ECOTOXICOLOGIA

AULA TP 6

Planeamento das Aulas

Aula 1 – Introdução aos Testes de Ecotoxicologia. Biomarcadores e Delineamento Experimental

Aula 2 – Efeitos do Glifosato no crescimento dos organismos teste (taxas de inibição e constantes de inibição IC50). Marcadores biofísicos de toxicidade I

Aula 3 – Marcadores biofísicos de toxicidade II

Aula 4 – Marcadores bioquímicos de toxicidade I – Pigmentos vegetais

Aula 5 – Marcadores bioquímicos de toxicidade II – Pigmentos vegetais II

Aula 6 - Marcadores bioquímicos de toxicidade III – Dano Membranar

Aula 7 – Marcadores bioquímicos de toxicidade IV – Dano Membranar e Ácidos Gordos

Aula 8 - Marcadores bioquímicos de toxicidade III – SOD

Aula 9 – Técnicas de extração e análise de metais pesados

Aula 10 - Nanotoxicologia

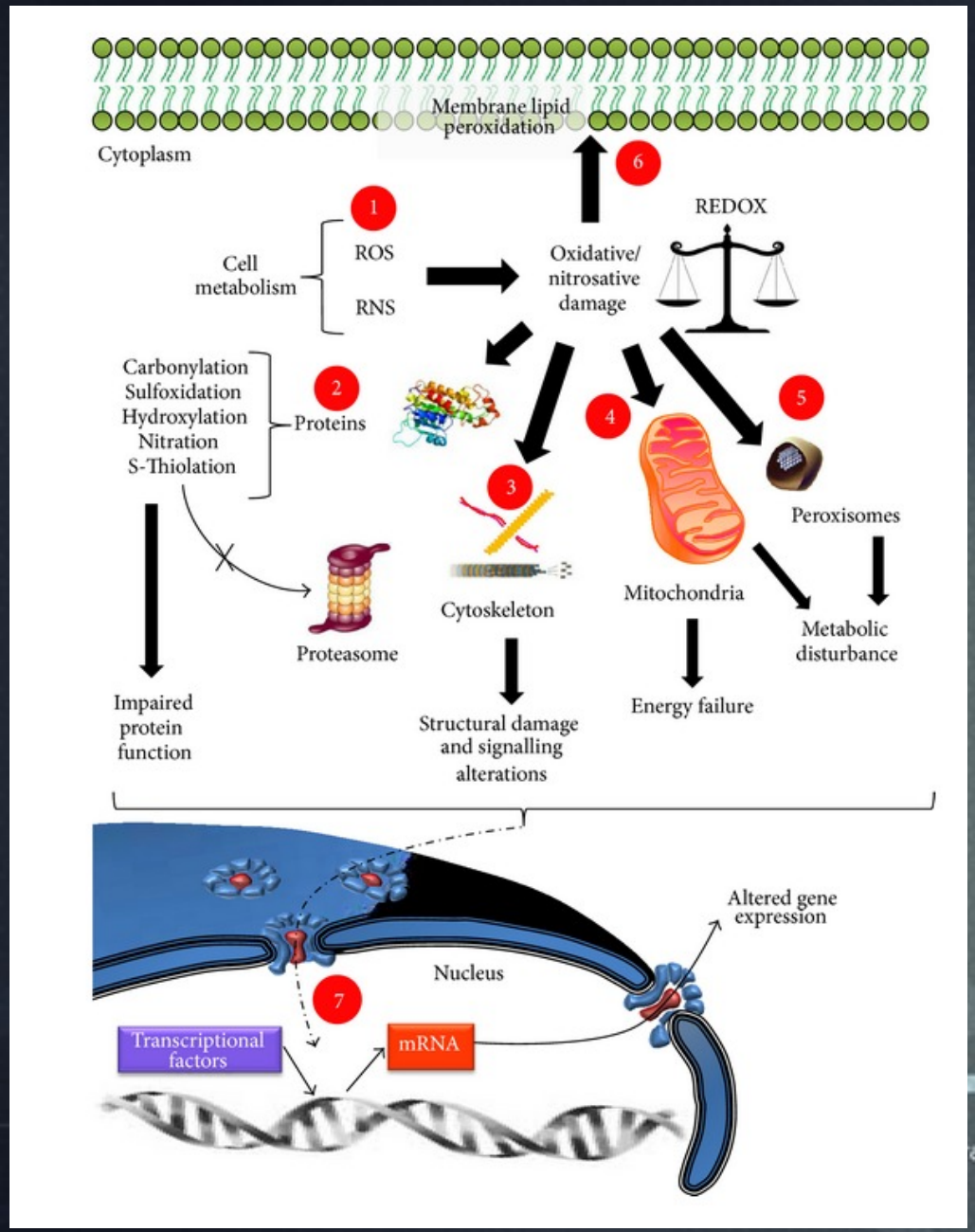
PEROXIDAÇÃO LÍPIDICA

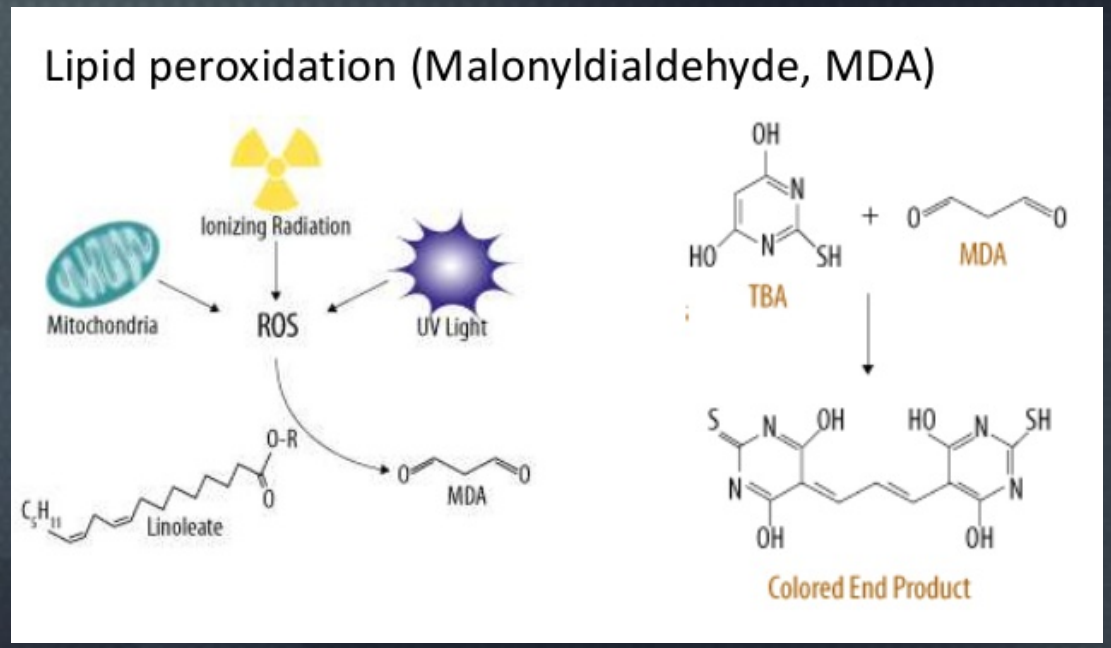
- Homogenizar o material vegetal numa proporção de 1 mL de solução TBA/TCA a cada amostra de pellet.
    - TBA 0.5 %
    - TCA 20%

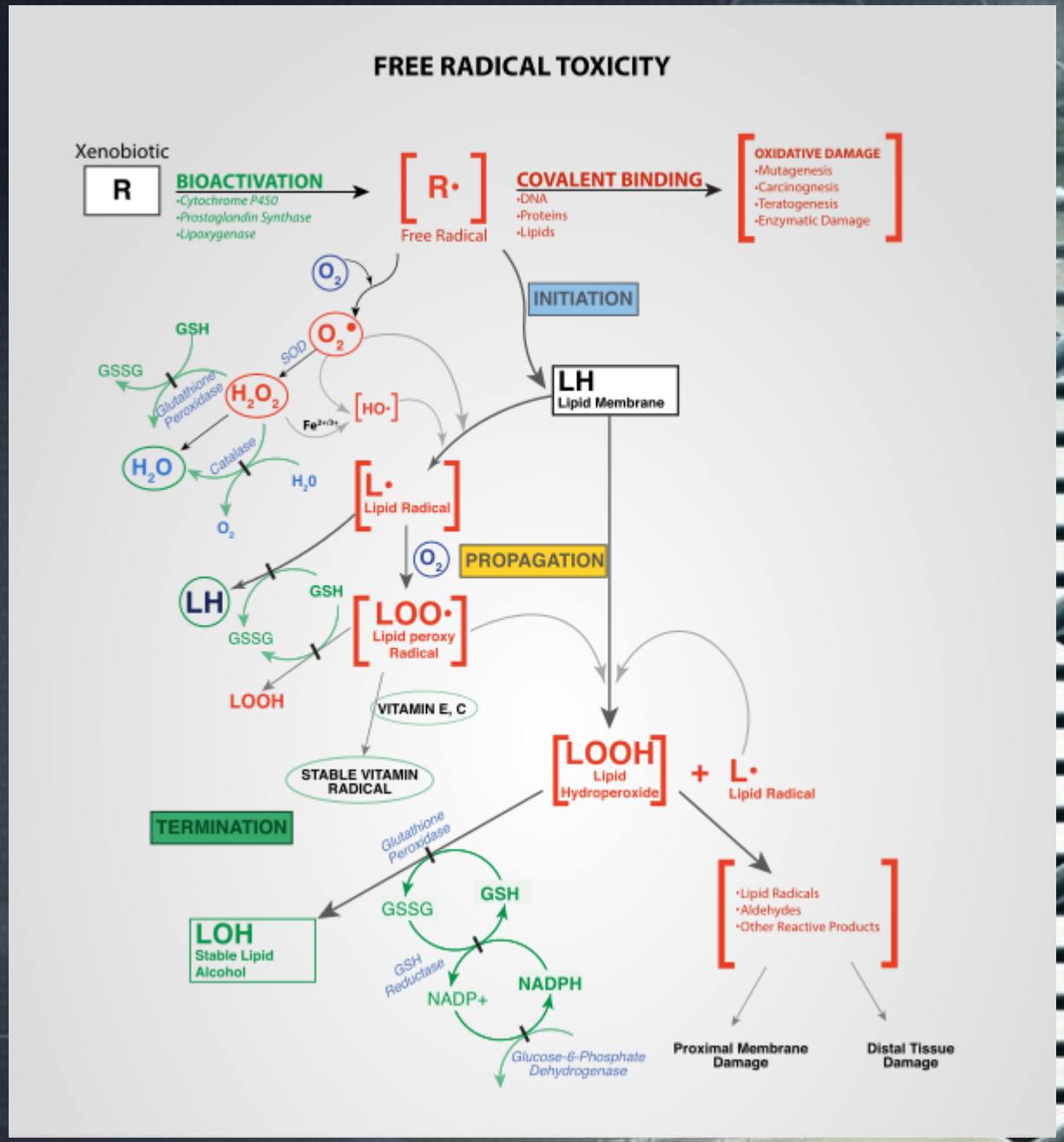
- Colocar o extrato a incubar durante 30 min a 95 ºC.

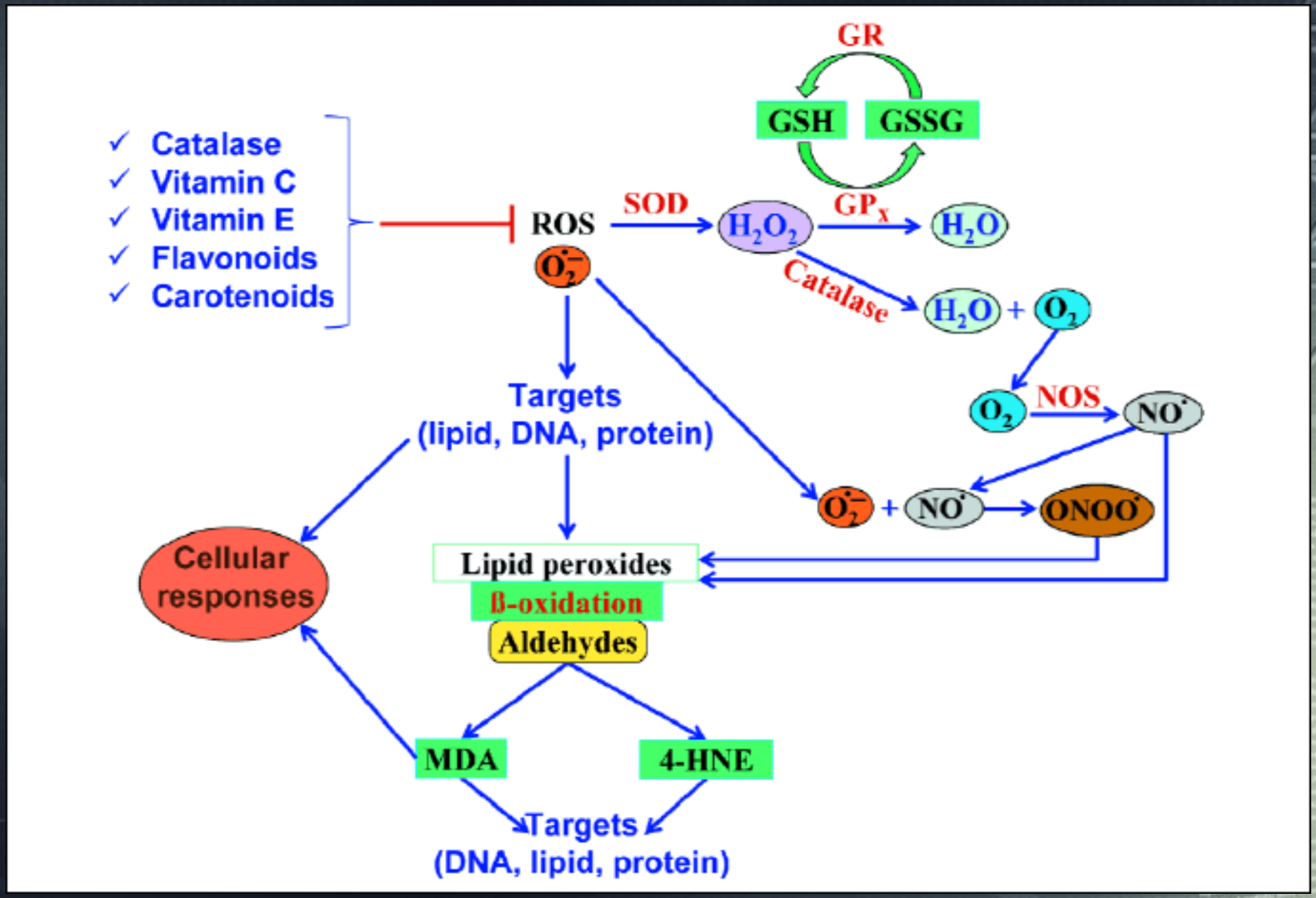
- Ler a absorvância a 532 nm e 600 nm e aplicar a equação (ε [MDA] = 155 mM^{-1} cm^{-1}):

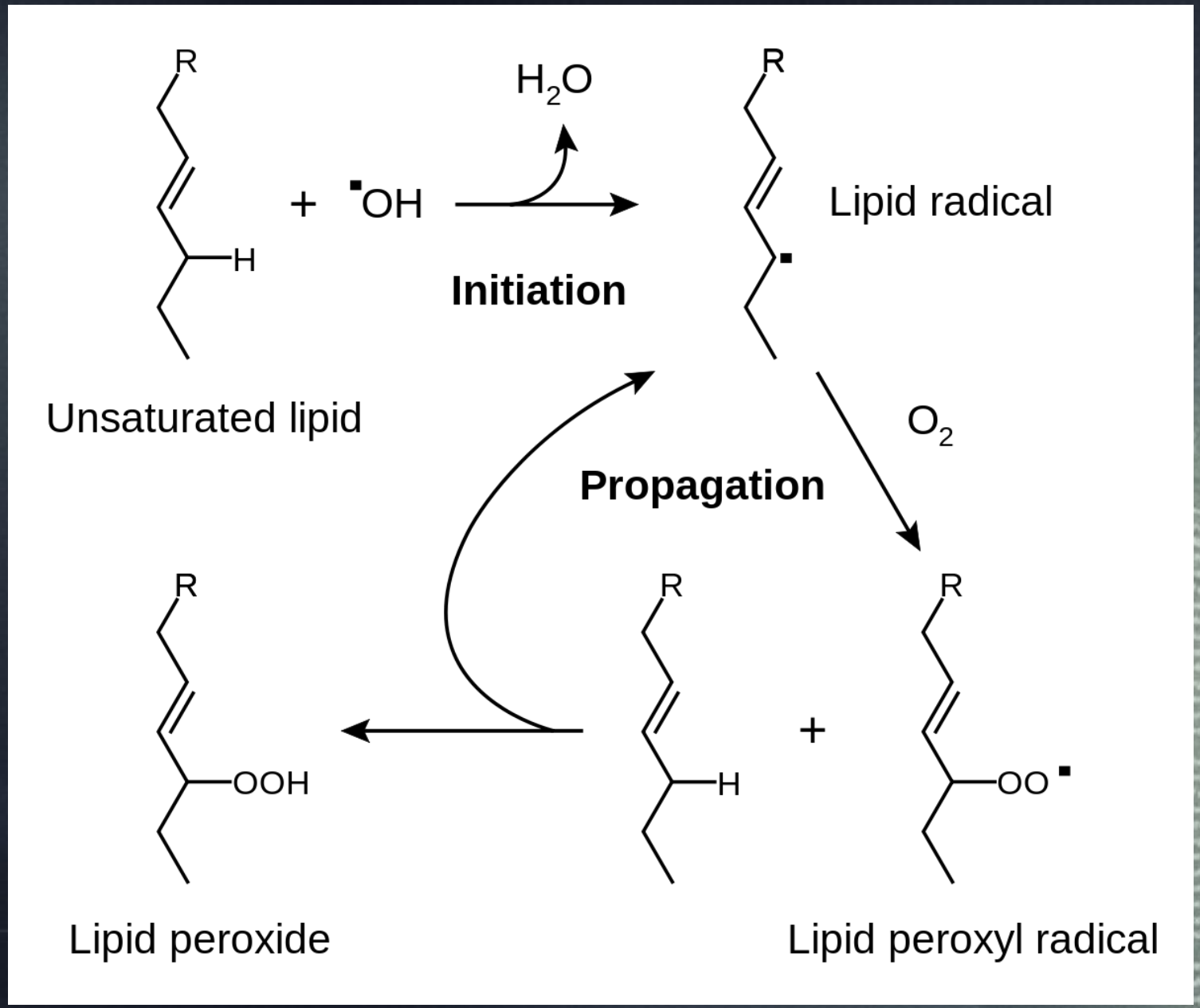
$$A_{532\,nm} - A_{600\,nm} = [MDA]mM \times \varepsilon MDA$$

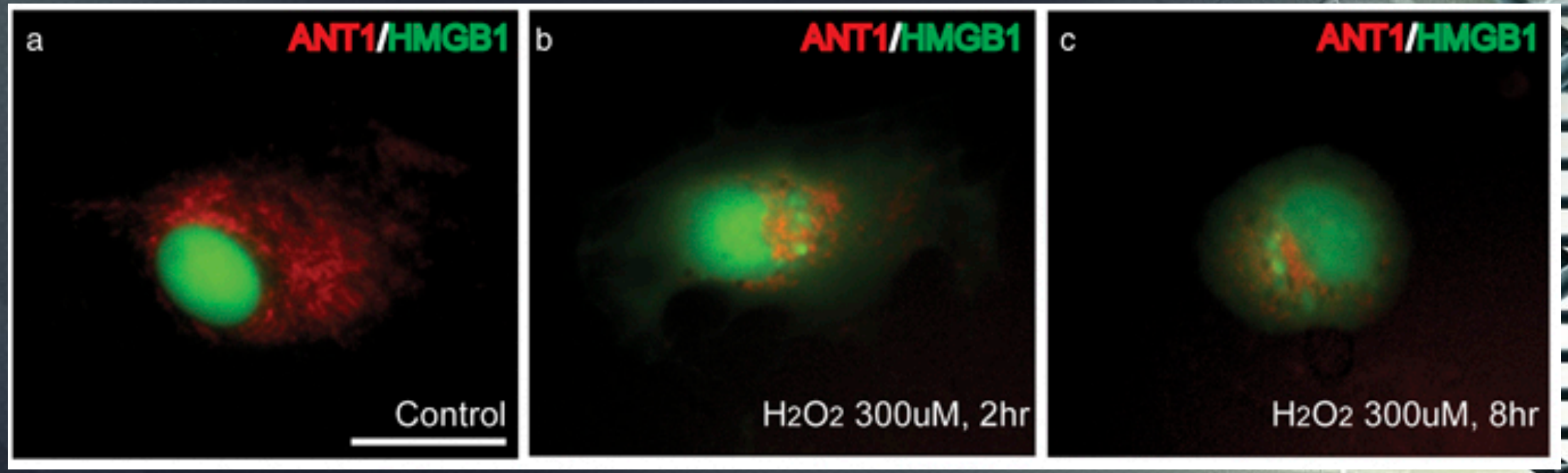

Membrane lipid peroxidation
Cytoplasm
6
1
Cell metabolism
ROS
RNS
Oxidative/ nitrosative damage
REDOX
Carbonylation
Sulfoxidation
Hydroxylation
Nitration
S-Thiolation
2
Proteins
4
5
3
Peroxisomes
Mitochondria
Cytoskeleton
Proteasome
Metabolic disturbance
Impaired protein function
Structural damage and signalling alterations
Energy failure
Altered gene expression
Nucleus
7
Transcriptional factors
mRNA

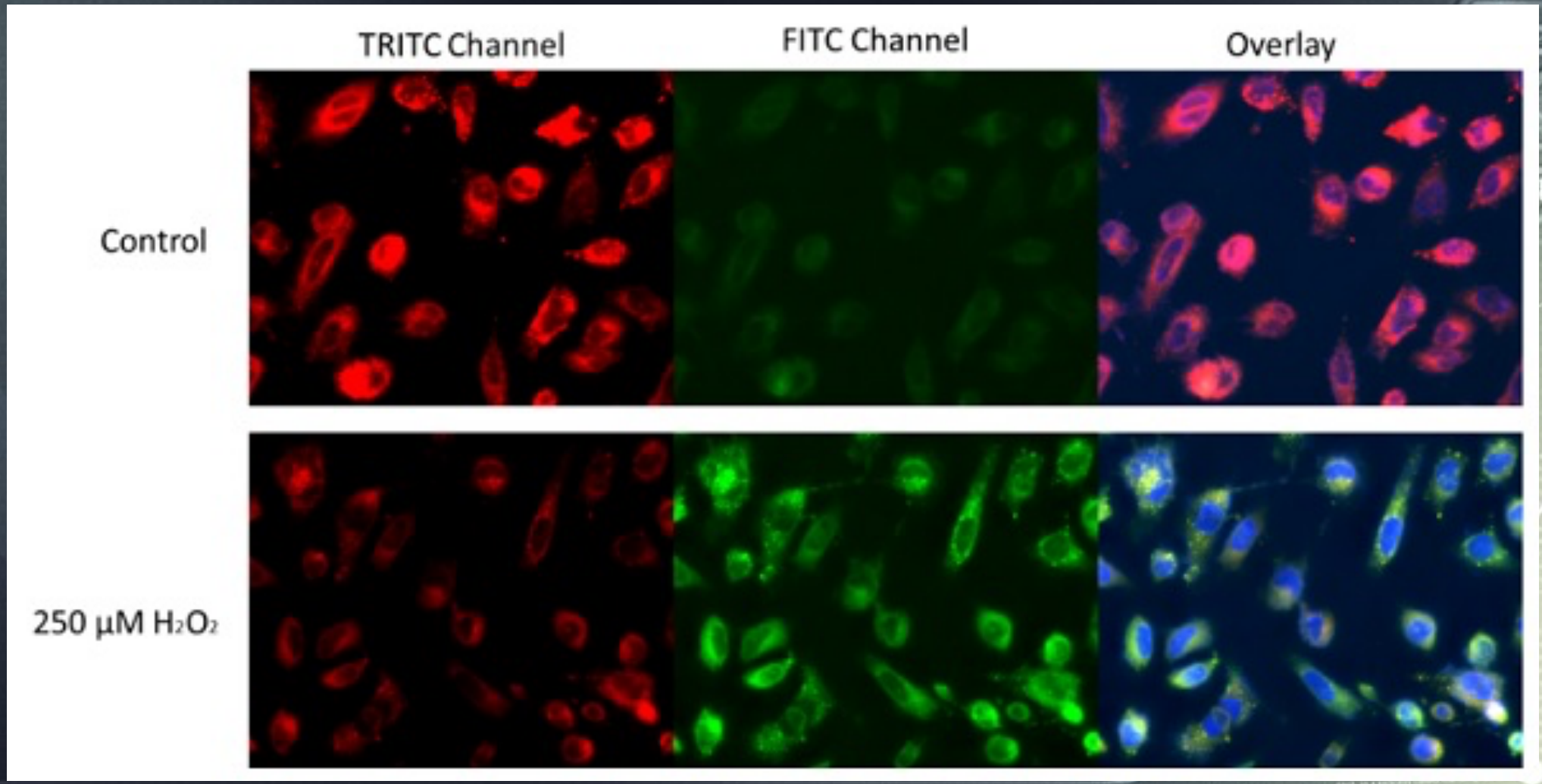

Lipid peroxidation (Malonyldialdehyde, MDA)
Ionizing Radiation
Mitochondria
ROS
UV Light
O-R
O
Linoleate
MDA
OH
HO
N
SH
TBA
+
MDA
S
N
OH
HO
N
SH
N
OH
OH
Colored End Product

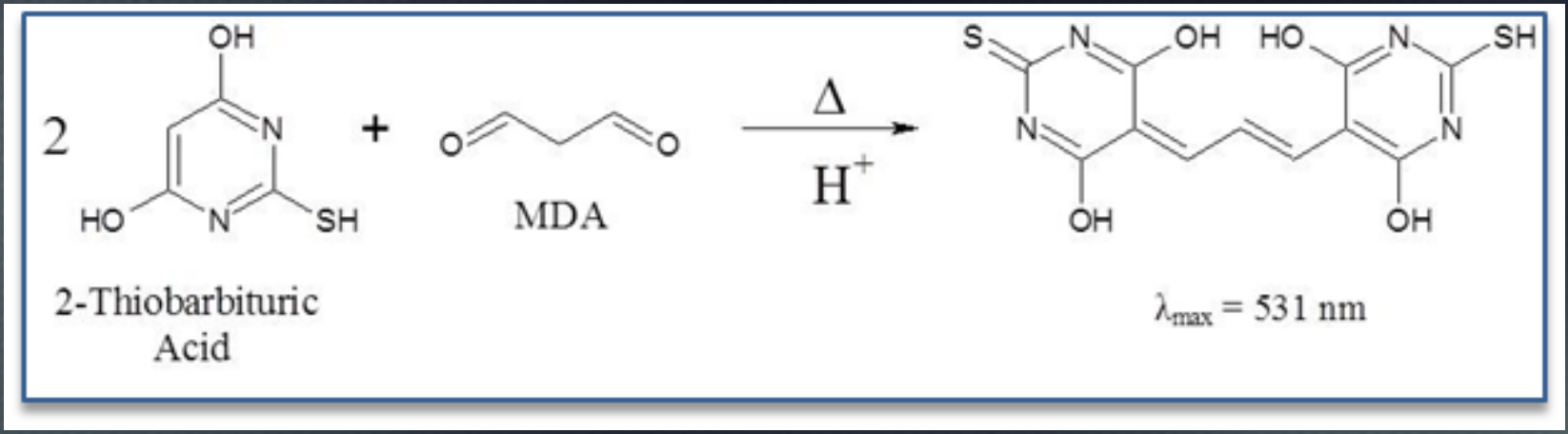

FREE RADICAL TOXICITY
Xenobiotic
R
BIOACTIVATION
•Cytochrome P450
•Prostaglandin Synthase
•Lipoxygenase
R•
Free Radical
COVALENT BINDING
•DNA
•Proteins
•Lipids
OXIDATIVE DAMAGE
•Mutagenesis
•Carcinogenesis
•Teratogenesis
•Enzymatic Damage
INITIATION
LH
Lipid Membrane
GSH
GSSG
Glutathione Peroxidase
SOD
H2O2
HO•
Fe2+/3+
H2O
Catalase
H2O
O2
L•
Lipid Radical
PROPAGATION
LH
GSH
GSSG
LOO•
Lipid peroxy Radical
LOOH
VITAMIN E, C
STABLE VITAMIN RADICAL
TERMINATION
LOOH
Lipid Hydroperoxide
+
L•
Lipid Radical
Glutathione Peroxidase
GSH
GSSG
LOH
Stable Lipid Alcohol
GSH Reductase
NADPH
NADP+
Glucose-6-Phosphate Dehydrogenase
•Lipid Radicals
•Aldehydes
•Other Reactive Products
Proximal Membrane Damage
Distal Tissue Damage

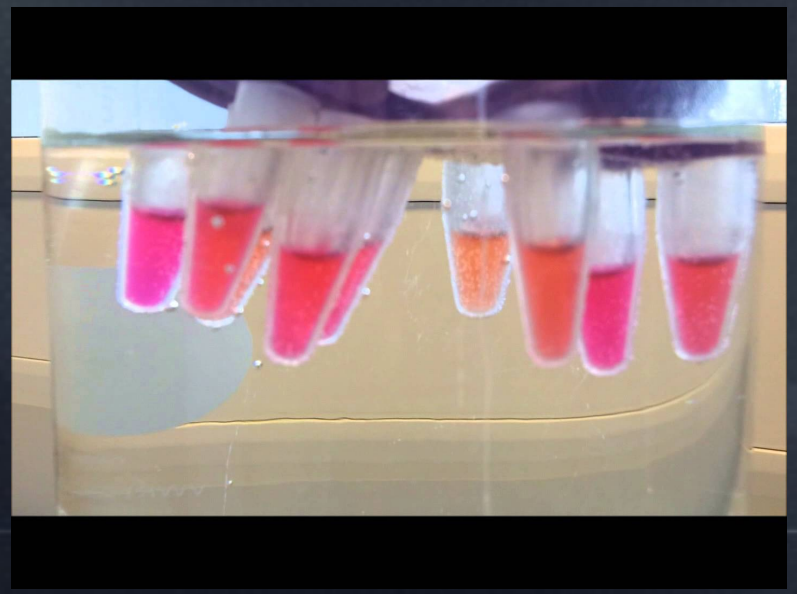

GR
GSH
GSSG
✓ Catalase
✓ Vitamin C
✓ Vitamin E
✓ Flavonoids
✓ Carotenoids
ROS
SOD
H_2O_2
GP_x
H_2O
$O_2^{\cdot-}$
Catalase
$H_2O + O_2$
Targets
(lipid, DNA, protein)
O_2
NOS
NO˙
$O_2^{\cdot-} + NO^{\cdot}$
ONOO˙
Cellular responses
Lipid peroxides
ß-oxidation
Aldehydes
MDA
4-HNE
Targets
(DNA, lipid, protein)

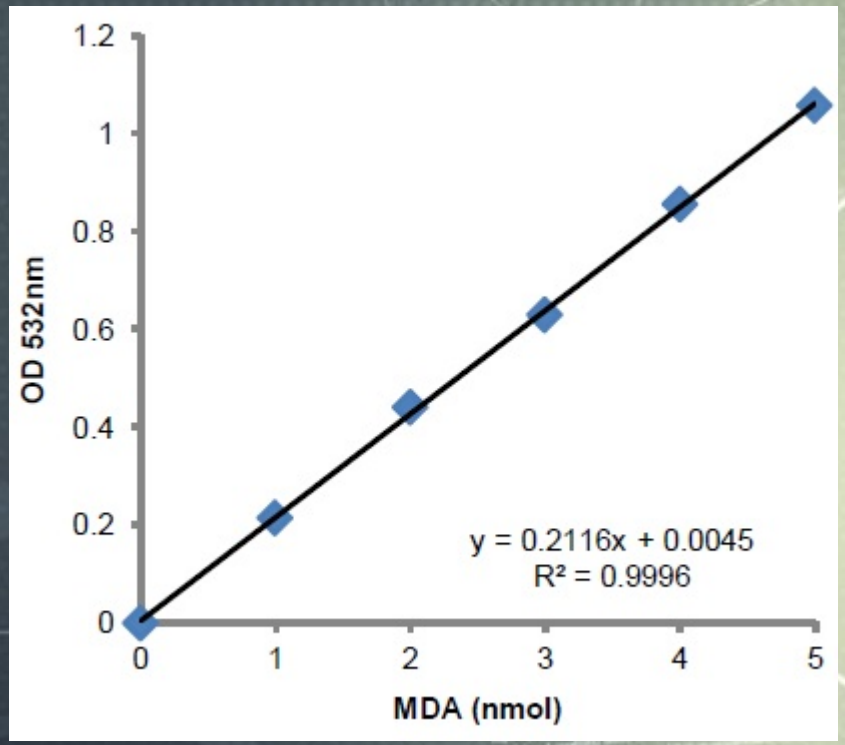

R
H
+ ·OH
H2O
Initiation
R
Lipid radical
Unsaturated lipid
O2
Propagation
R
OOH
Lipid peroxide
R
H
+
R
OO·
Lipid peroxyl radical


a
ANT1/HMGB1
Control
b
ANT1/HMGB1
H2O2 300uM, 2hr
c
ANT1/HMGB1
H2O2 300uM, 8hr


TRITC Channel
FITC Channel
Overlay
Control
250 µM H_2O_2


2
OH
N
HO
N
SH
+
O
O
MDA
Δ
H+
S
N
OH
HO
N
SH
N
N
OH
OH
2-Thiobarbituric Acid
λmax = 531 nm




1.2
1
0.8
0.6
0.4
0.2
0
OD 532nm
0
1
2
3
4
5
MDA (nmol)
y = 0.2116x + 0.0045
R² = 0.9996