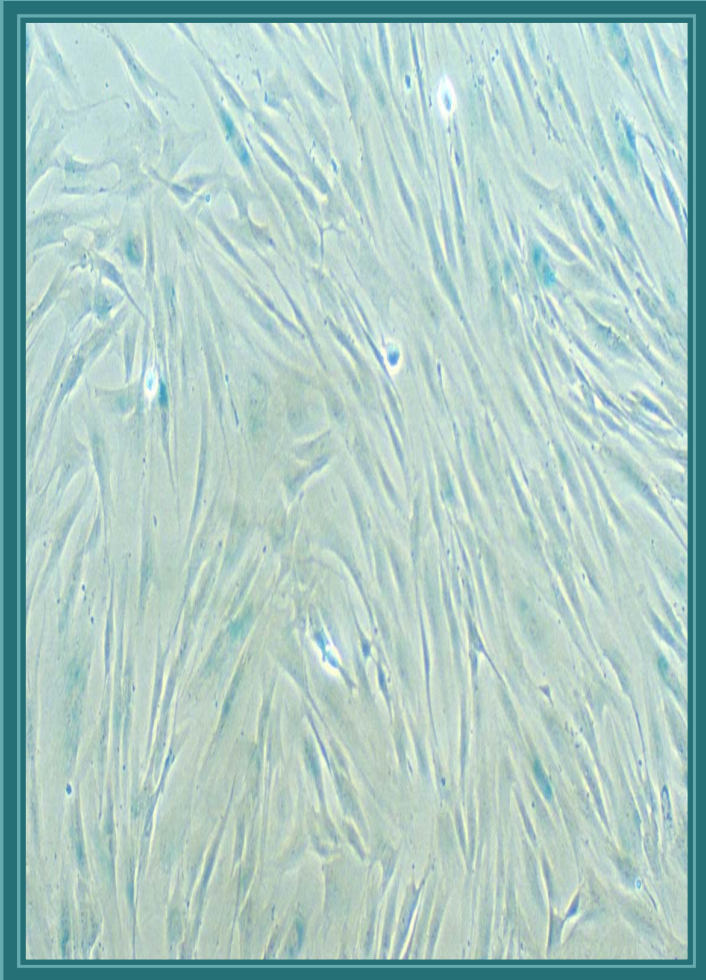
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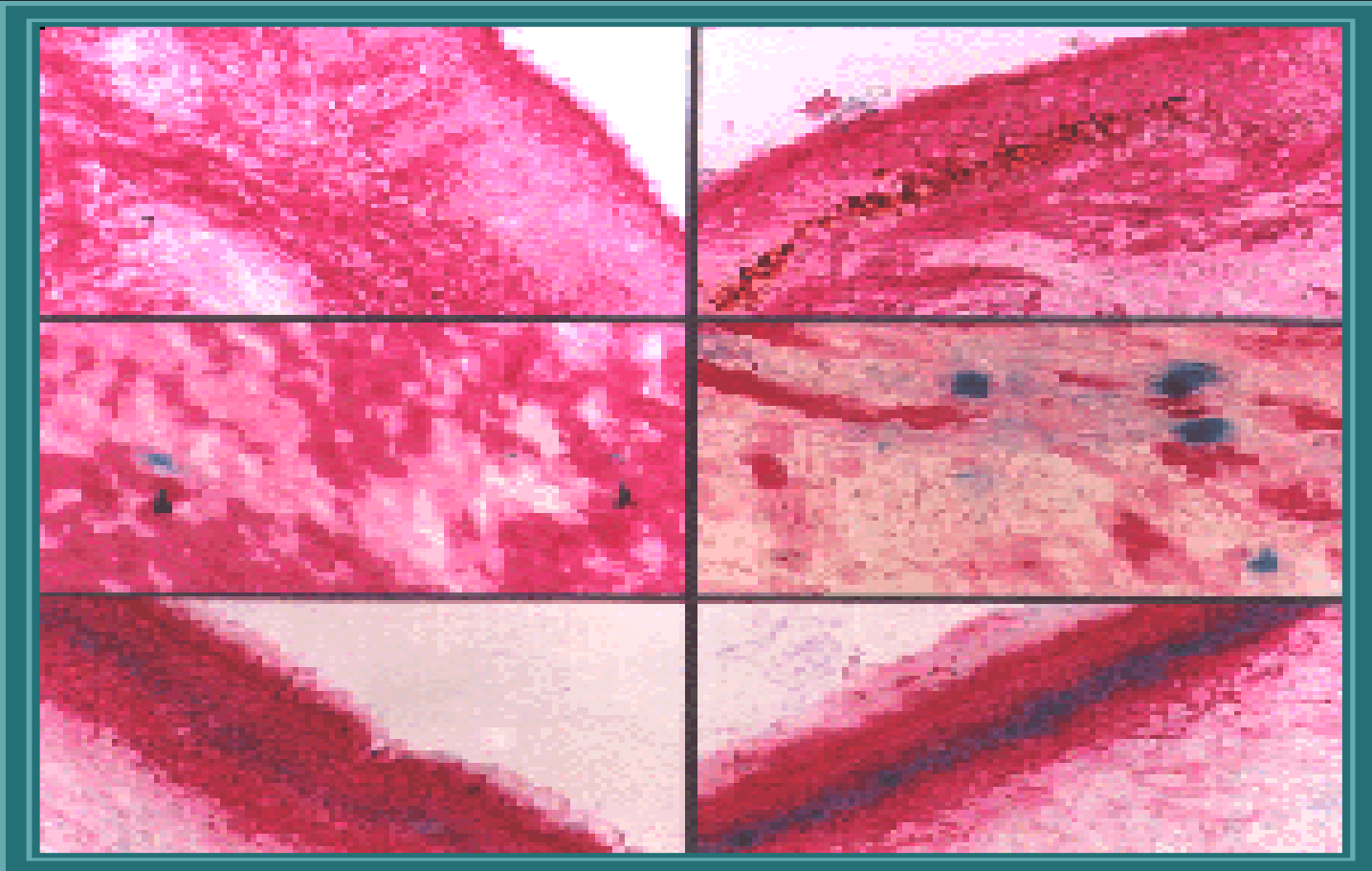
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# Senescence-associated $\beta$ -galactosidase

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# Ageing skin



# Root cause

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- **Oxidative damage**
- **'Wear and tear  
'phenomenon'**
- **Predisposes to disease**
- **Transplant damage/  
Stroke/  
Neurodegeneration/Cancer/  
Cardio-vascular disease**

# Mechanisms

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- **Oxidative damage accelerates biological clocks**
- **Damage to genes involved in cellular defences**
- **Platform for disease to develop**

# Mechanisms

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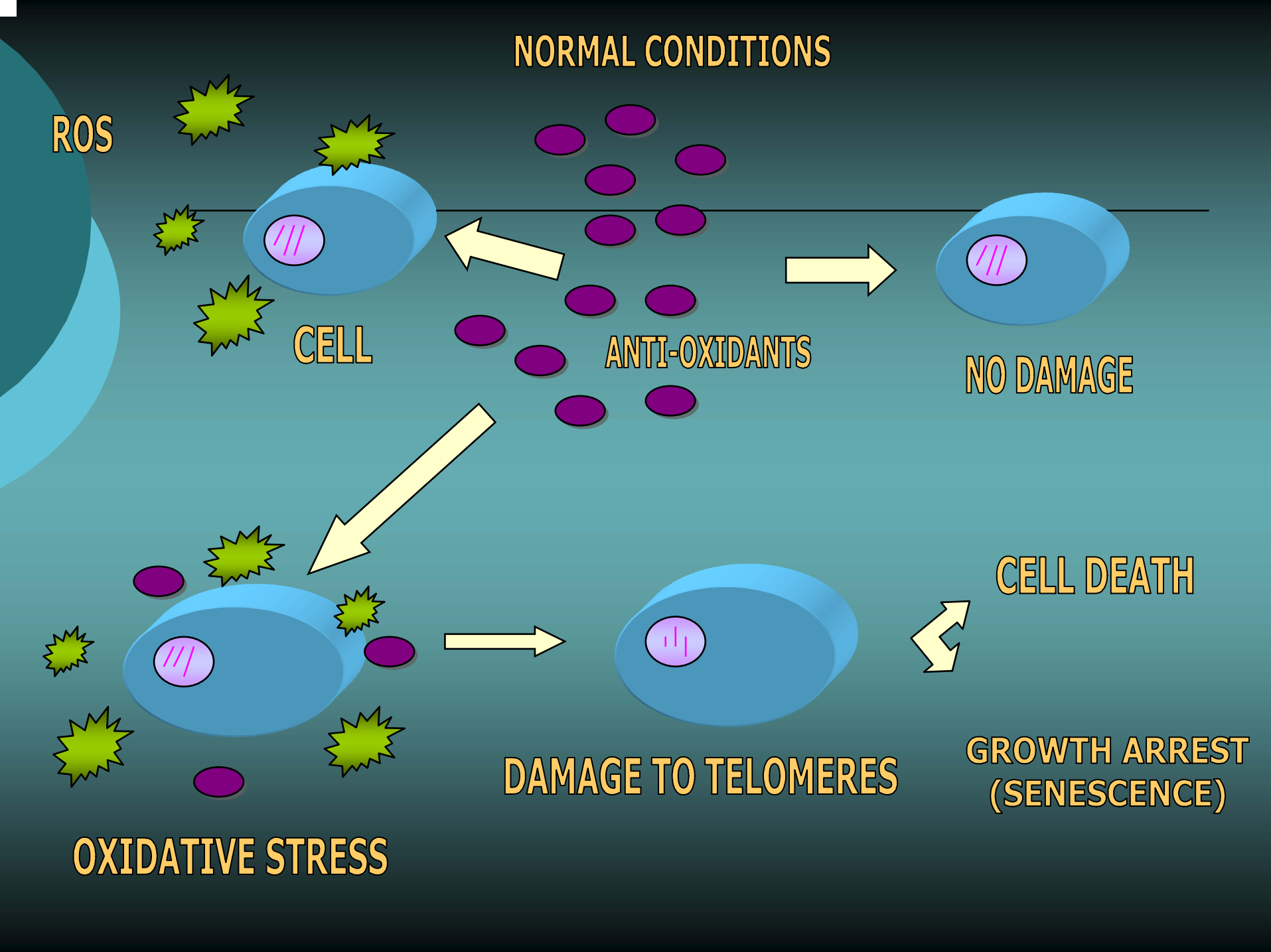
- **Clock (Telomere) linked to Energy production(Mitochondrion) and Fuel utilisation (Ribosome)**
- **MTR-** (Shiels PG and Davies RW (2003) In:The Molecular biology of the neurone (2nd Edition)435-464;  
Shiels PG and Jardine AG (2003), Cloning and Stem Cells 5(2) 157-161)
- **Biomarkers for ageing- senescence associated genes**
- **Exploit to study wide range of conditions**  
Kidney:Joosten et al Am J Pathol. 2003 Apr;162(4):1305-12;  
Cancer: Ashraf et al Br J Cancer. 200623;95(8):1056-61;  
Cognitive function:Harris et al 2006; Neurosci Lett. 9;406(3):260-4  
CVD; Starr et al 2006 Exp.Geron.

# Telomeres

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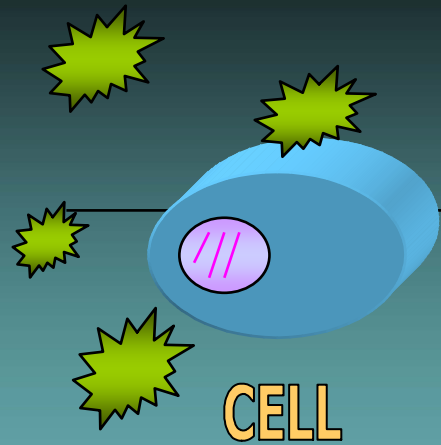
- Ends of chromosomes
- Sense and signal damage
- Home of repair systems
- Integrate energy production and utilisation
- Anti-cancer
- Telomere length in PBLs predictive for likelihood of mortality





# NORMAL CONDITIONS

ROS

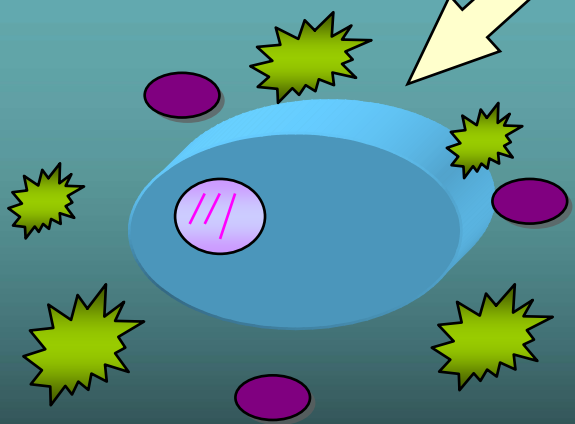
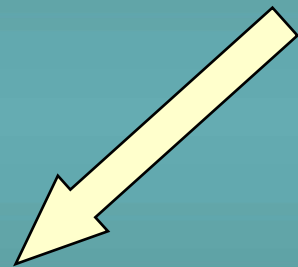


CELL

ANTI-OXIDANTS



NO DAMAGE



OXIDATIVE STRESS

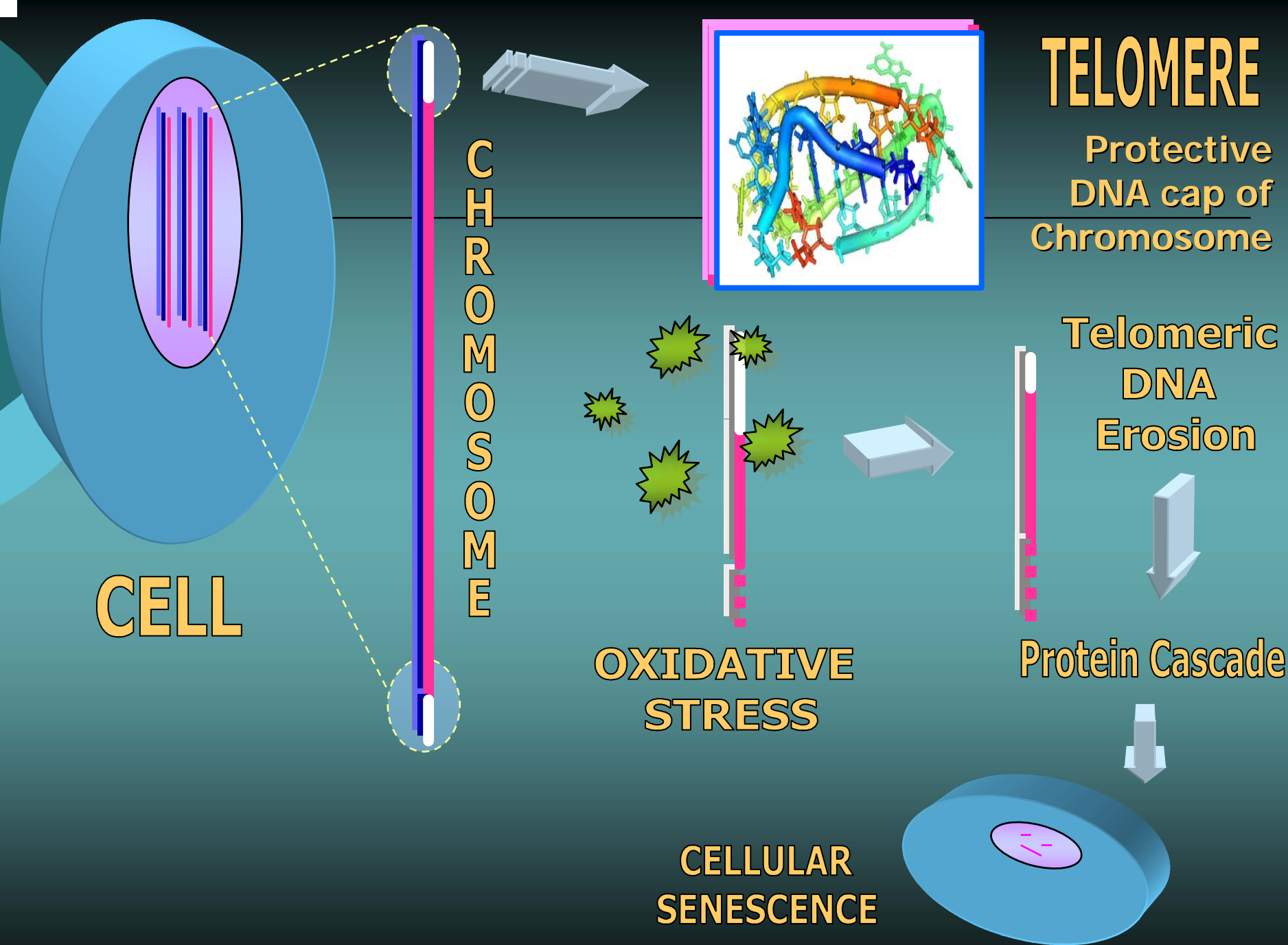


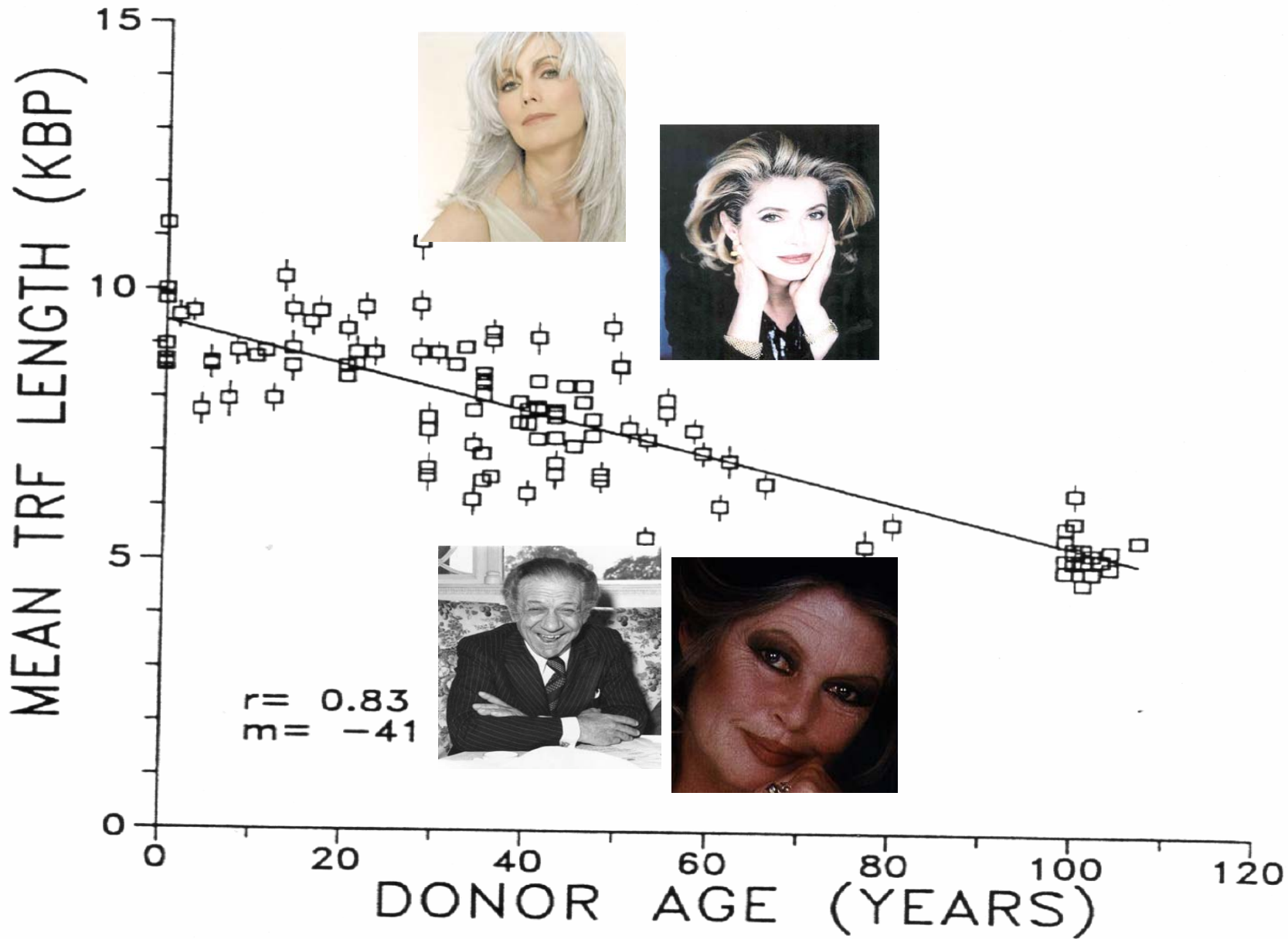
DAMAGE TO TELOMERES



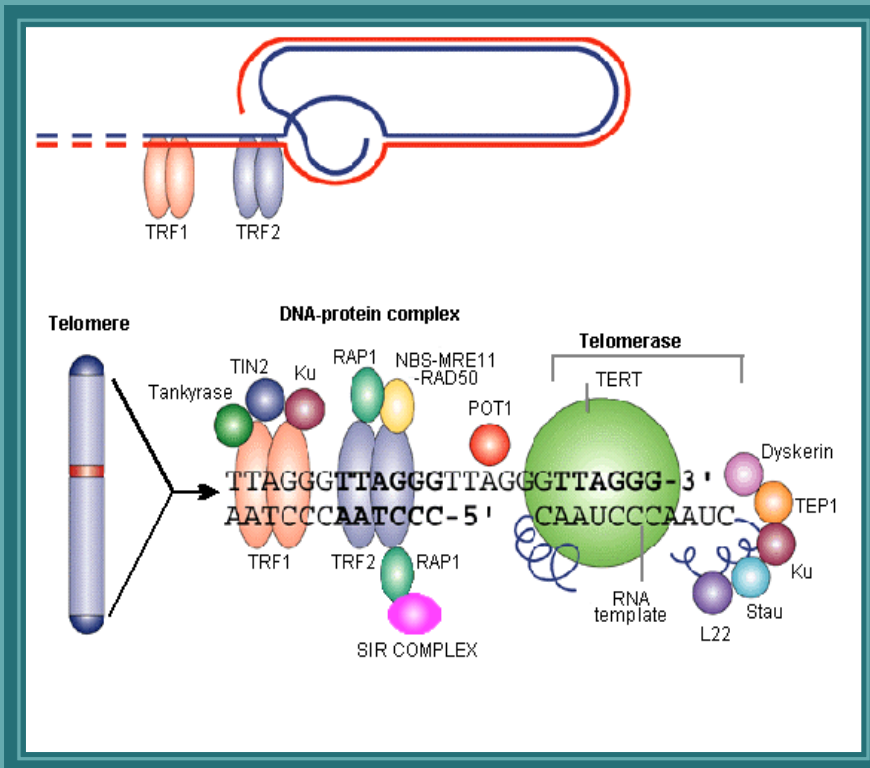
CELL DEATH

GROWTH ARREST (SENESCENCE)





# Telomeric nucleoprotein complex



- DNA repair components
- Damage sensing and signalling components

Figure 1. Schematic of proposed telomere structure with senescence associated proteins (Adapted from Nature Reviews Immunology 2002;2:699-706).

# Telomere nucleoprotein complexes

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**Disruption leads to:**

- **Progeric syndromes**
- **Predisposition to disease:**
  - **Cancer**
  - **Osteoporosis**
  - **Cardiovascular Disease**

# Werner's syndrome



**WS patient age 15 yrs**



**WS patient age 48 yrs**

# How do we assess biological age?

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- **SAGs**
- **Cytological markers  
(e.g. SA beta Gal)**
- **Telomere lengths**

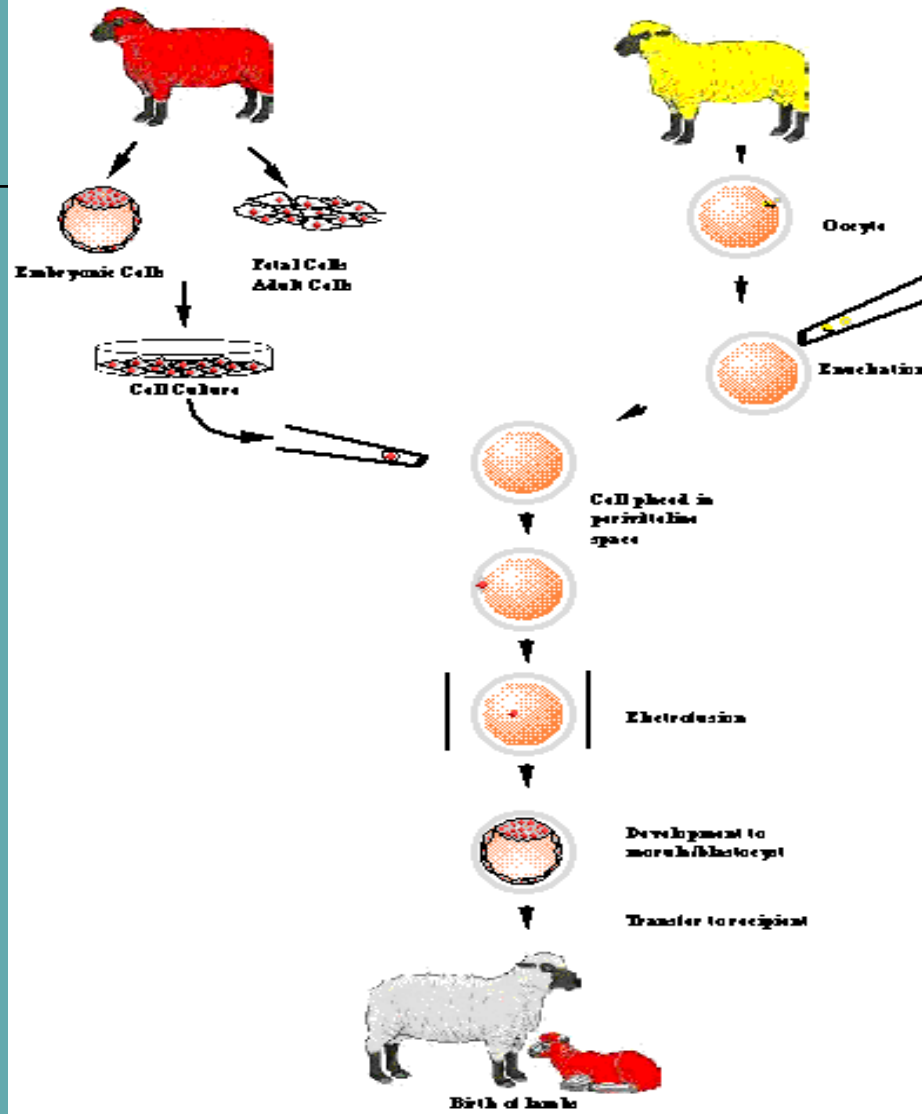
# Why the MTR?

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- NT animals
- Epithelial cell donor nuclei derived animals show accelerated senescence
- Replicated across species
- MTR

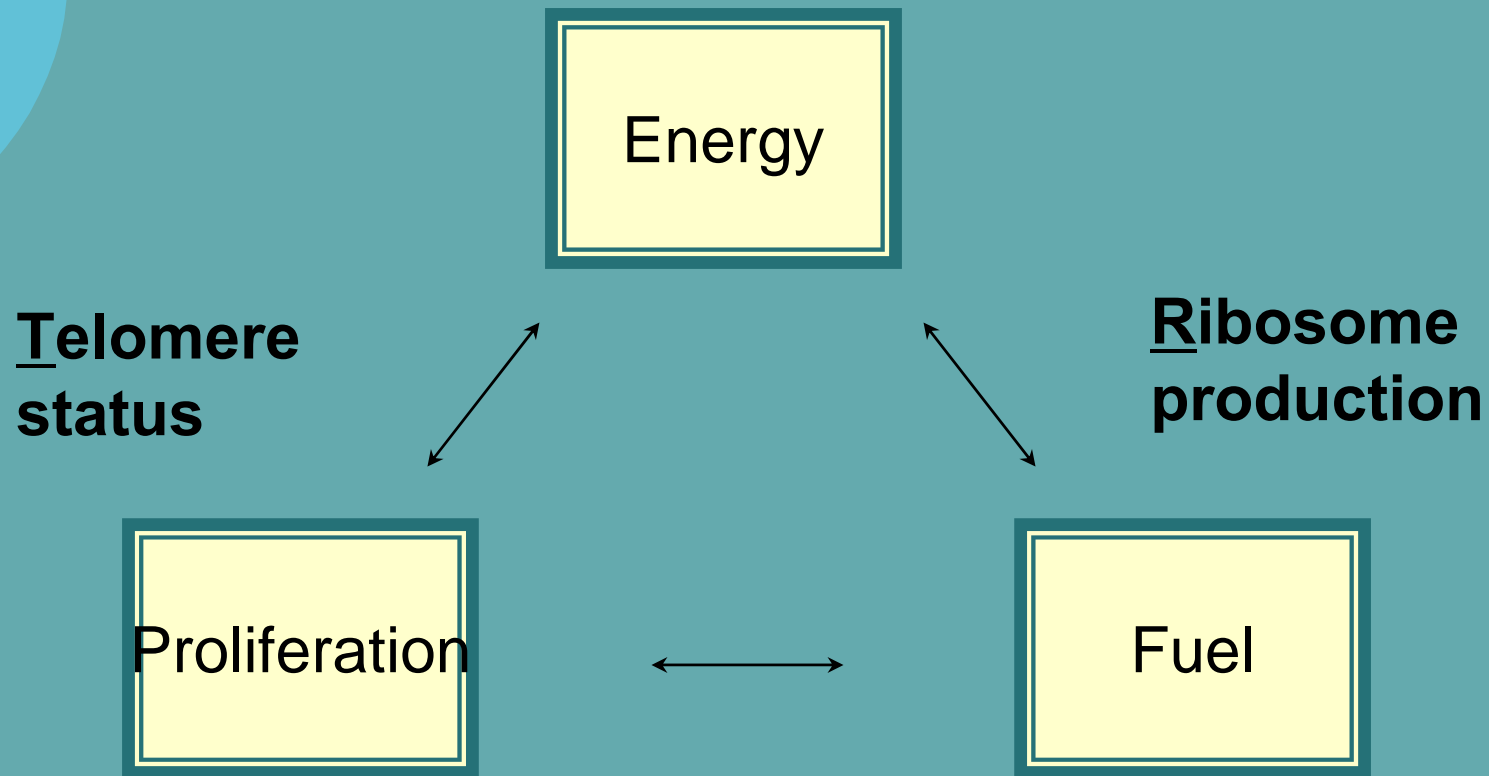
# Nuclear Transfer



# MTR-why the link?

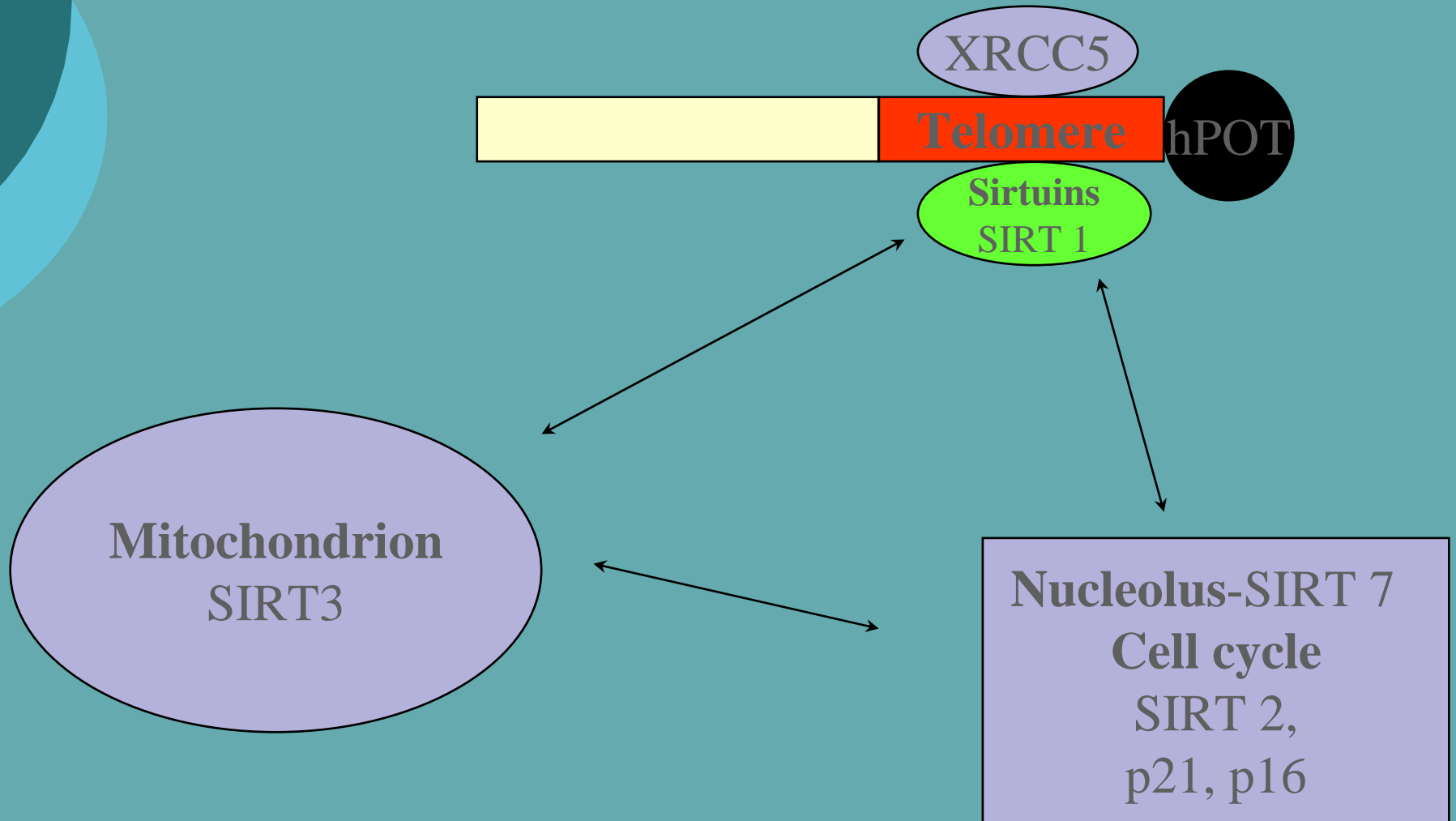
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## Mitochondrial function



# MTR associated genes

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# Is the MTR an informative system for disease analysis?

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- **Yes for Renal disease (organ senescence)**

Joosten SA, et al Am J Pathol. 2003 162(4):1305-12

- **Increased oxidant stress**
- **How does cell respond to acute oxidant insult?**

# Evaluate using MTR system

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- Do you see telomere shortening?
- Do you see change in MTR associated gene expression?
- Is this reflected in function post insult?
- Is it associated with a predisposition to disease?

# What happens in Primary cells following acute oxidant insult?

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- **Lots of equivocal data**
- **P16 dependent or independent**
- **Telomere dependent or independent**
  
- **Check ourselves**

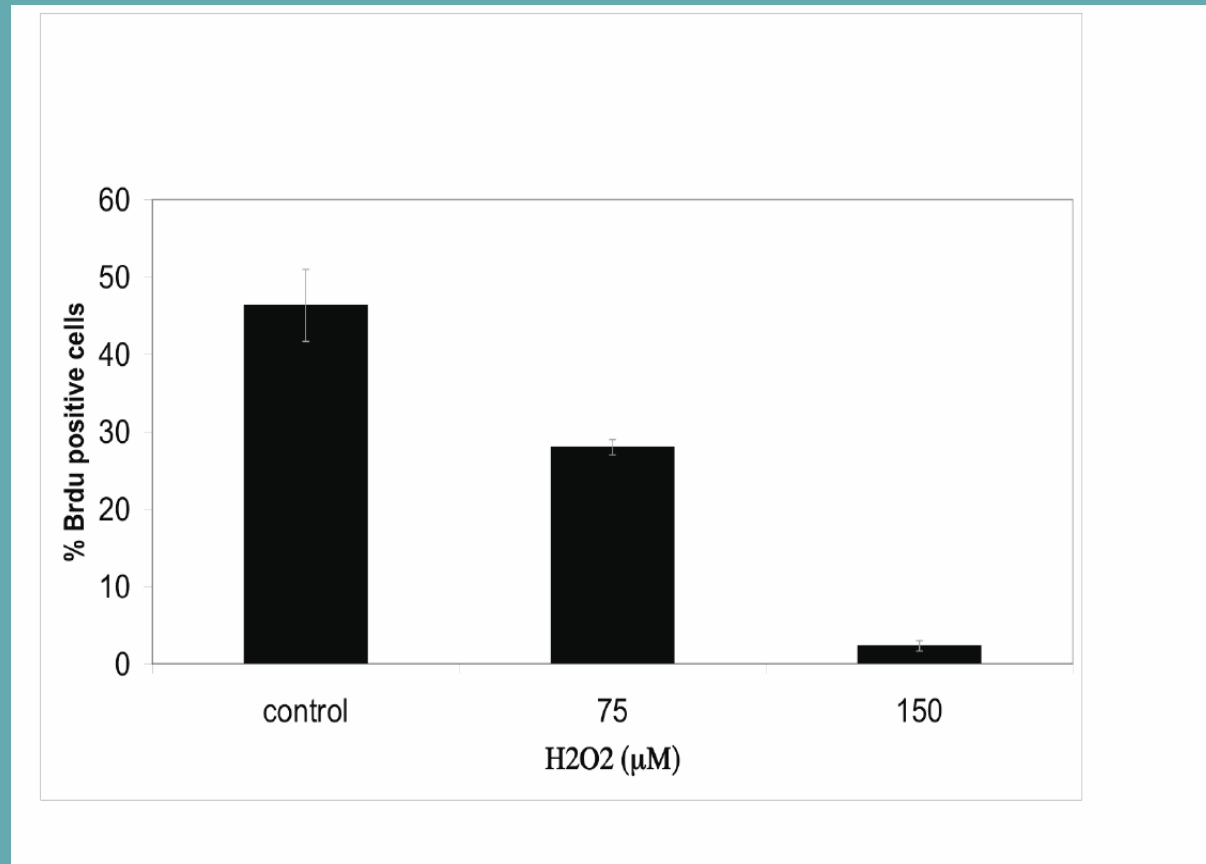
# Model

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- **Human diploid fibroblasts (HDFs)**
- **H<sub>2</sub>O<sub>2</sub> insult**
- **Look at MTR status**

# Growth arrest induced by H<sub>2</sub>O<sub>2</sub>?

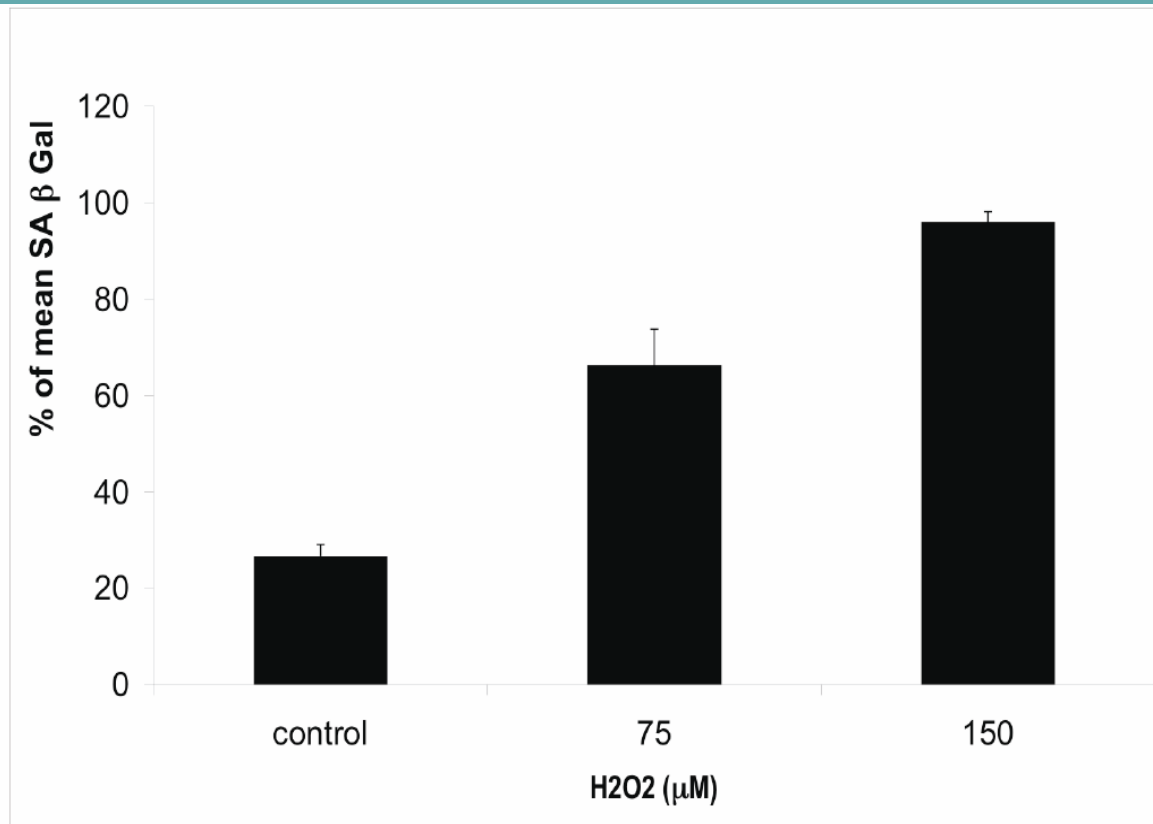
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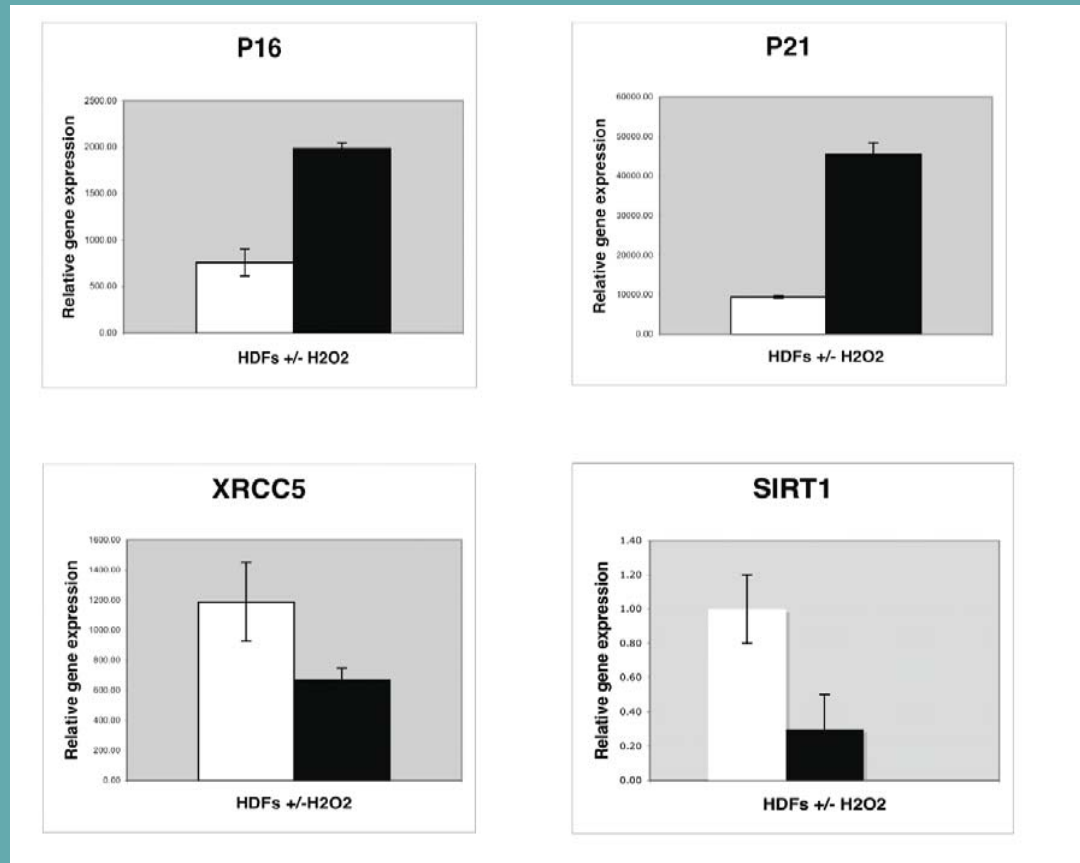
BrdU incorporation in HDFs as measurement of DNA synthesis

# SA $\beta$ -Gal analyses in HDFs.

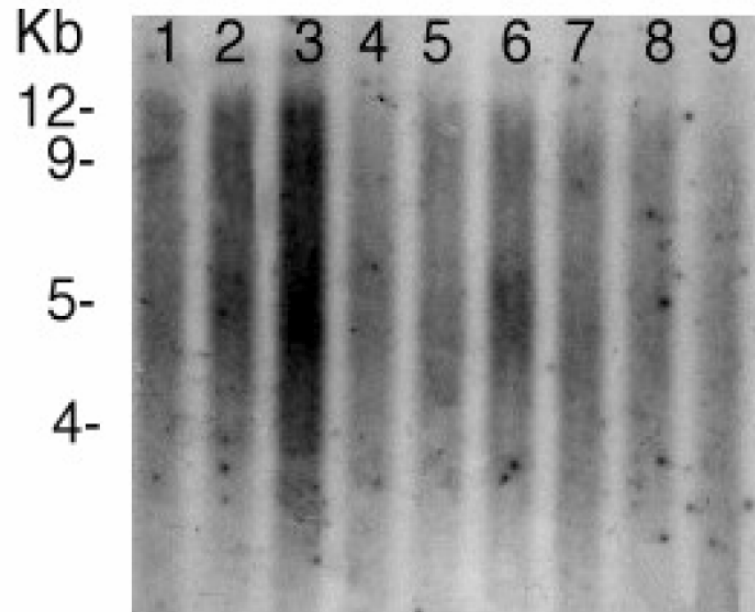
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# Senescence associated gene expression in HDFs.



# Telomere analysis



Lane	1	2	3	4	5	6	7	8	9
H <sub>2</sub> O <sub>2</sub> treatment	0	75 $\mu$ M	150 $\mu$ M	0	75 $\mu$ M	150 $\mu$ M	0	75 $\mu$ M	150 $\mu$ M
mTRF [kb]	7.02	6.96	6.91	6.71	6.64	6.43	6.47	6.49	5.92

# Status

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- **Telomere independent**
- **P16 dependent**
- **P21 dependent**
  
- **STASIS-stress and stimulation induced senescence**
- **Not replicative senescence**

# Utility

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- **Screen NCEs for biological stress**
- **Screen NCEs for anti-oxidant efficacy**
- **Use same biomarkers to assess pathologies**

# System

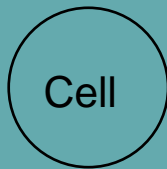
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- **Expand to evaluate all telomere associated genes (37) in a similar system**
- **Which are most effective reporters**
- **Use as biological chemistry tool**

# SenTest

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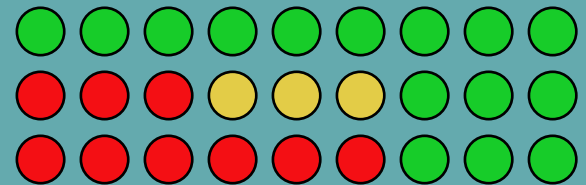
Stress



NCE

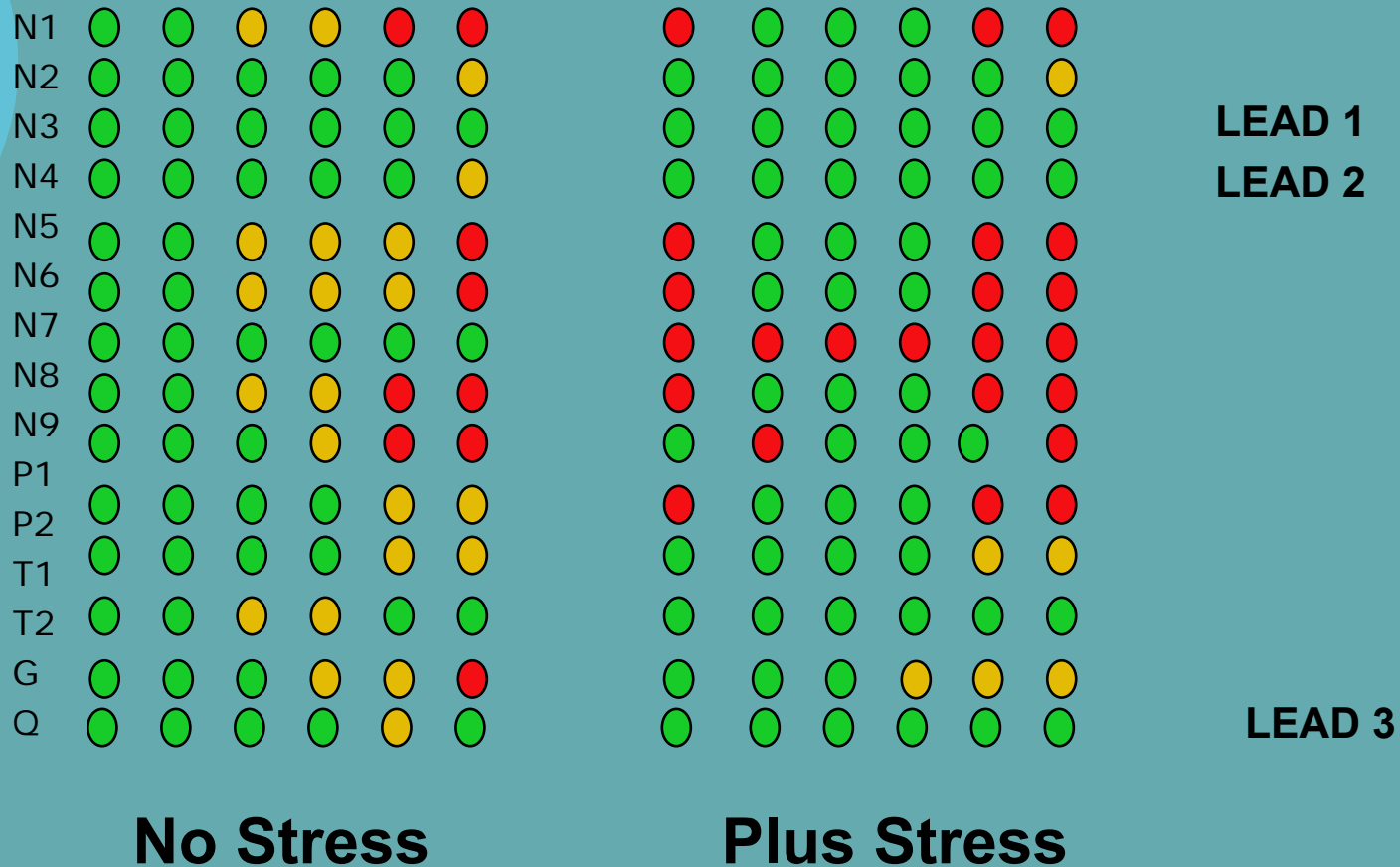


Safe



Danger

# Antioxidant testing



# Technical success

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- **SenTest** established as low throughput assay
- 37 genes in Biological clock paired down to core set (6 genes)
- NCE anti oxidants- 5 classes developed-15 compounds
- 3 lead compounds

# Technical success

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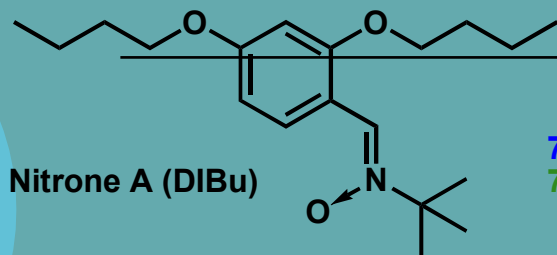
- External compound validation  
7 compounds - Inflammation,  
Ischaemia (clinically trial-FAILED!)

**SENTEST PREDICTS PROBLEM  
WITH THIS AGENT!**

- *In vivo* validation -Transplantation
- High throughput assay in development
- New IP to be filed

# Stress Testing

Max. nitron concentration at which HDFs are viable  
(no oxidant and challenged with 150  $\mu\text{M}$   $\text{H}_2\text{O}_2$ )



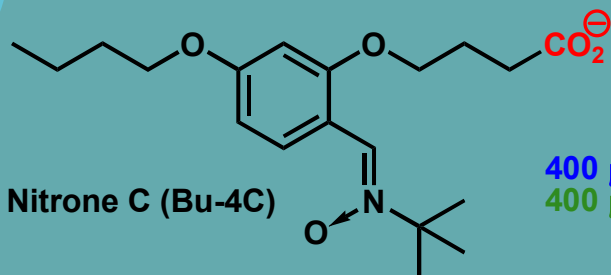
Nitron A (DIBu)

75  $\mu\text{M}$   
75  $\mu\text{M}$



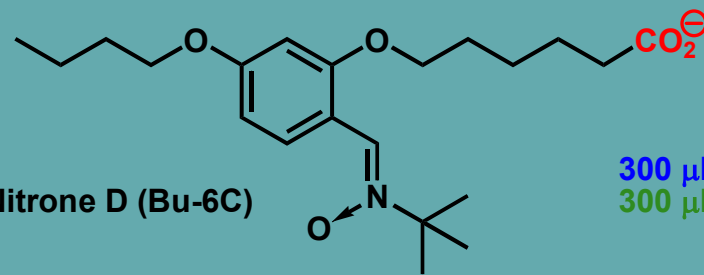
Nitron B (DIDOD)

200  $\mu\text{M}$   
200  $\mu\text{M}$



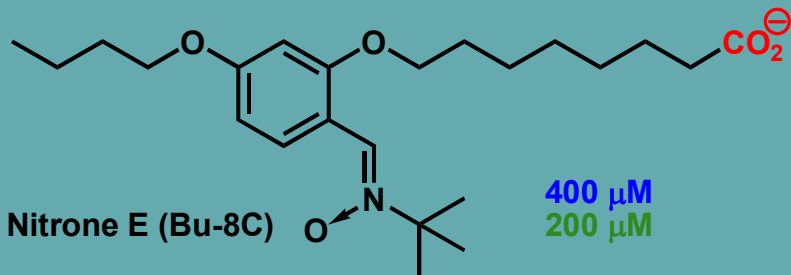
Nitron C (Bu-4C)

400  $\mu\text{M}$   
400  $\mu\text{M}$



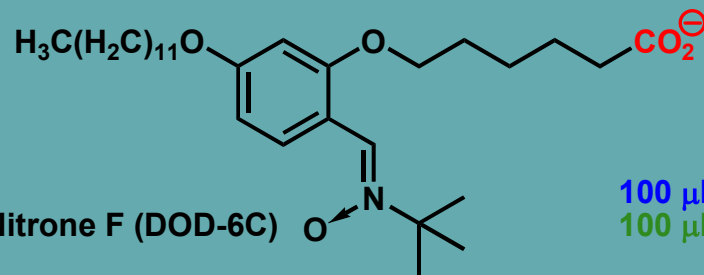
Nitron D (Bu-6C)

300  $\mu\text{M}$   
300  $\mu\text{M}$



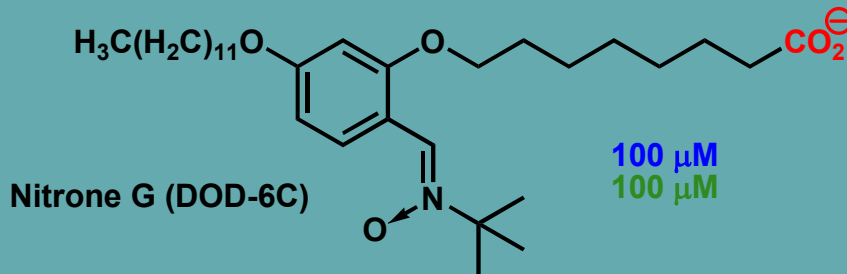
Nitron E (Bu-8C)

400  $\mu\text{M}$   
200  $\mu\text{M}$



Nitron F (DOD-6C)

100  $\mu\text{M}$   
100  $\mu\text{M}$

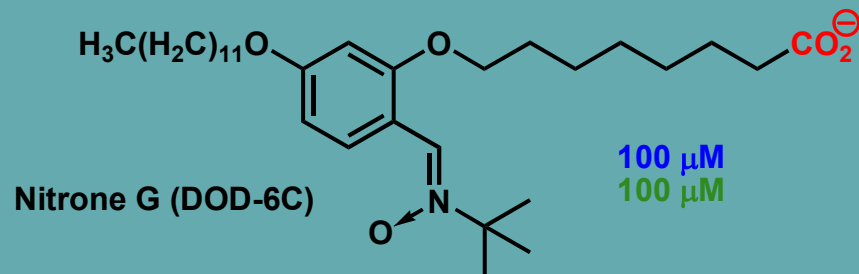
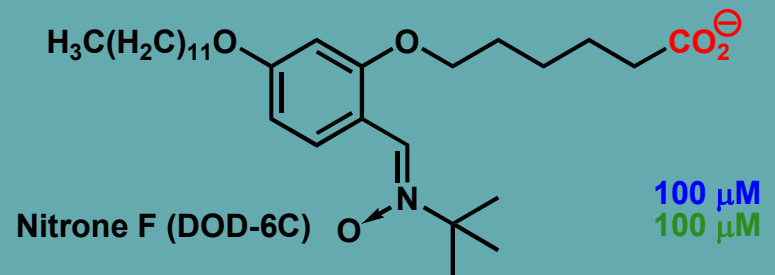
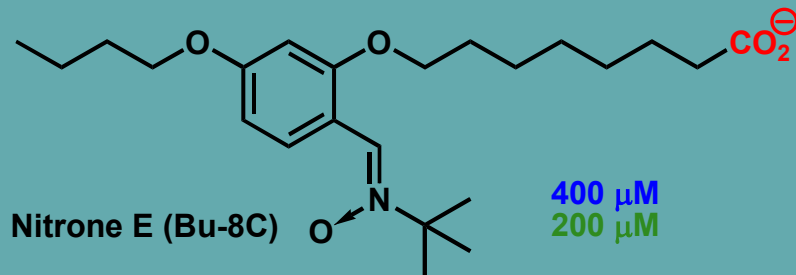
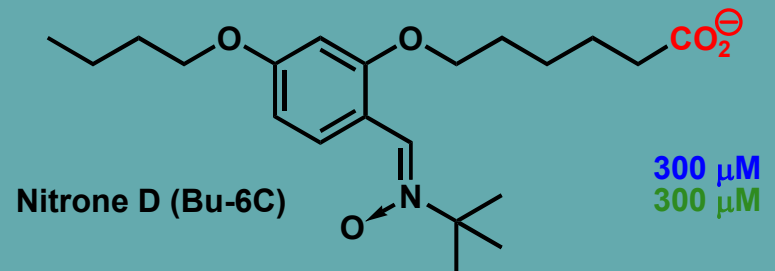
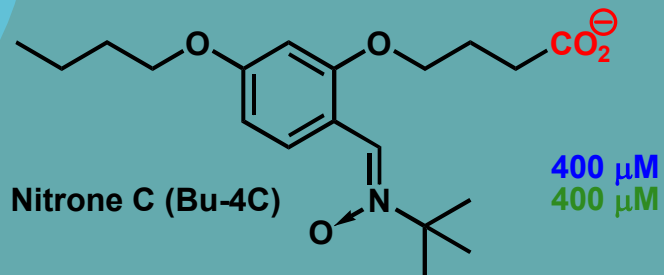
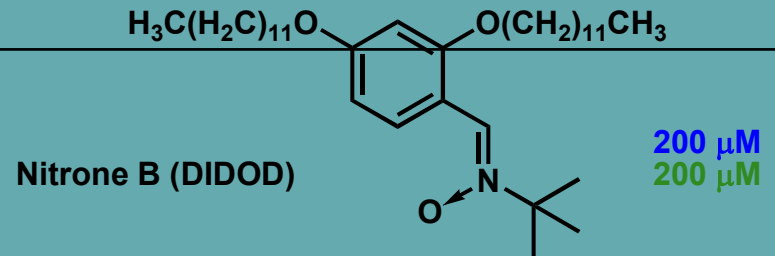
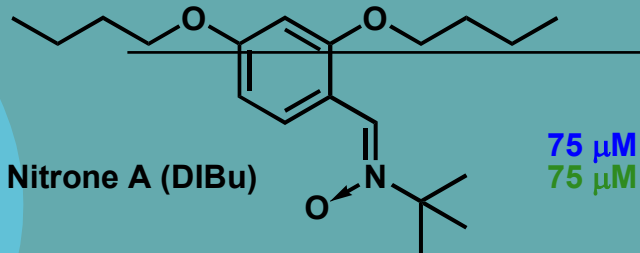


Nitron G (DOD-6C)

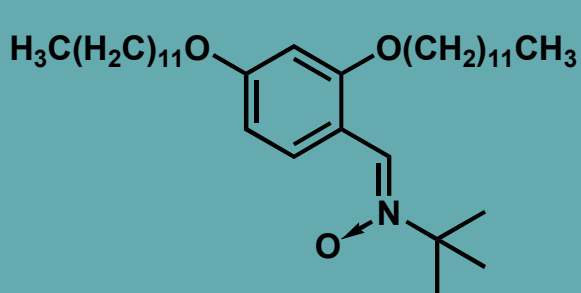
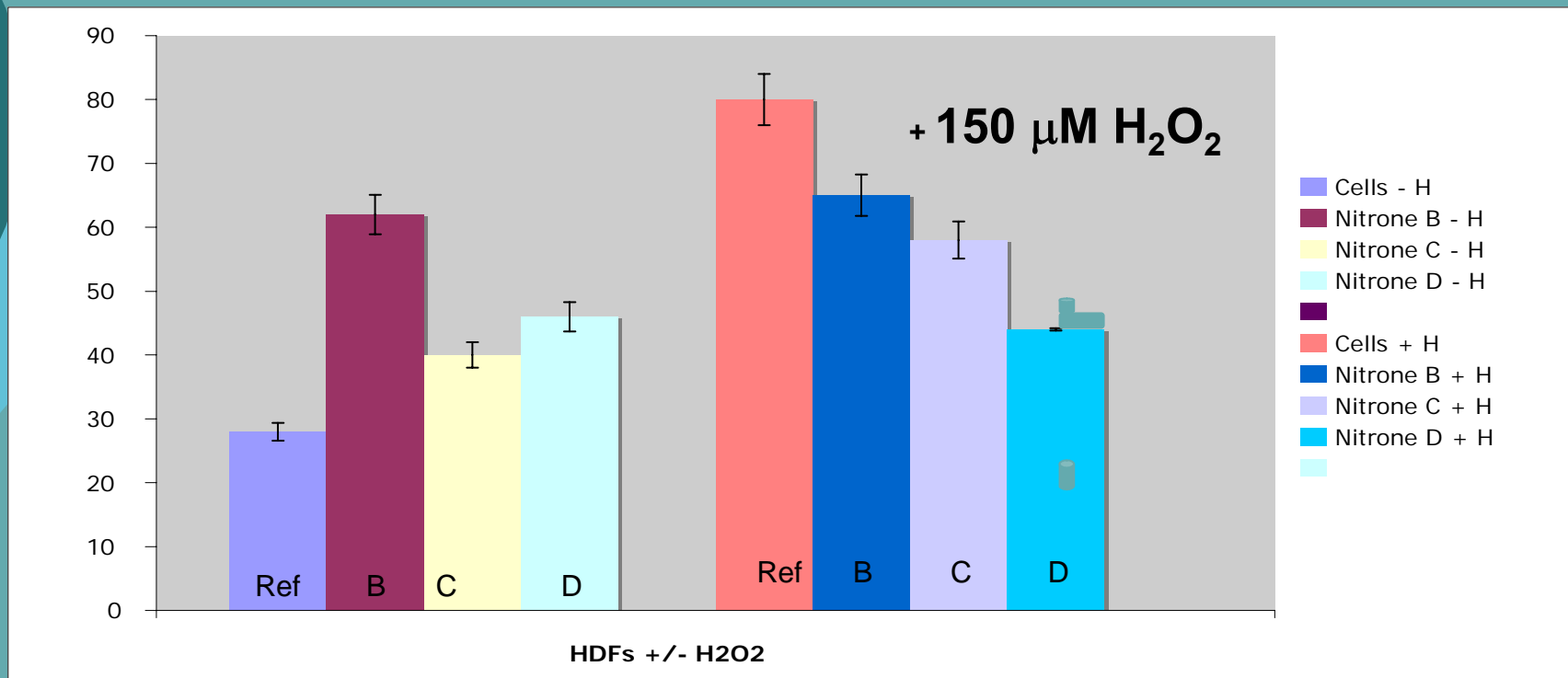
100  $\mu\text{M}$   
100  $\mu\text{M}$

# Toxicity Testing

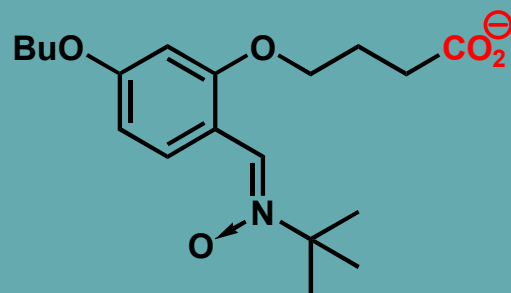
Max. nitron concentration at which HDFs are viable  
(no oxidant and challenged with 150  $\mu\text{M}$   $\text{H}_2\text{O}_2$ )



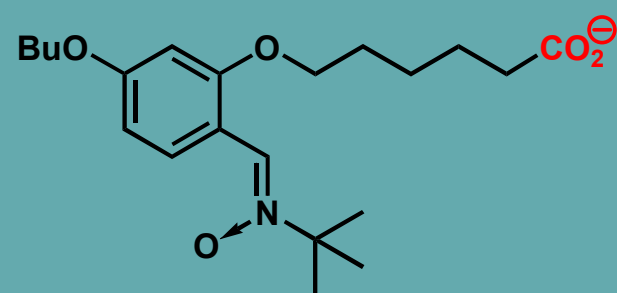
# SA beta-galactosidase expression



Nitrone B (200  $\mu\text{M}$ )

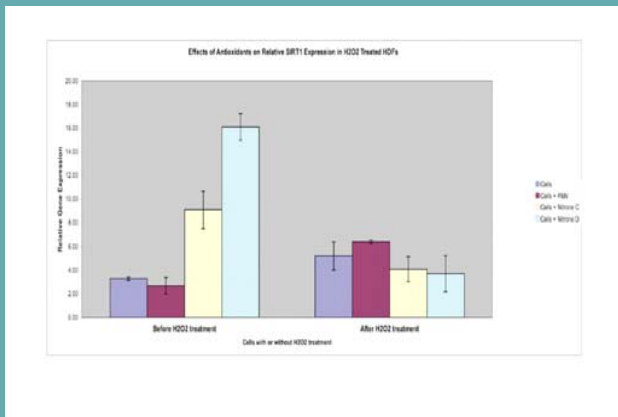
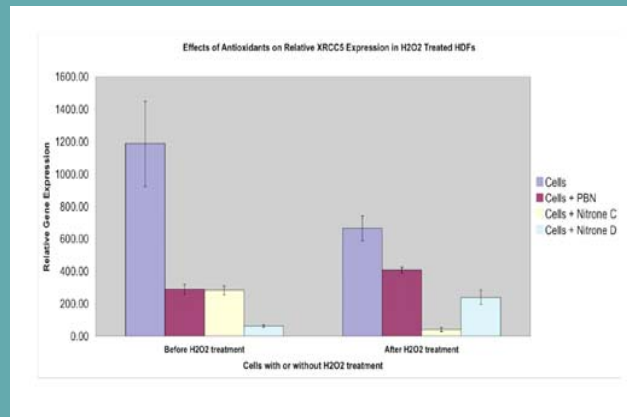
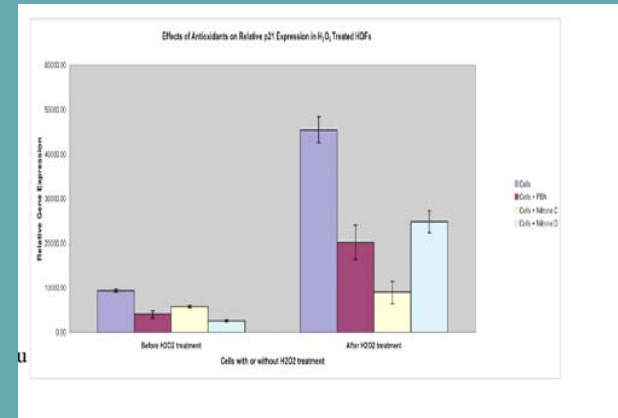
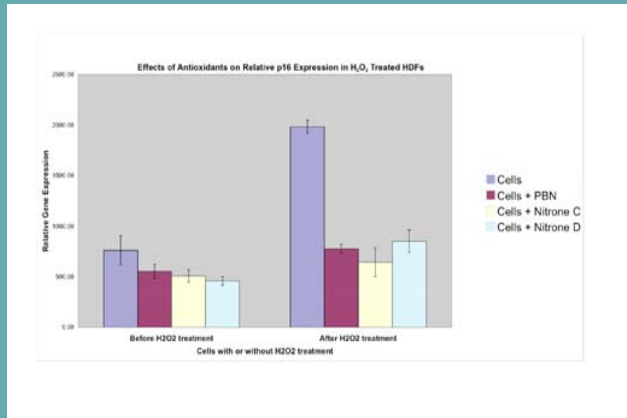


Nitrone C (400  $\mu\text{M}$ )

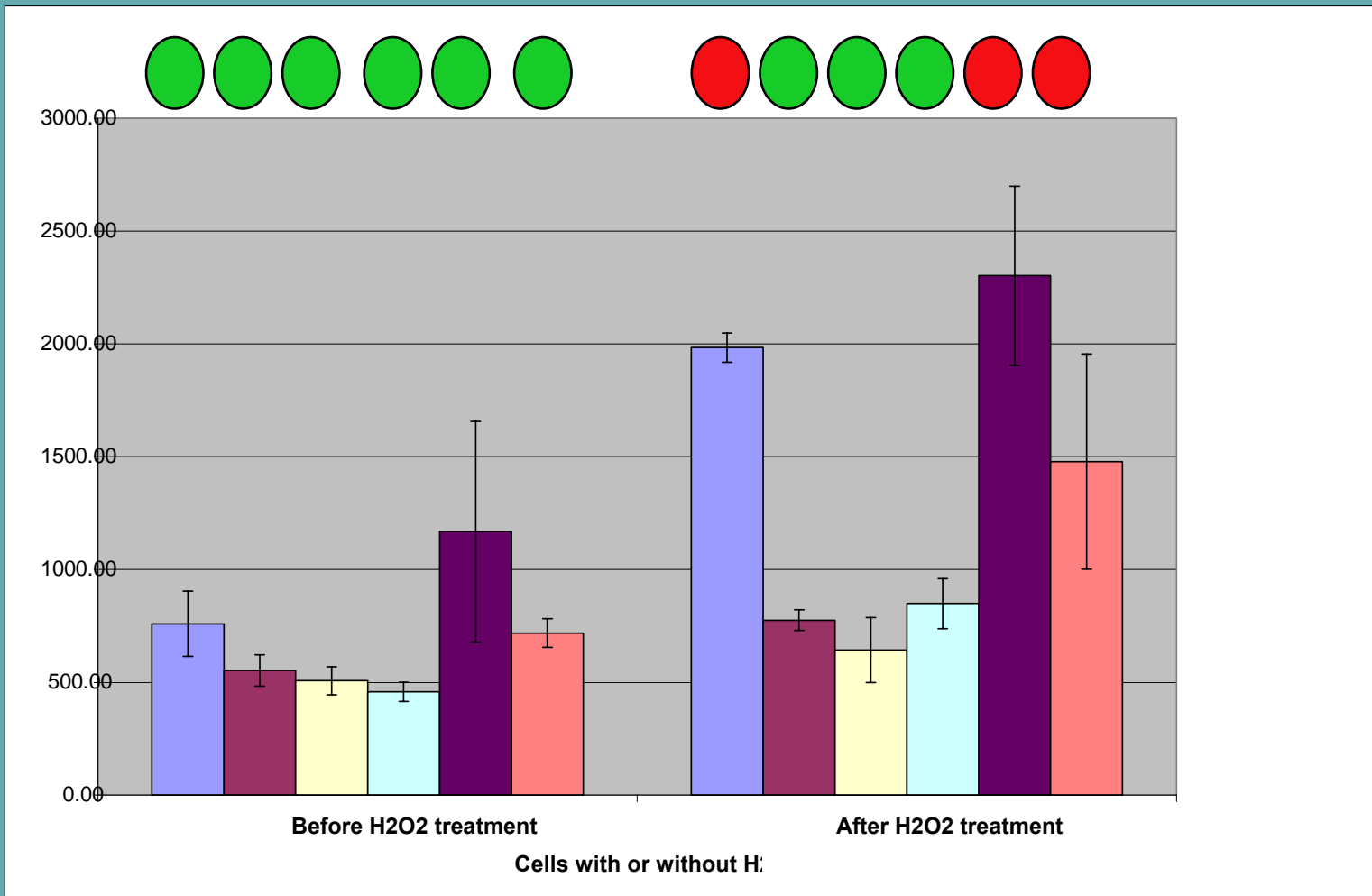


Nitrone D (300  $\mu\text{M}$ )

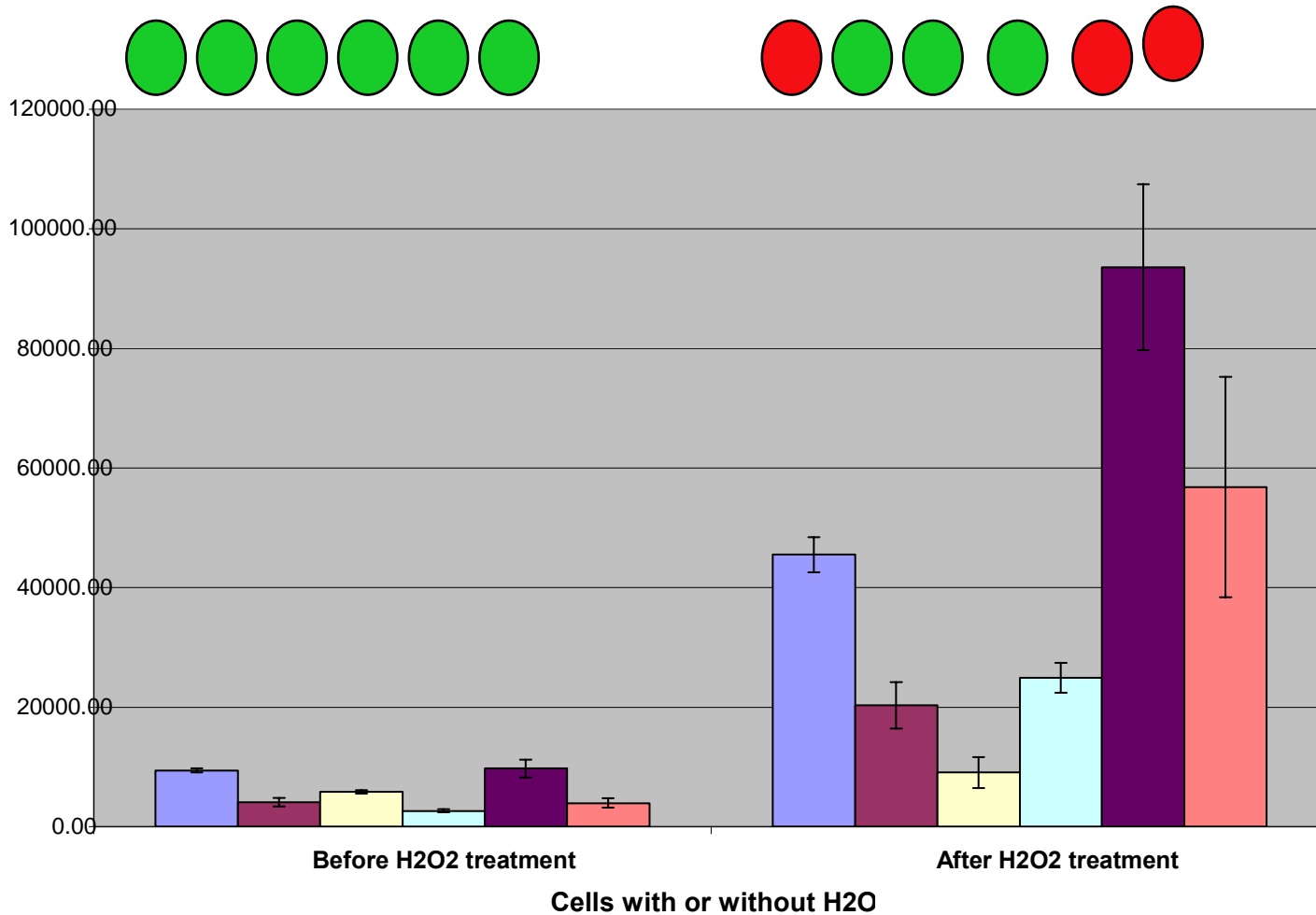
# QPCR analyses



# Superior antioxidants 1



# Superior antioxidants 2



# Other applications

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- **Diseases of ageing or accelerated ageing**
  - **Renal Tx, CVD, Neurodegeneration**
- **Cancer -**
  - **prognosis/disease state/progression**
  - **Therapy**

Ashraf N, Zino S, Macintyre A, Kingsmore D, Payne AP, George WD, Shiels PG.

Br J Cancer. 2006 95(8):1056-61

# Acknowledgements

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- **Shiels Group**
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**Richard Hartley**  
**Alison Hay**

**Tony Payne**  
**Wayne Davies**



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Questions?