Interactions between light and matter - Light for medicine

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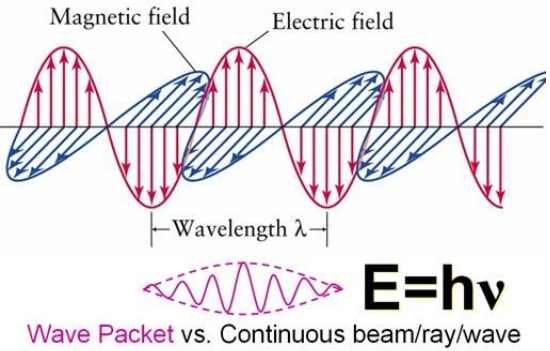
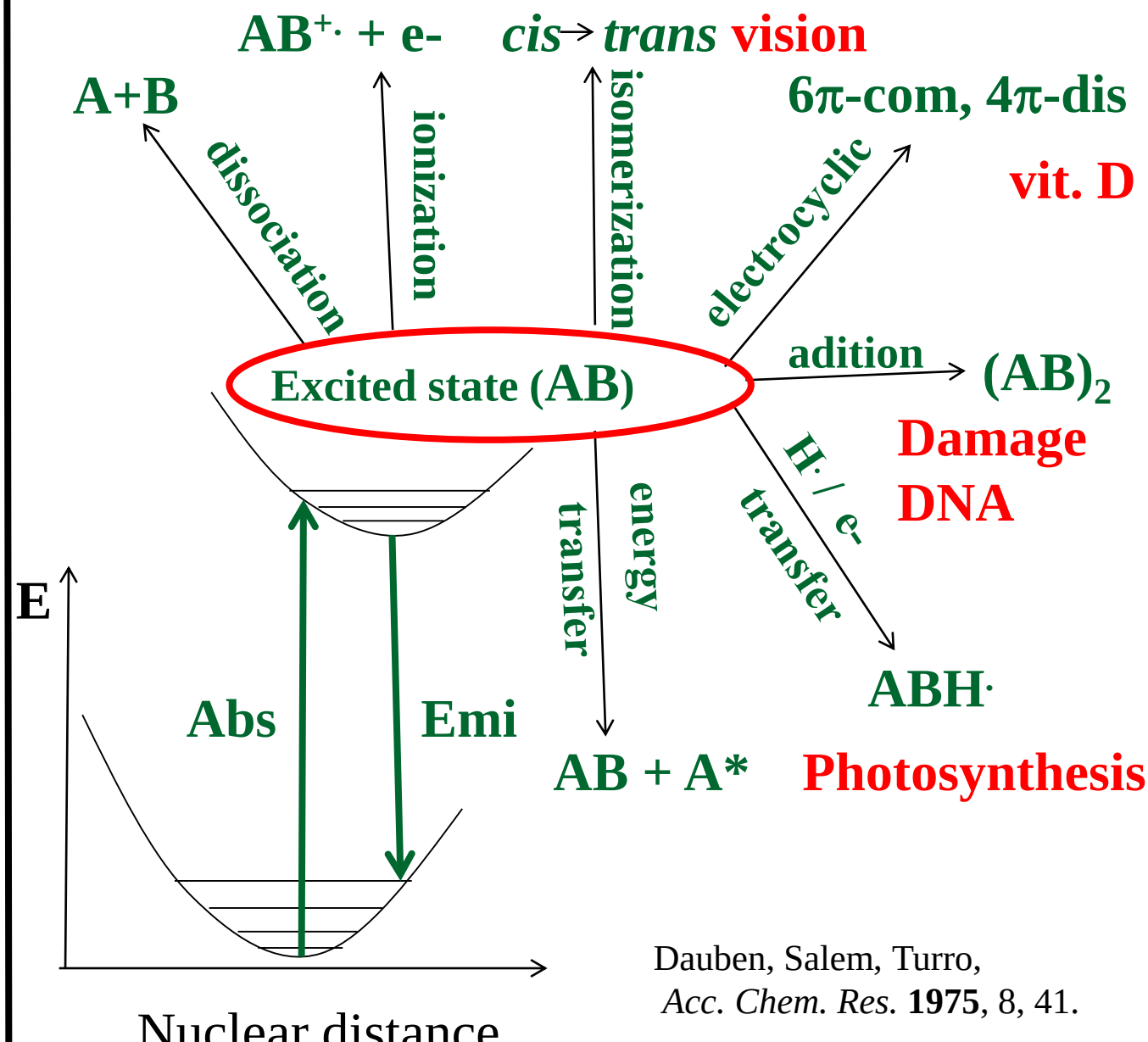
FAPESP

PhotoTech
The USP Research Consortium for Photochemical Technology

Photons



Excited states: ↑reactivity



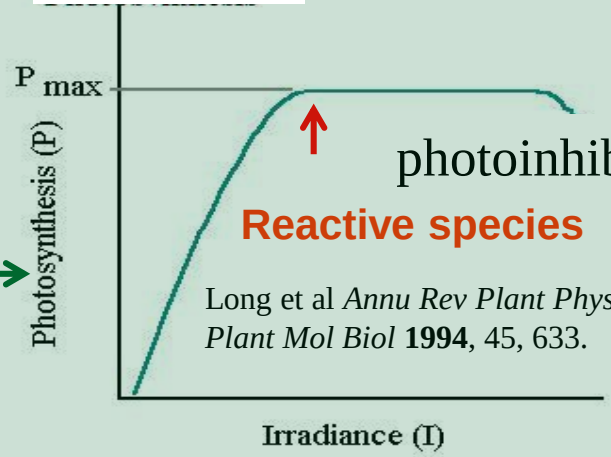
Dauben, Salem, Turro,
Acc. Chem. Res. **1975**, 8, 41.

Photosynthesis

Food chain

Bioenergy

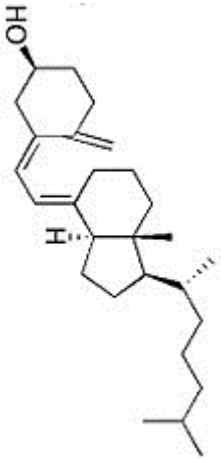
Efficiency



Long et al *Annu Rev Plant Physiol Plant Mol Biol* **1994**, 45, 633.

Food oxidation

Health of skin and body: Vitamin D



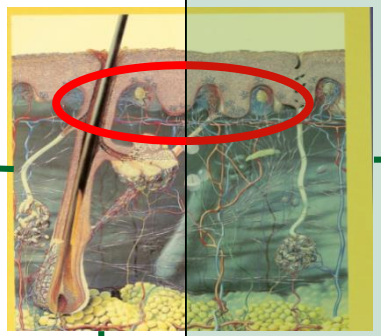
7-dehidrocolesterol

↓ UVB

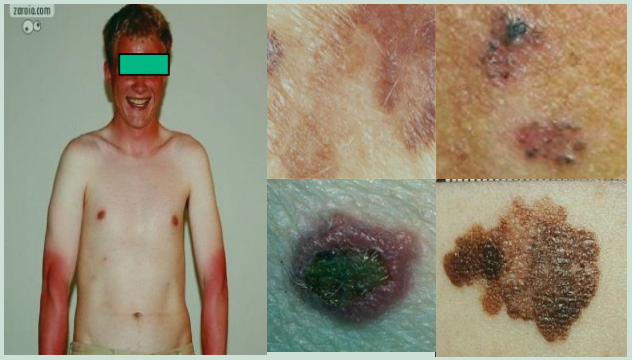
Pre-vitamin D3

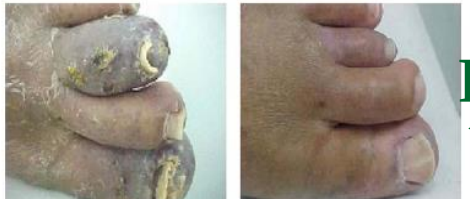
↓ [1,7]-H migration

Vitamin D3



Photoaging, cancer

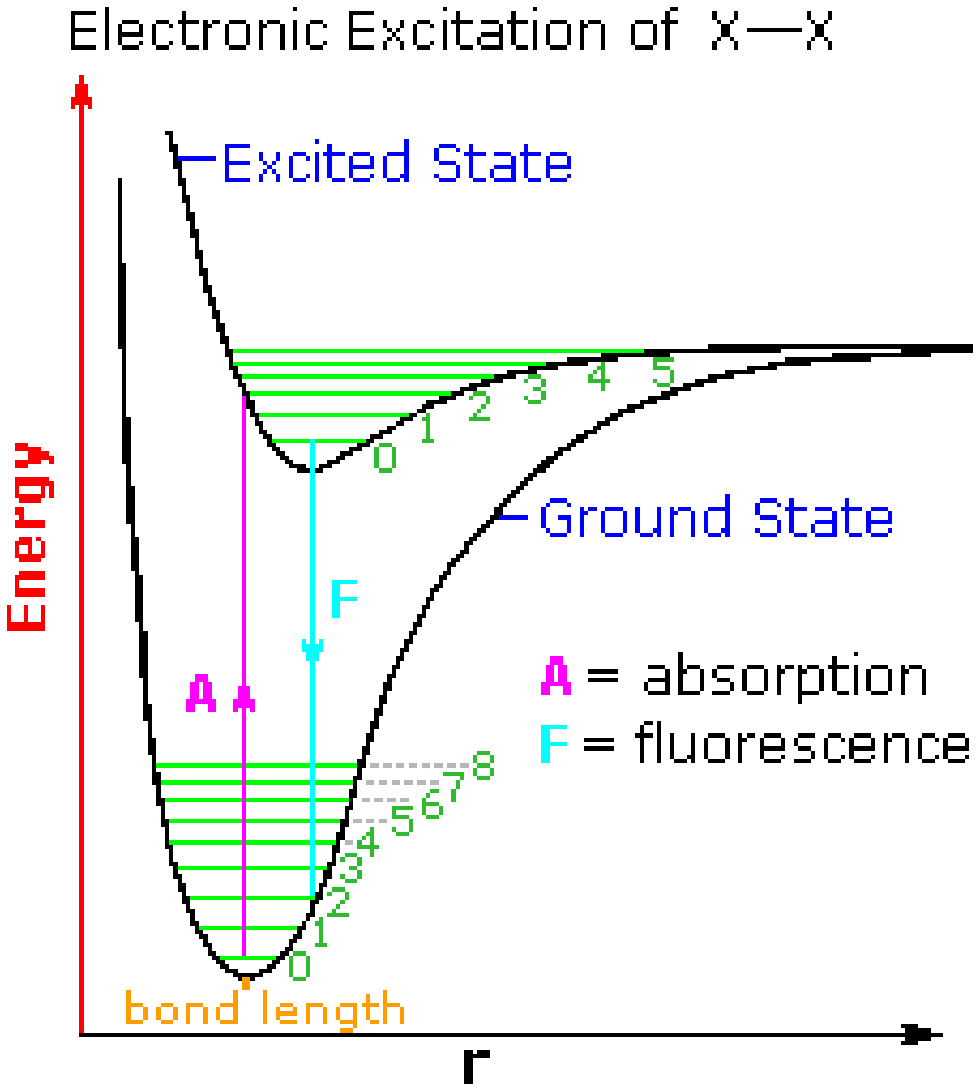




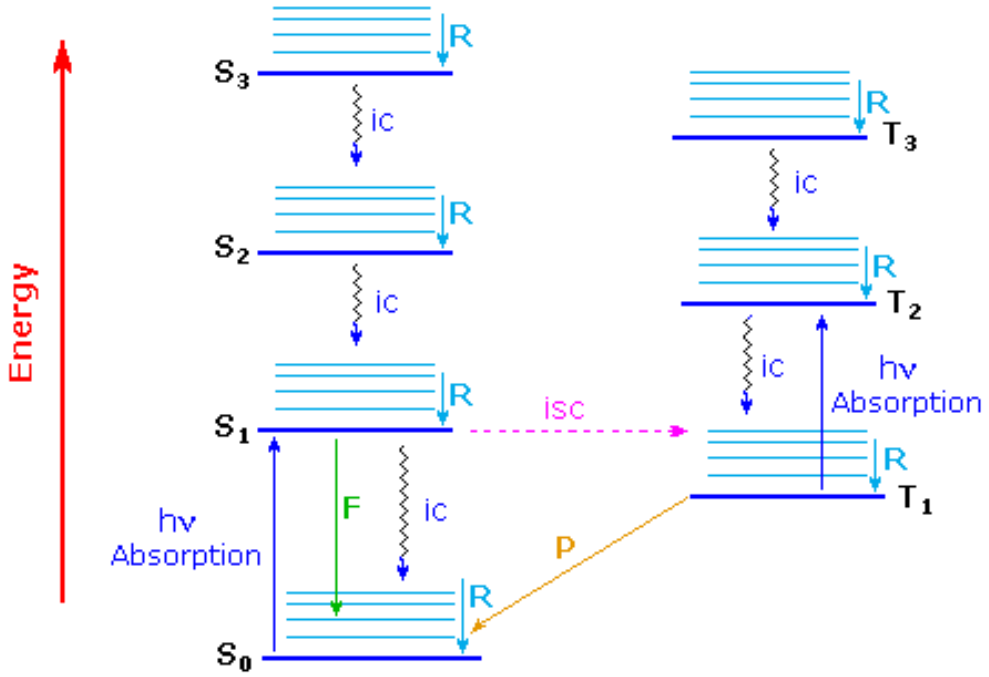
Photomedicine

The dark side of the sun

Light absorption



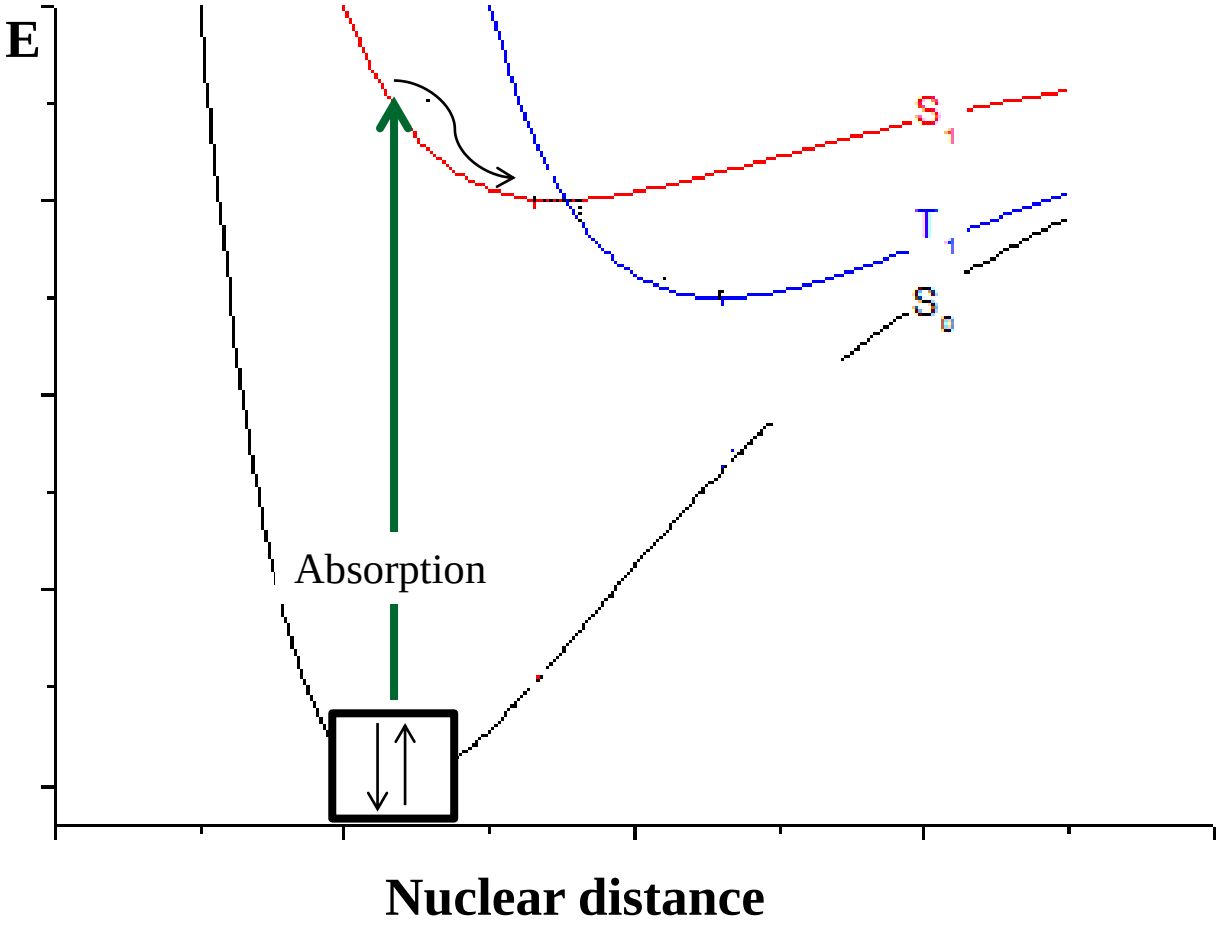
A Jablonski Diagram



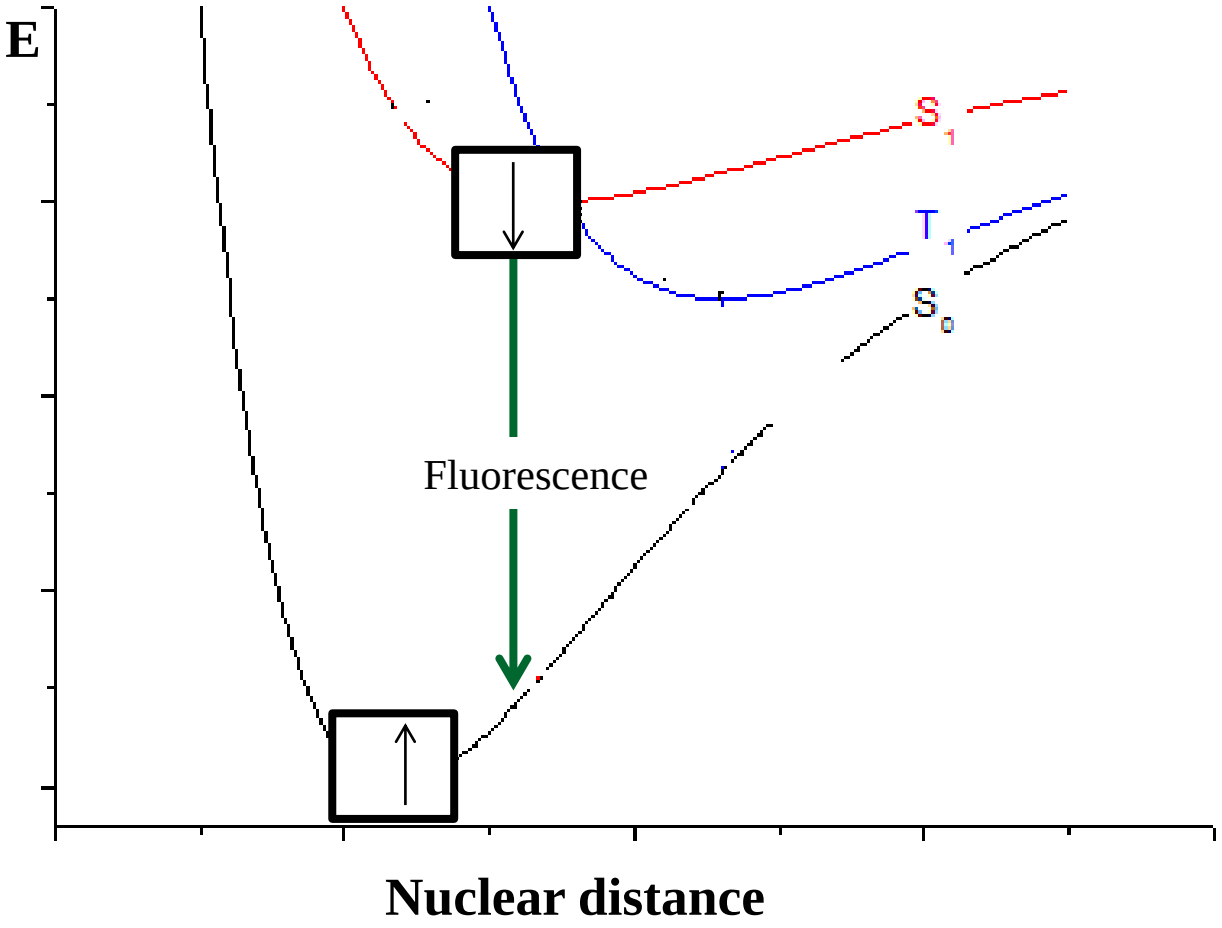
S = singlet state
T = triplet state
ic = internal conversion
isc = intersystem crossing
R = relaxation
F = fluorescence
P = phosphorescence

Process	Transition	Timescale (sec)
Light Absorption (Excitation)	$S_0 \rightarrow S_n$	ca. 10^{-15} (instantaneous)
Internal Conversion	$S_n \rightarrow S_1$	10^{-14} to 10^{-11}
Vibrational Relaxation	$S_n^* \rightarrow S_n$	10^{-12} to 10^{-10}
Intersystem Crossing	$S_1 \rightarrow T_1$	10^{-11} to 10^{-6}
Fluorescence	$S_1 \rightarrow S_0$	10^{-9} to 10^{-6}
Phosphorescence	$T_1 \rightarrow S_0$	10^{-3} to 100
Non-Radiative Decay	$S_1 \rightarrow S_0$ $T_1 \rightarrow S_0$	10^{-7} to 10^{-5}

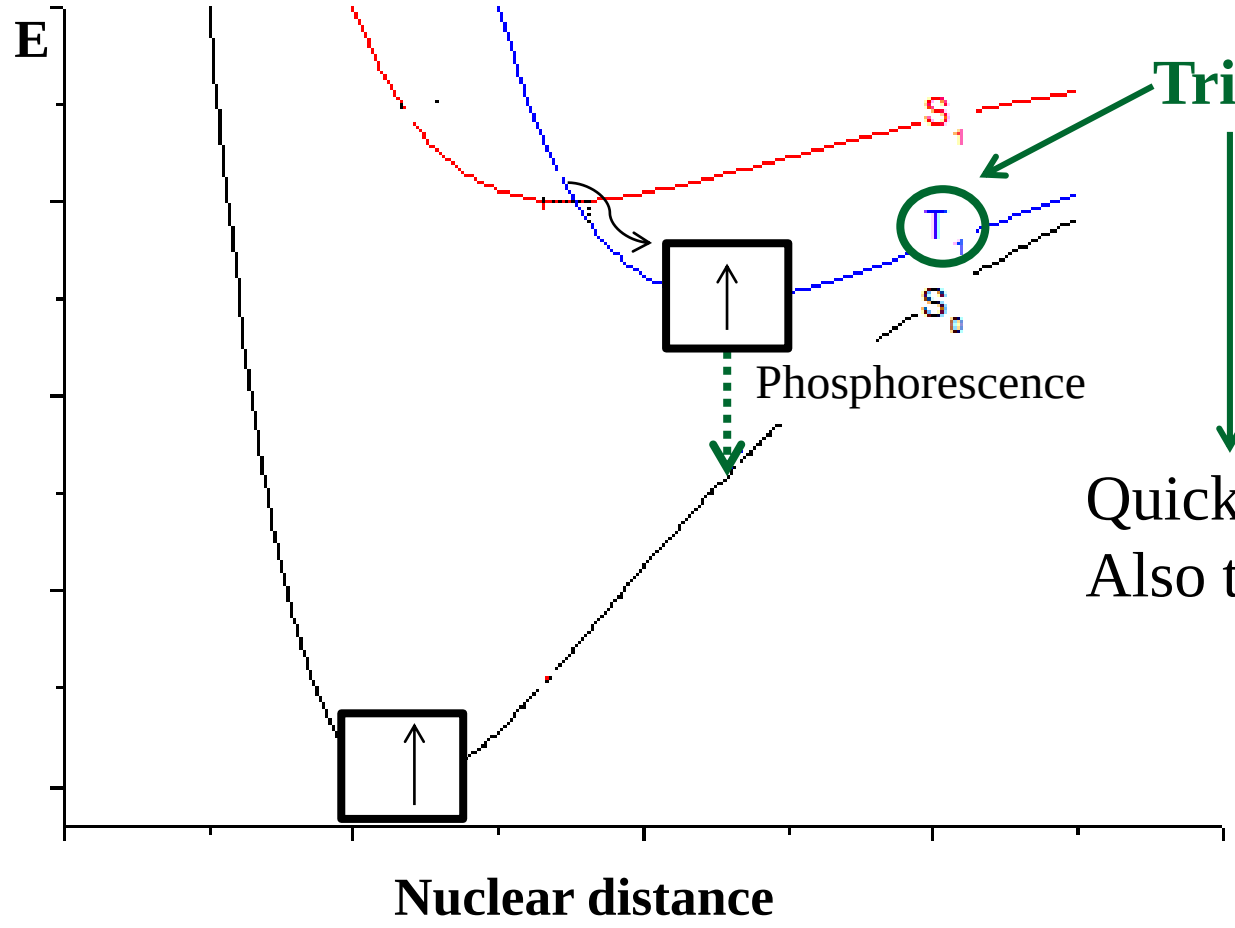
The role of the triplet species in photosensitization processes: absorption



The role of the triplet species photosensitization processes: Singlet state



The role of the triplet species photosensitization processes: Intersystem crossing



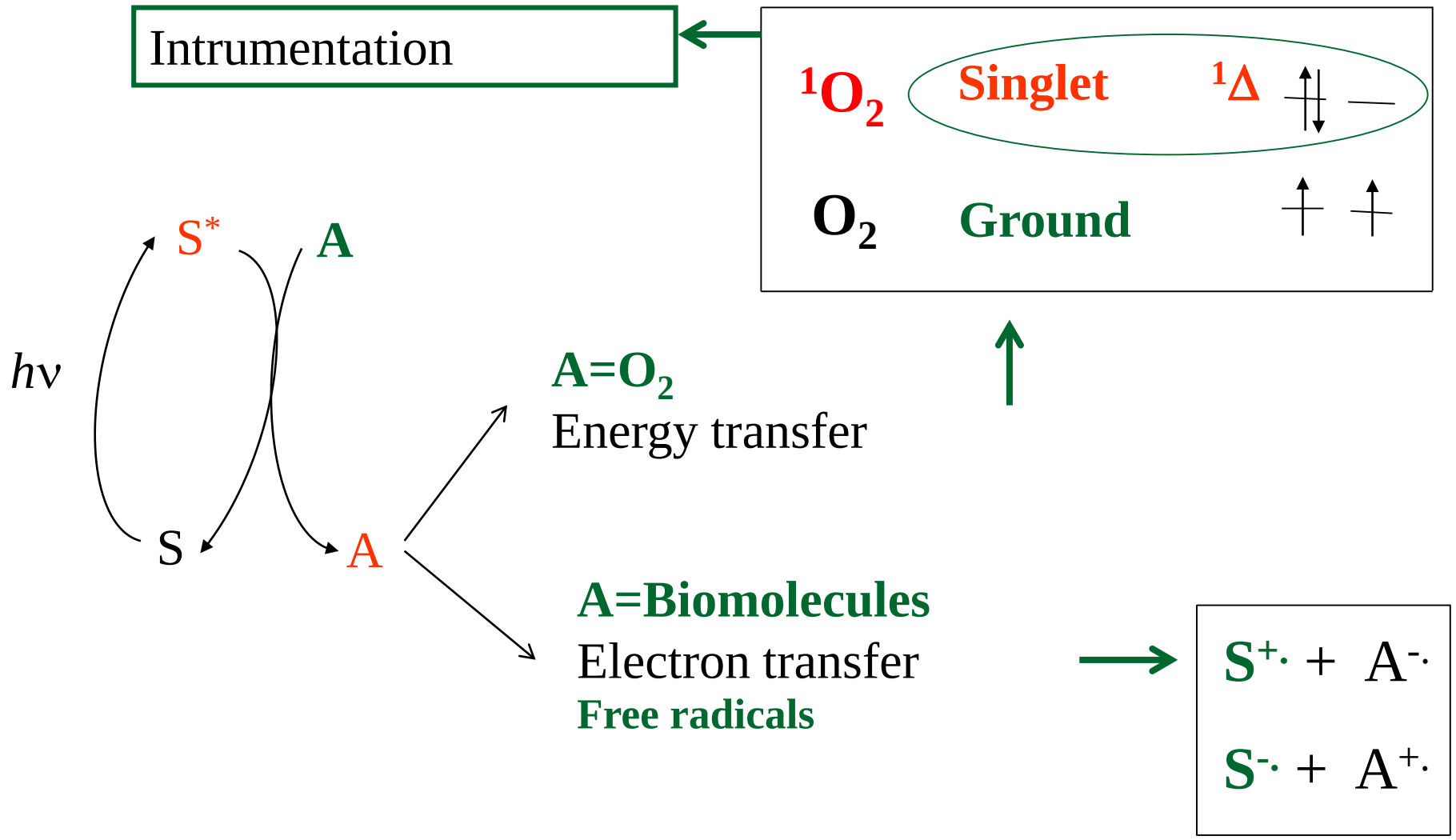
Triplet

Lives longer and is reactive

Quickly reacts with O_2

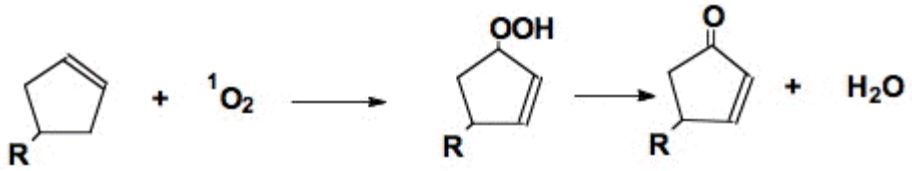
Also transfer of e^-, e^-/H^+, $H\cdot$

During the process of **photosensitization** light energy absorbed by the photosensitizer (S) is transferred to acceptor (A) and "S" returns to the ground state and can start another cycle of photosensitization

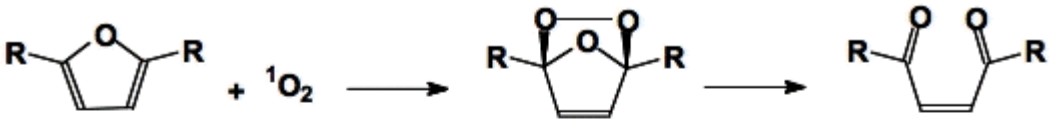


Singlet oxygen reacts with double bonds

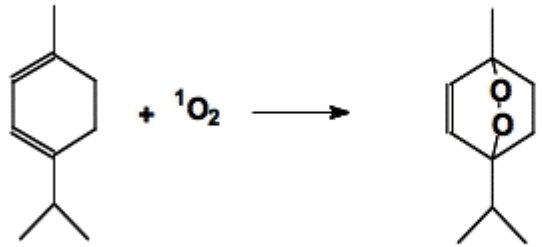
1. Ene reaction



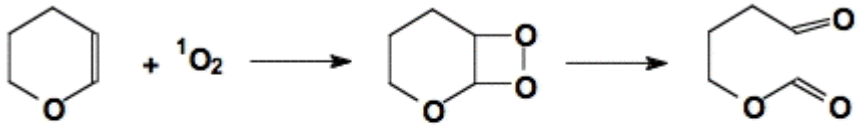
2. Diels-Alder Type Addition



1:4 addition



3. Addition to Activated Double Bonds



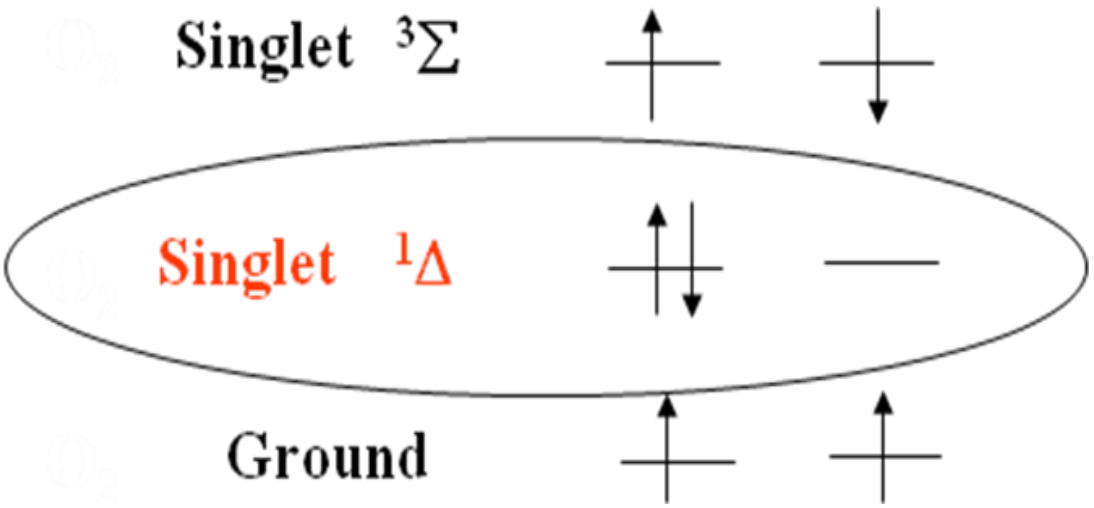
Single activated double bonds are also susceptible to addition of $^1O_2$

4. Electron Transfer:

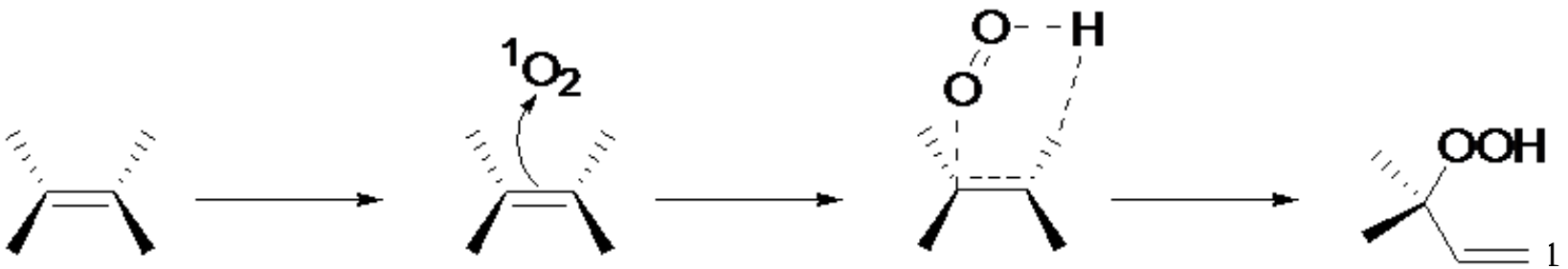
$$^1O_2 + e^- \longrightarrow O_2^{\cdot -}$$

One-electron reduction potential of $^1O_2$ to form superoxide is +650 mV

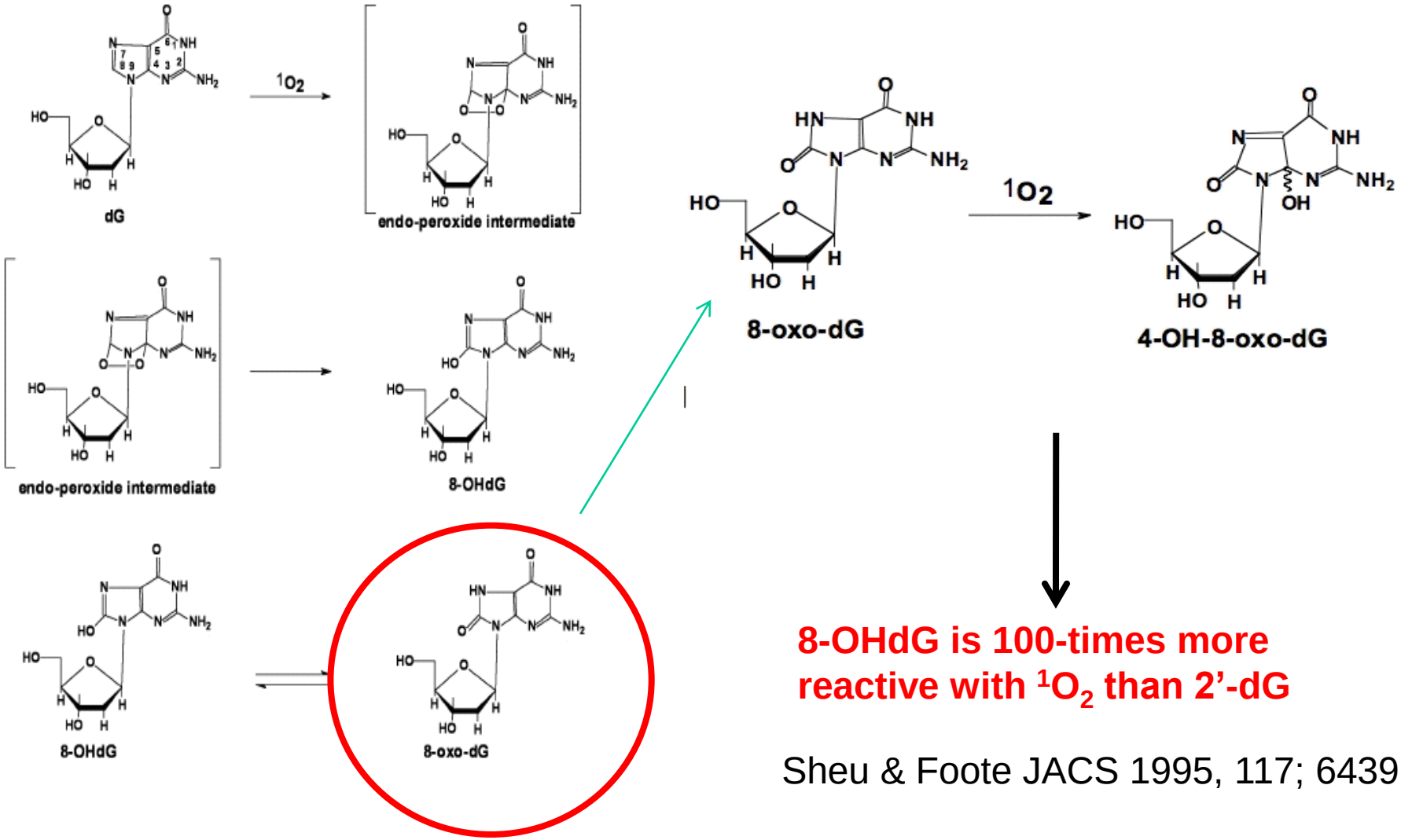
Singlet oxygen



Eletrophilic attack to double bonds



DNA and $^1O_2$

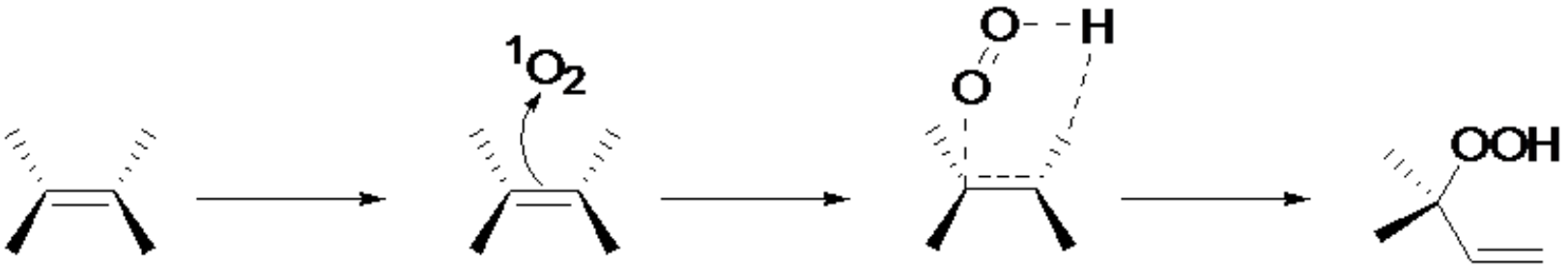


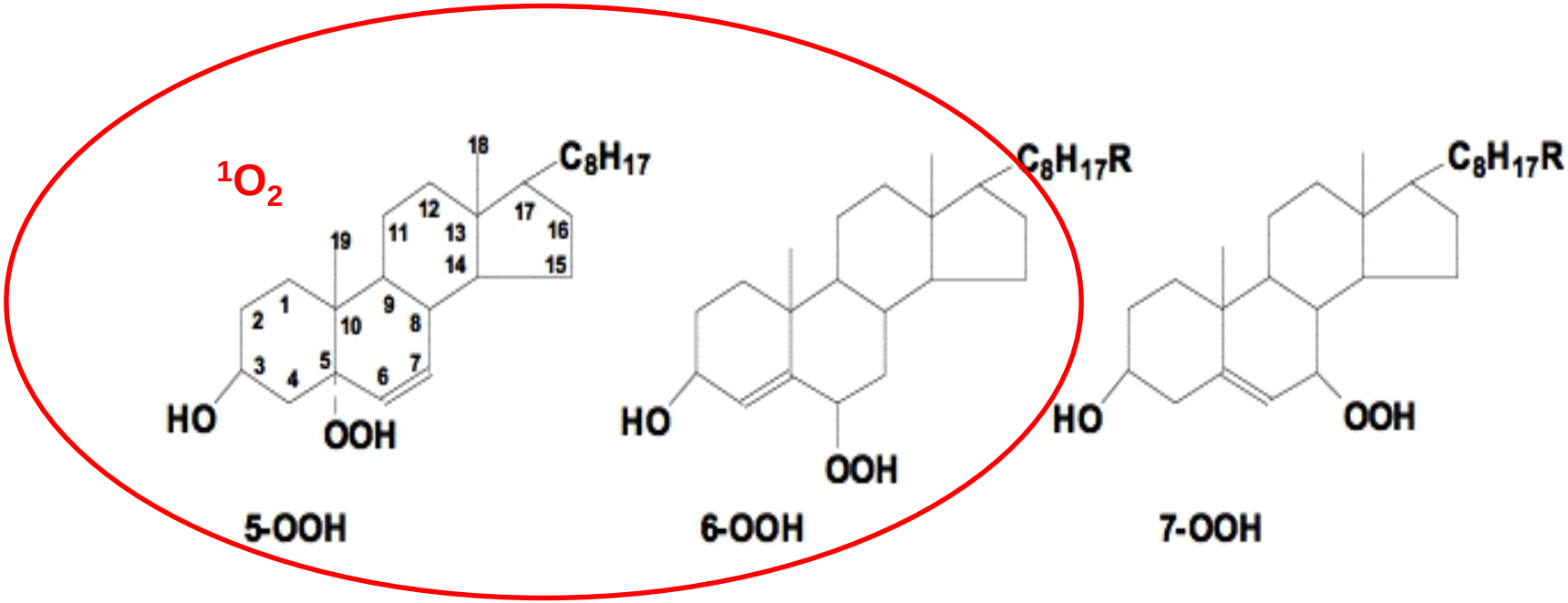
8-OHdG is 100-times more reactive with $^1O_2$ than 2'-dG

Sheu & Foote JACS 1995, 117; 6439.

7,8-dihydro-8-oxo-2'-deoxyguanosine

Lipids and $^1O_2$

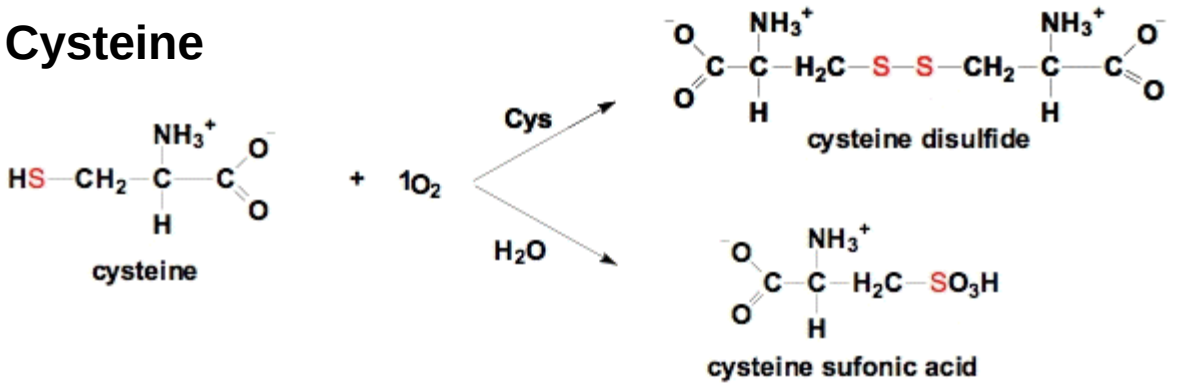




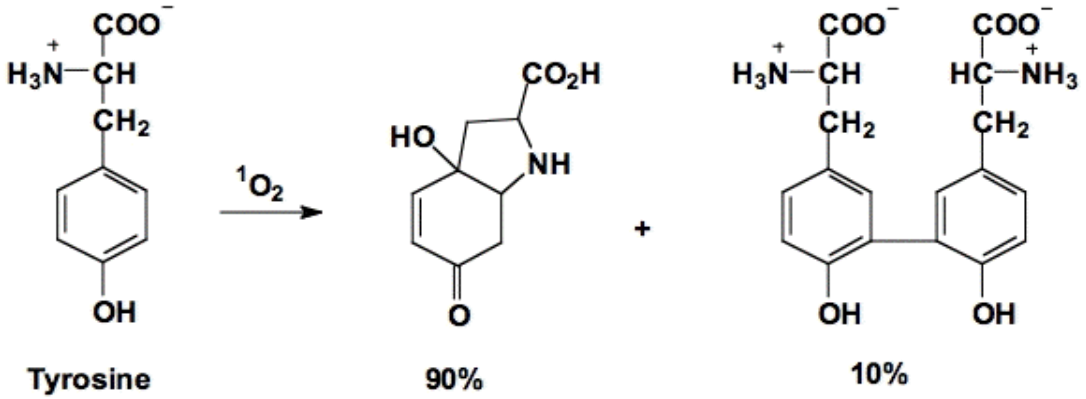
Proteins and $^1O_2$

Cysteine, Tyrosine, Tryptophan, Methionine, Histidine

Cysteine

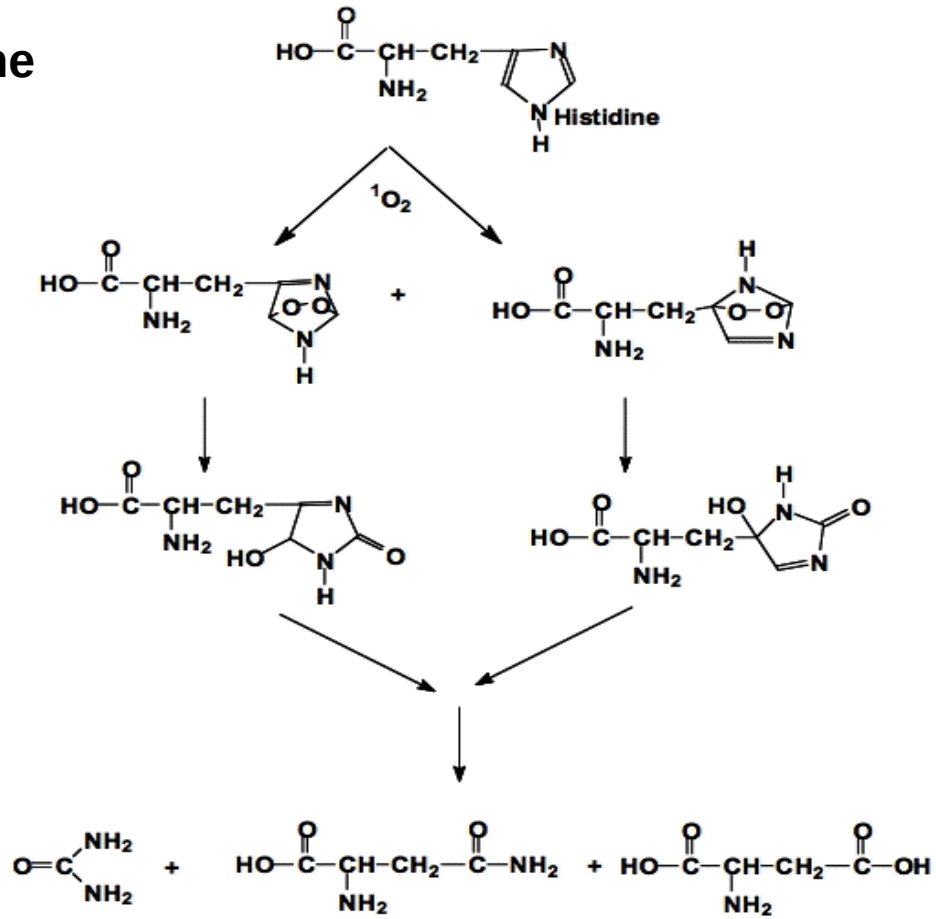


Tyrosine



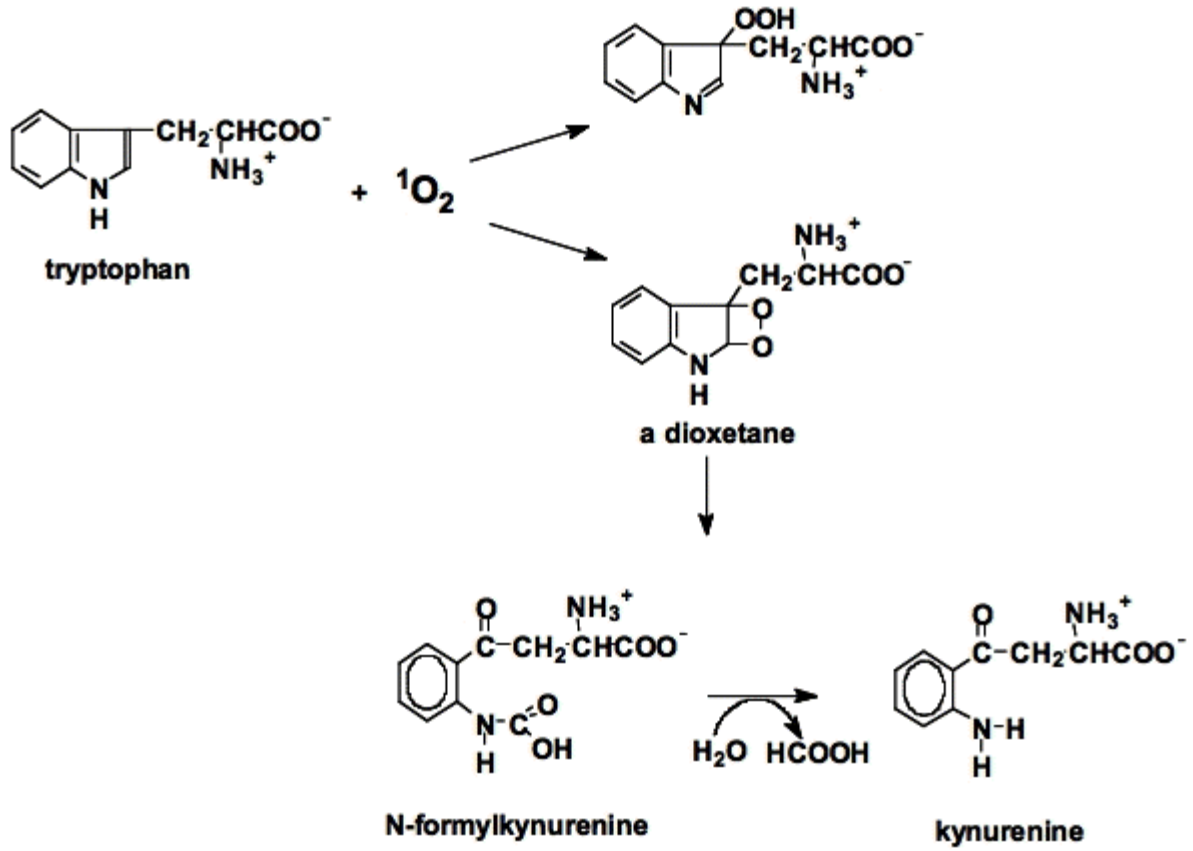
Proteins and $^1O_2$

Histidine



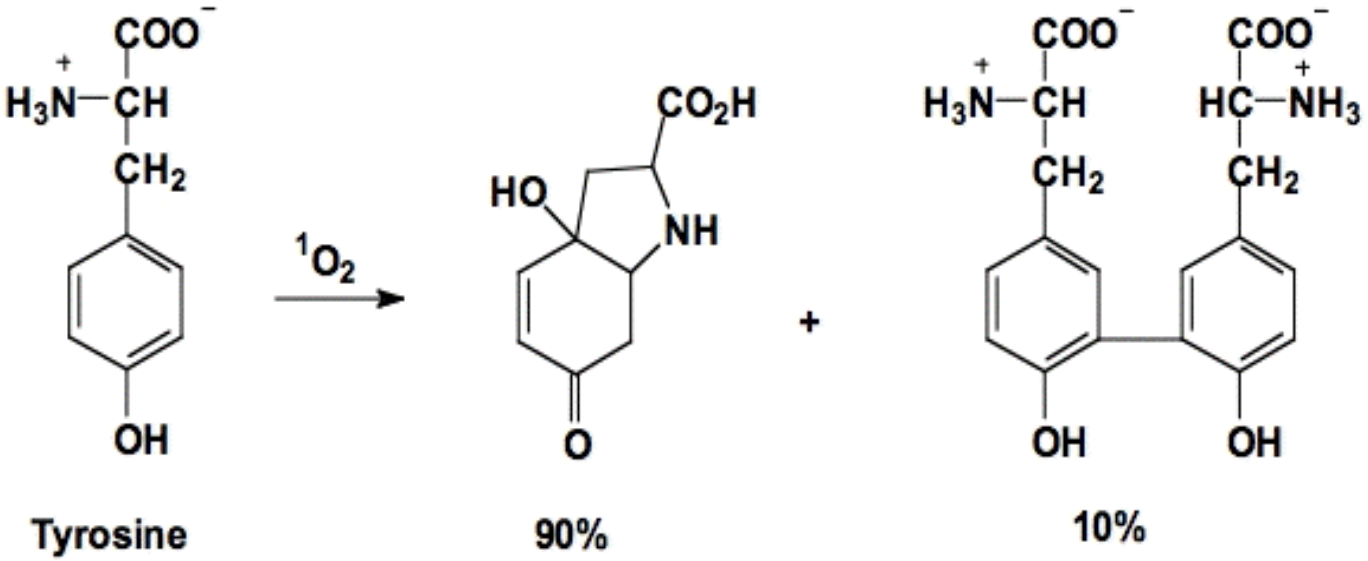
Proteins and $^1O_2$

Tryptophan



Proteins and $^1O_2$

Tyrosine



ESPÉCIES REATIVAS DE OXIGÊNIO (EROs)
ESPÉCIES REATIVAS DE NITROGÊNIO (ERNs)

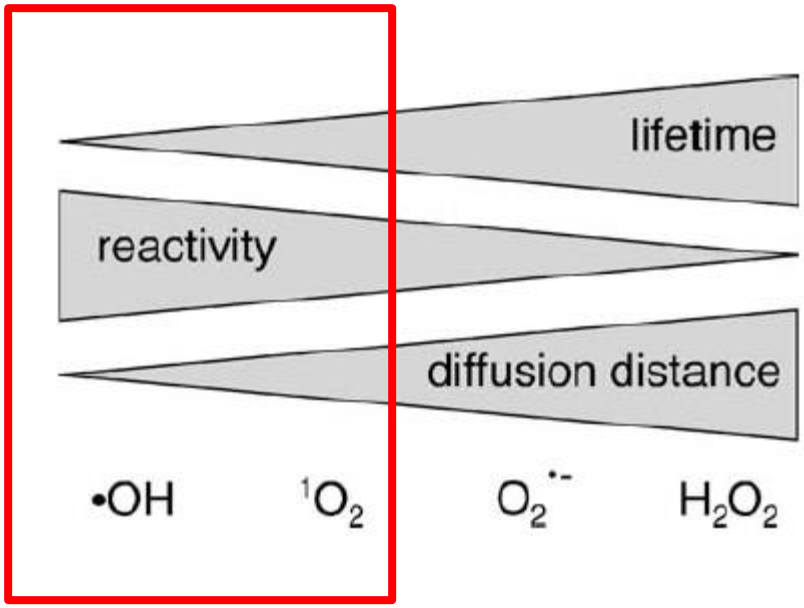
Oxigênio Excitado $^1\Delta$ — $^1O_2$ (Adiciona na dupla ligação)

Oxigênio Fundamental — O_2 —(e)→ Ânion radical super-oxido $O_2^{\cdot -}$

$O_2^{\cdot -}$ —(e, $2H^+$)→ H_2O_2 Peróxido de hidrogênio

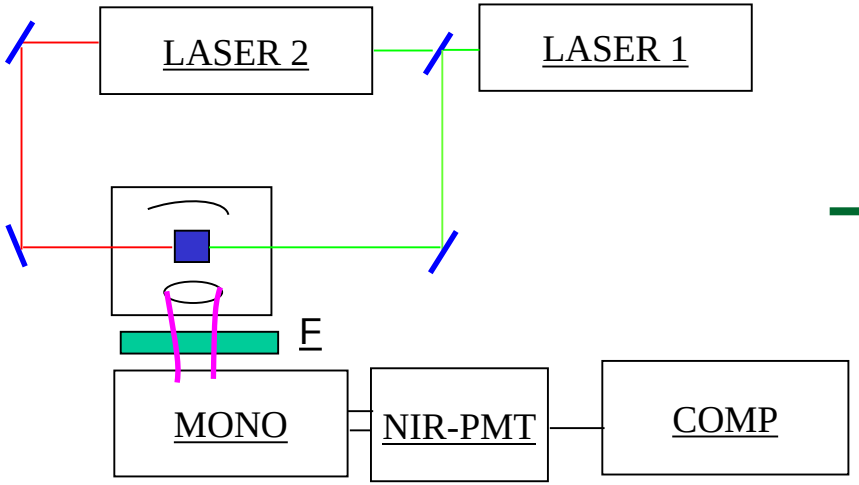
H_2O_2 —(Fe^{2+}, Fe^{3+})→ (Abstrai H) $OH^{\cdot}$ Radical Hidroxila

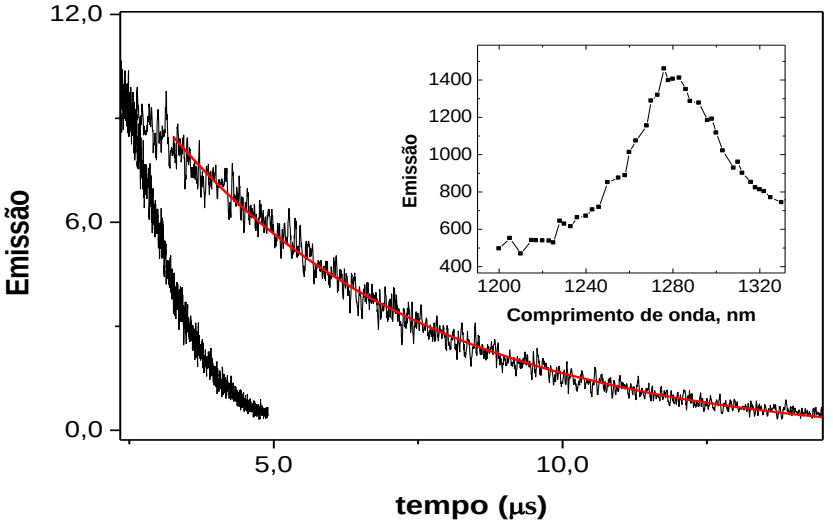
$O_2^{\cdot -}$ —($NO^{\cdot}$)→ Peroxidonitrito $OONO^-$ —(H^+)→ $OH^{\cdot} + NO_2$



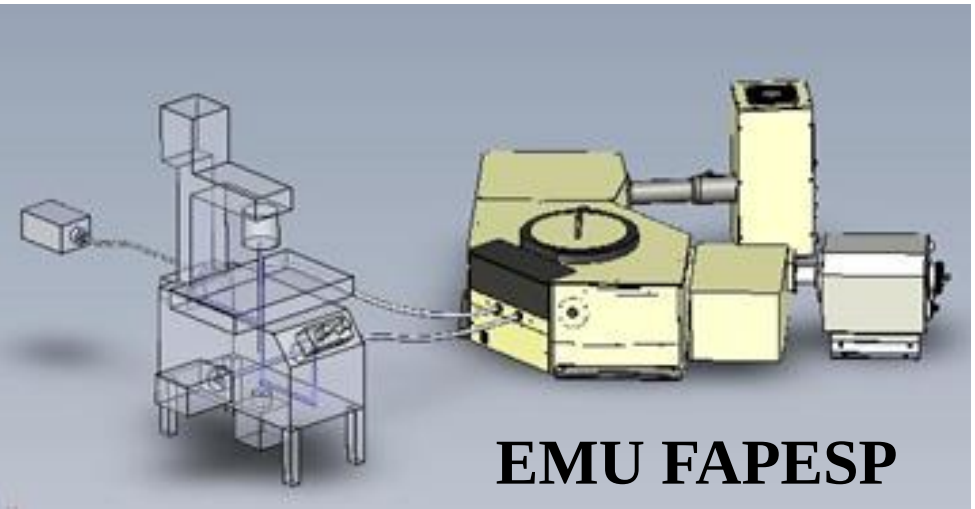
How can we make sure singlet oxygen is being produced?

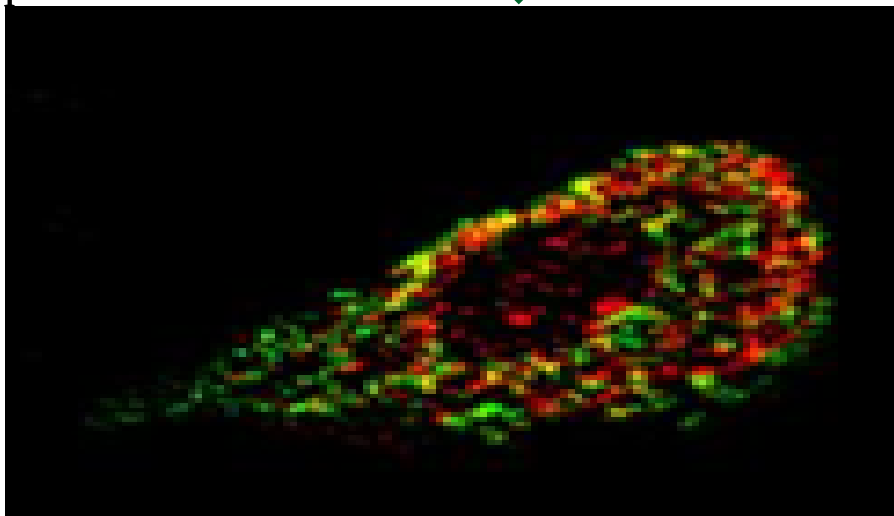
Instrumentation development do characterize and quantify $^1O_2$

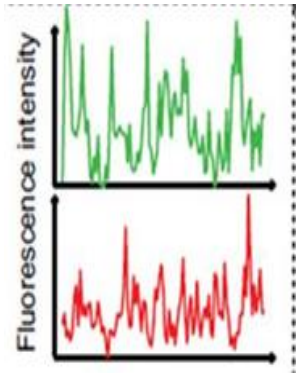




P. Di Mascio, M. Madeiros, S. Miyamoto
+ 20 pesquisadores

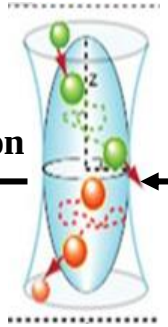




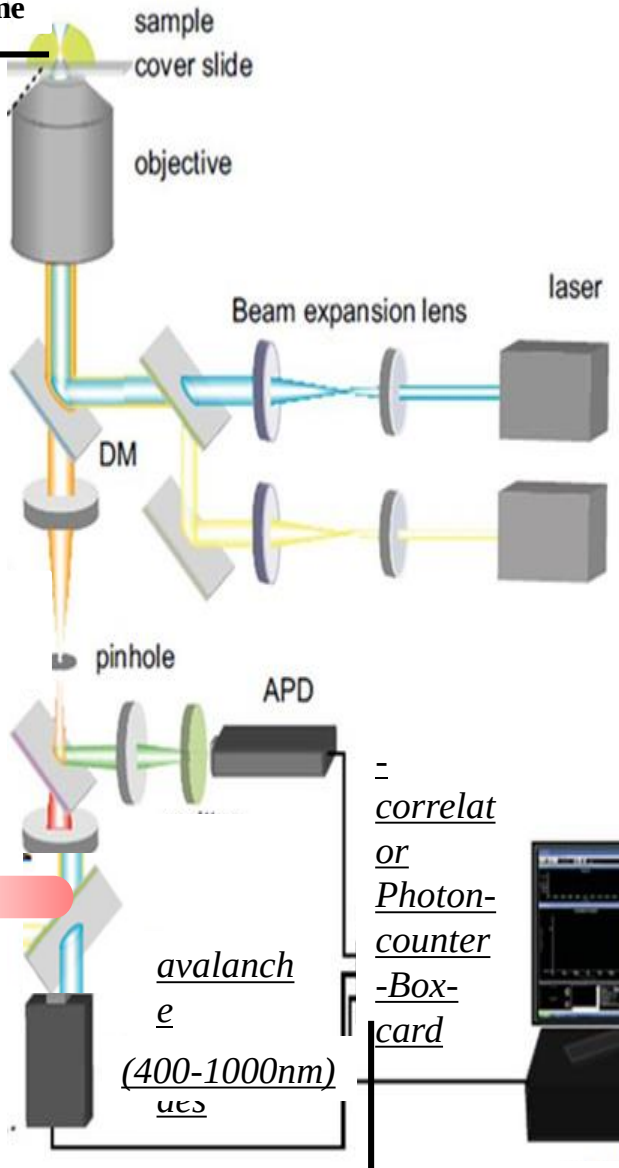


Fluorescence correlation

spectroscopy (FCS)

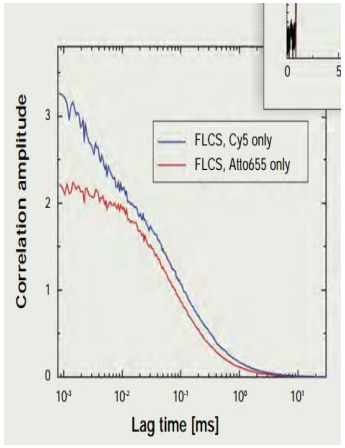


Confocal volume



Diffusion of fluorophores

Reactivity and lifetime of triplet



Fluorescence microscopy and phosphorescence

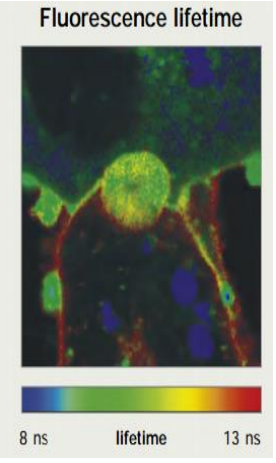
time-resolved (ps, ns, us, ms)

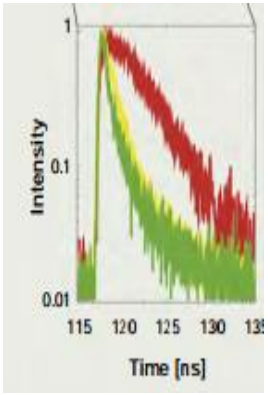
FLIM, FRET, FRAP, Glow in the NIR.

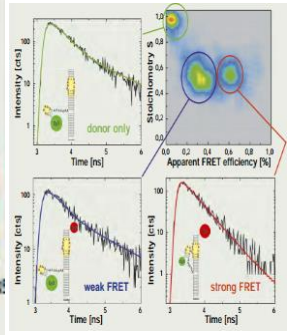
Intermediates generated by light

New probes and new markers for oxidative stress

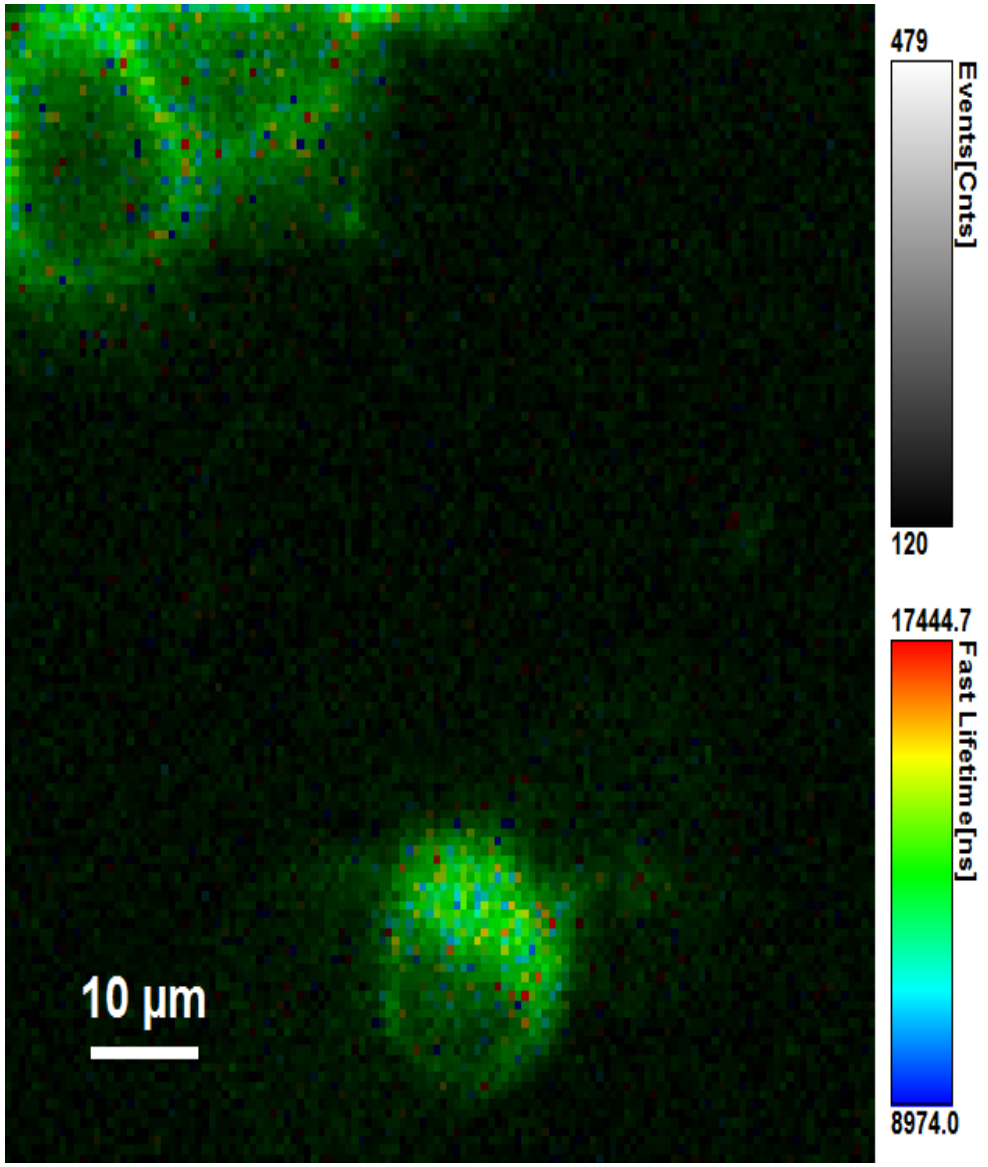
Molecular partners in the signaling cascade







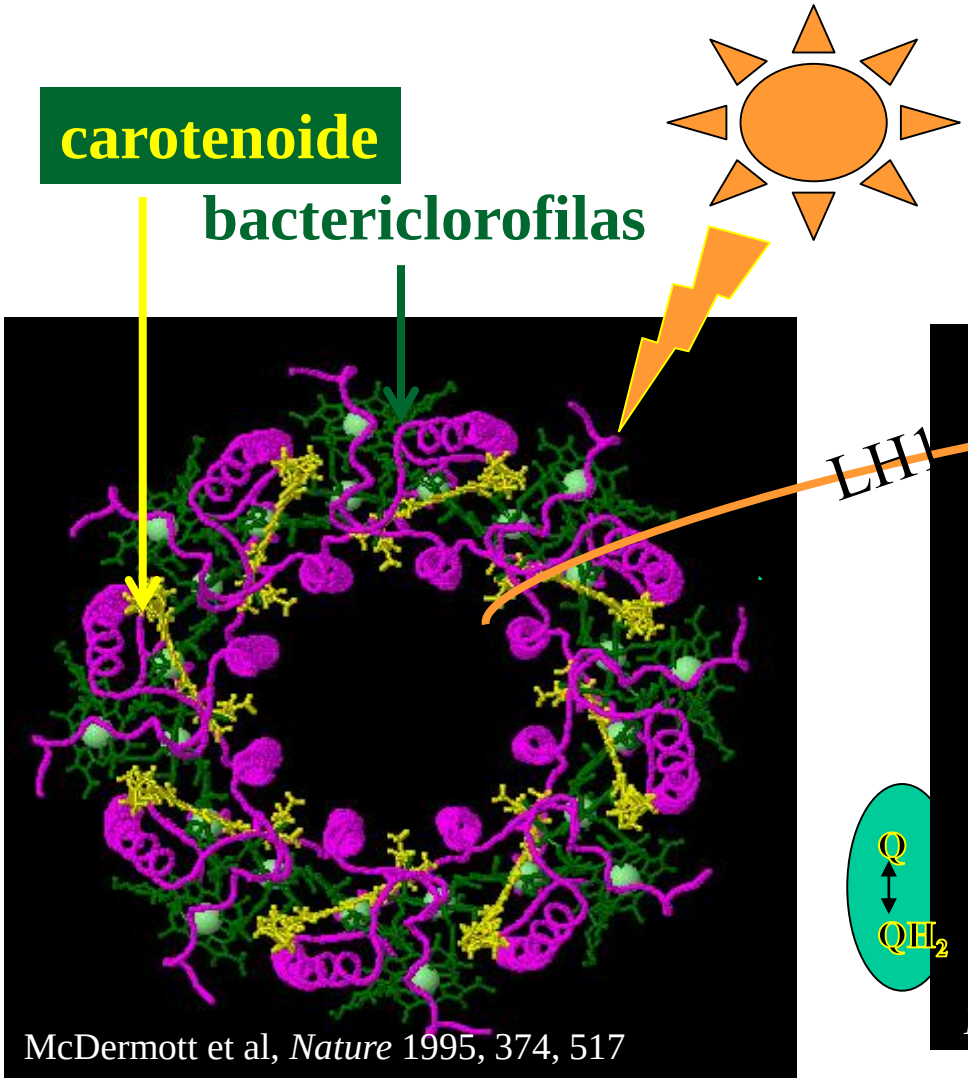
Time resolved confocal fluorescence and phosphorescence microscopy (visible and NIR)



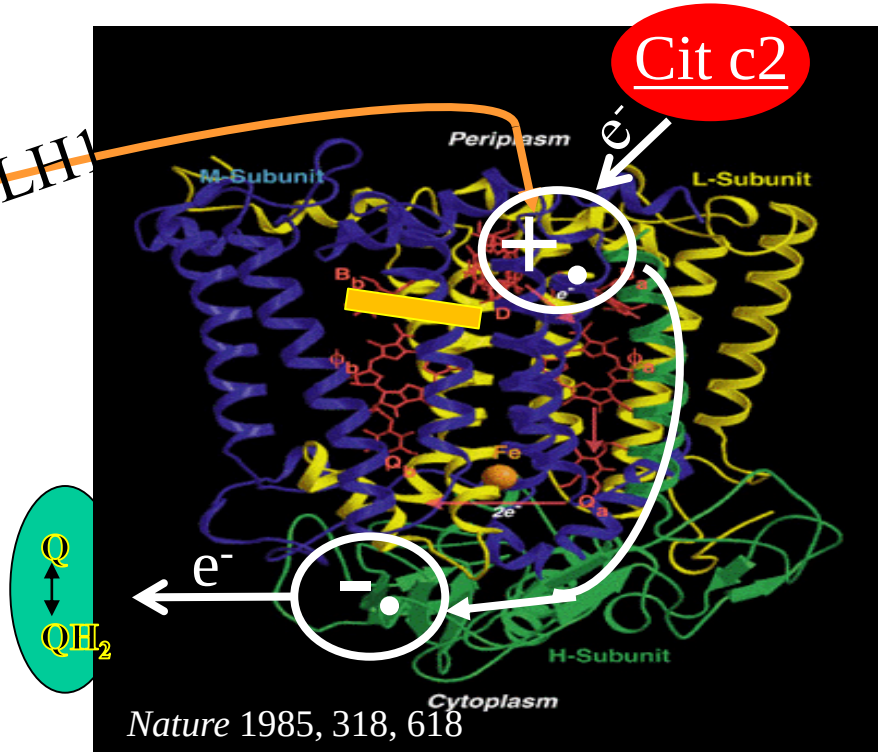
HaCaT cells treated with porphyrin. NIR image!

Is the production of singlet oxygen common in nature?

Os organismos vivos aprenderam a controlar e a utilizar as reações com os estados excitados!

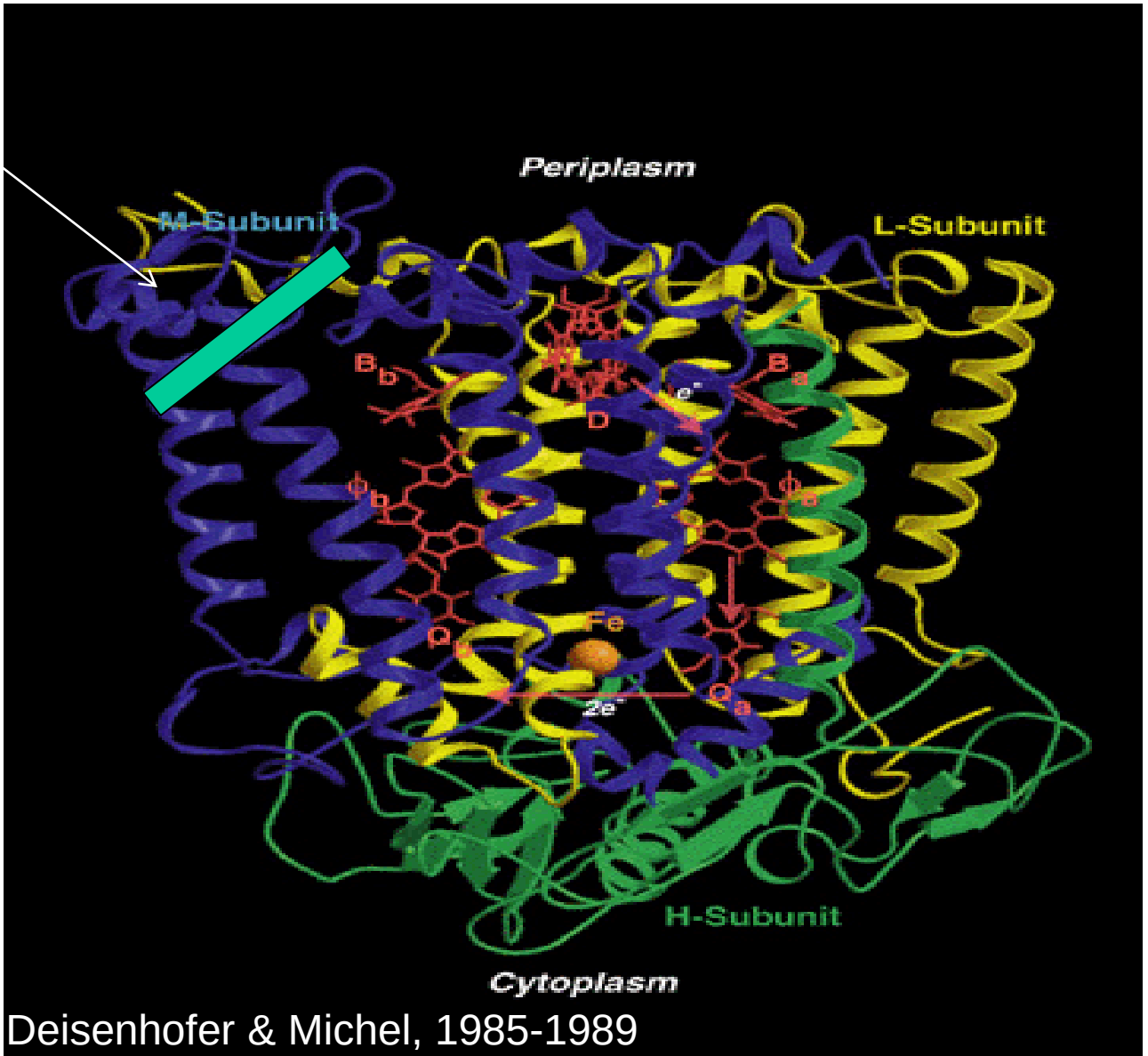


LH2 (light harvestng complex 2)
Complexos antena

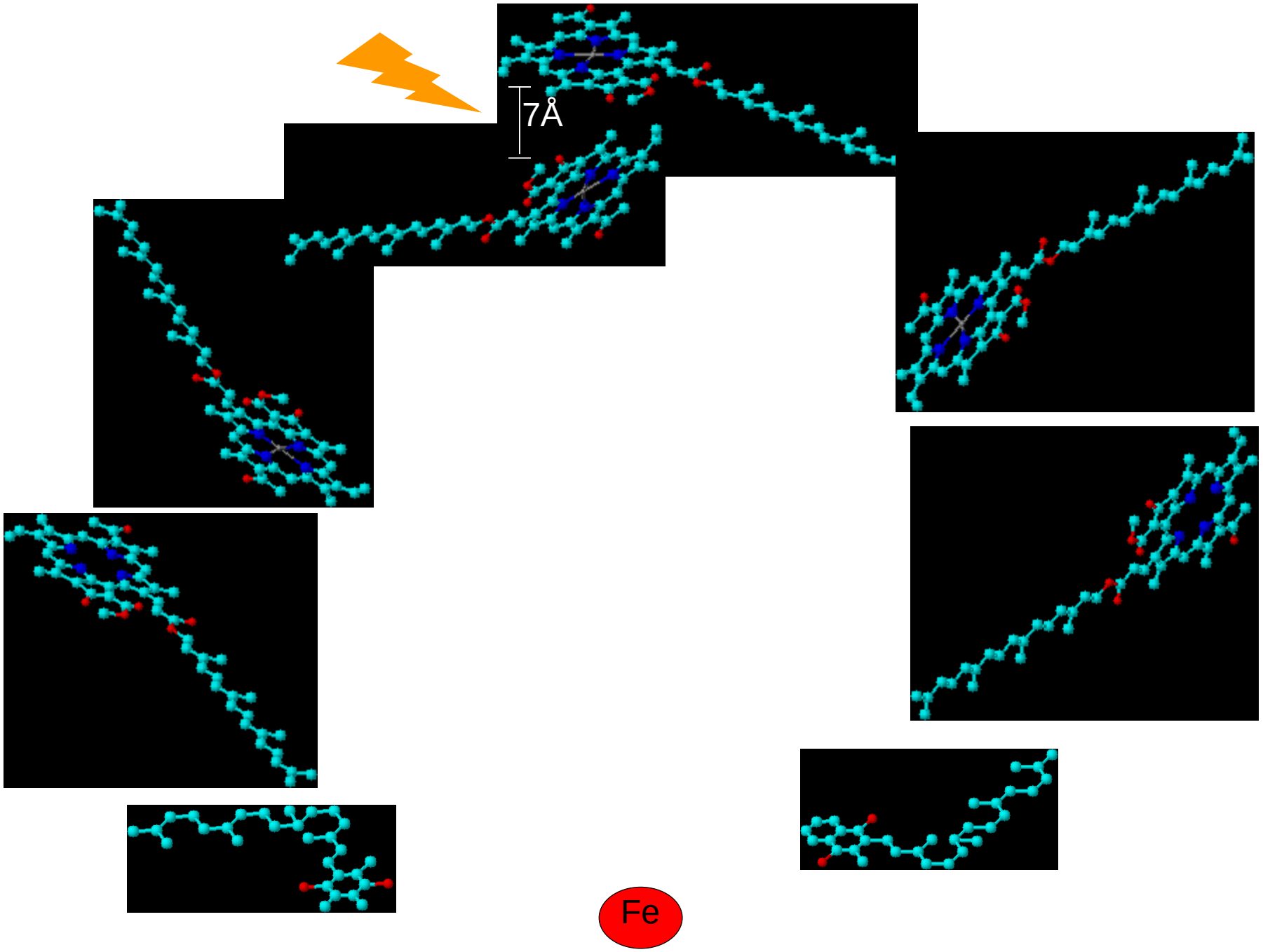


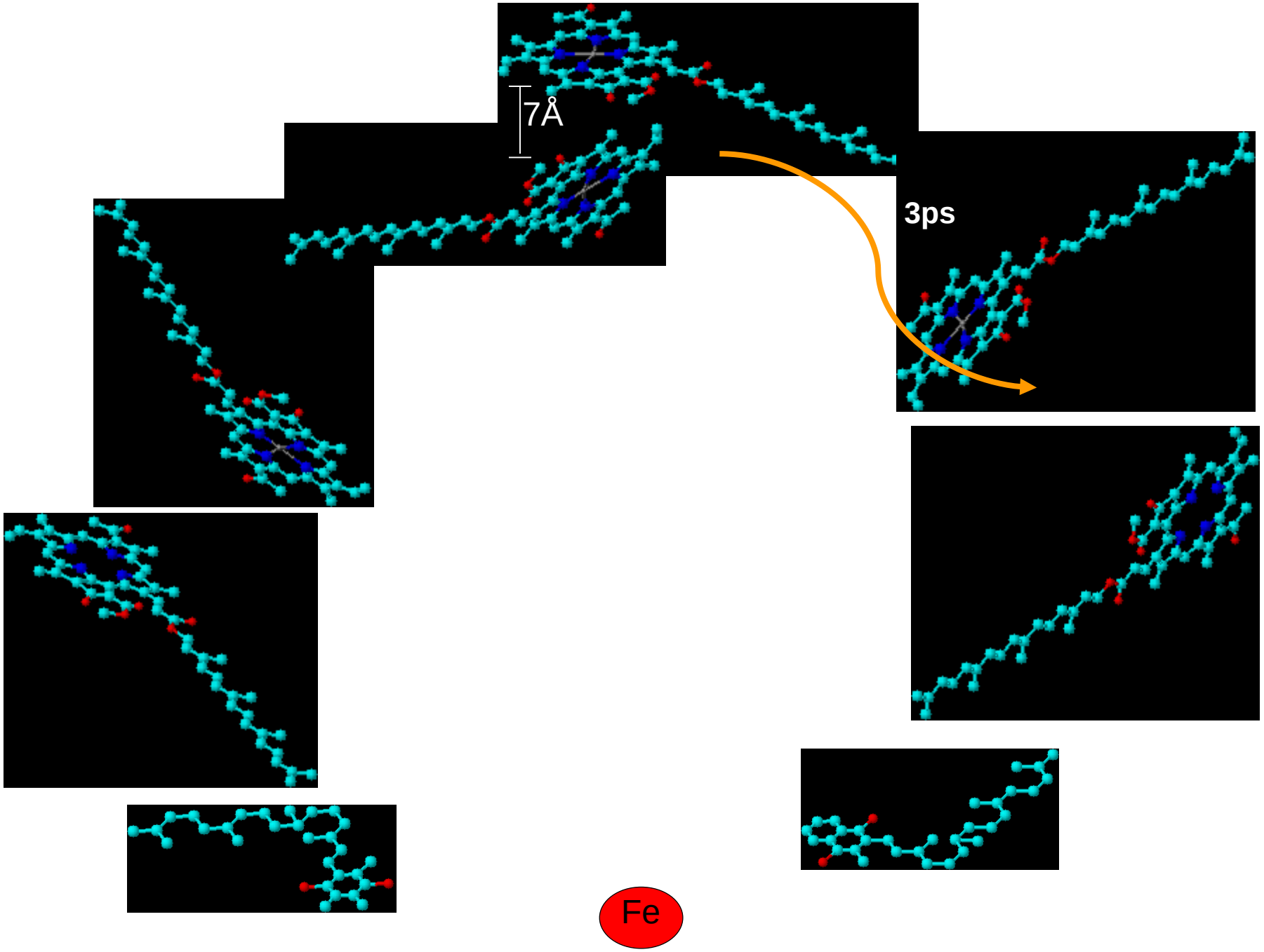
Deisenhofer, Huber & Michel,
Prêmio Nobel de Química 1988

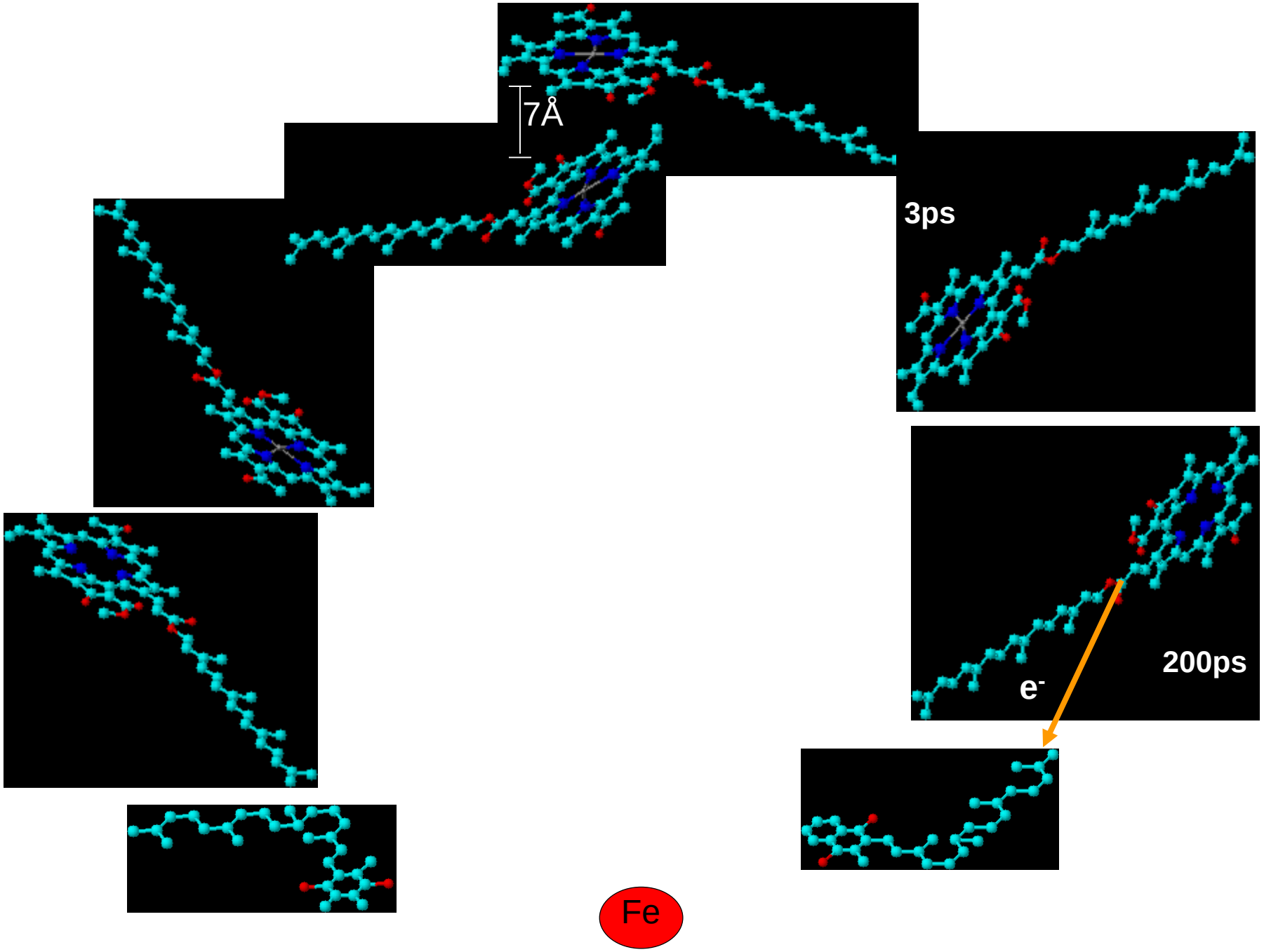
Reaction center of Rb. Sphaeroides

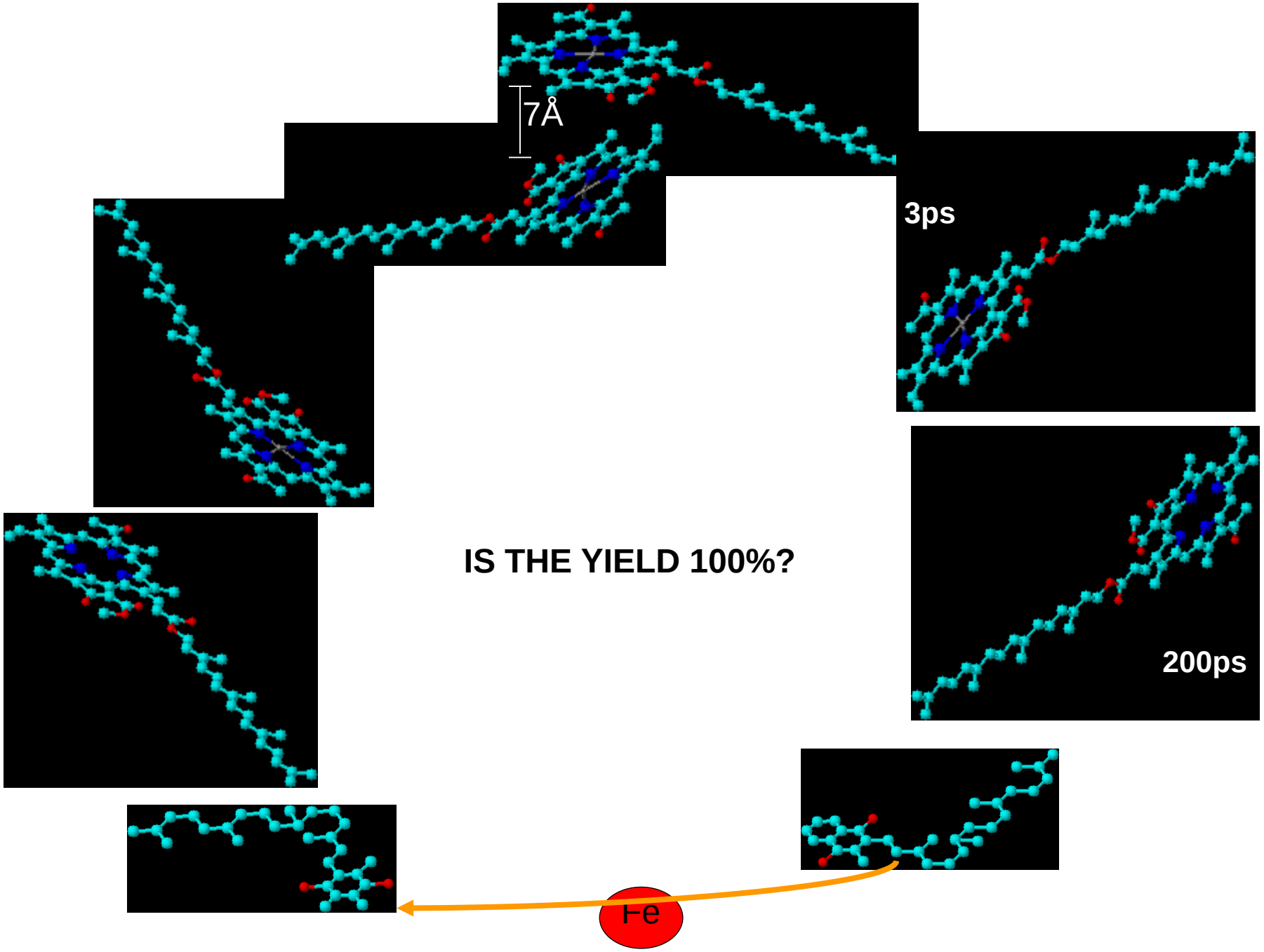


Deisenhofer & Michel, 1985-1989


7Å
Fe

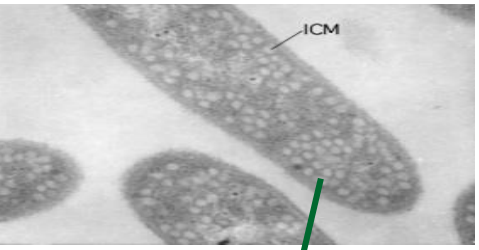

7Å
3ps
Fe

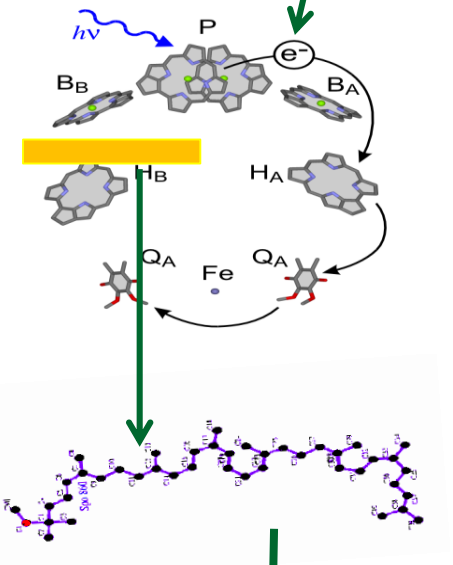

7Å
3ps
200ps
e-
Fe


7Å
3ps
200ps
IS THE YIELD 100%?
Fe
Citocromo C

Rhodobacter sphaeroids

Control of photoinduced reactions





Spheroidene/
carotenoide

Reduces ϕ_Δ

How to protect from the $^1O_2$?

PSII ⟶ $\phi\ ^1O_2 = 0.2$

Telfer et al. *J. Biol. Chem.* **1994**, 269, 13244.
de Weerd FL *J Phys Chem B* **2003**, 107, 6214.

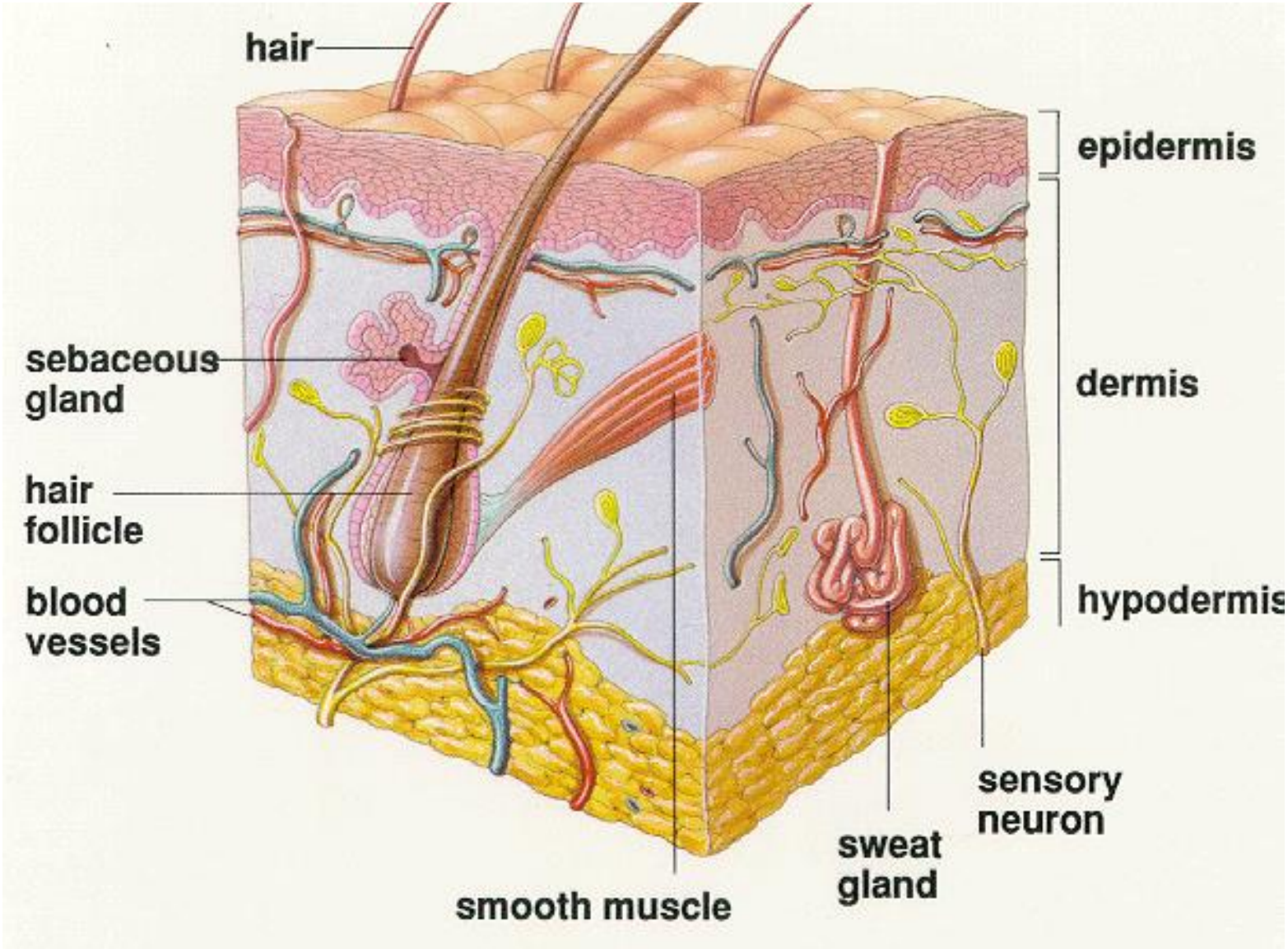
$\phi^1O_2 \sim 0.03$ (*Rhodobacter sphaeroids*)
$\phi^1O_2 \sim 0.05$ (*Rhodopseudomonas viridis*)
$\phi^1O_2 \sim 0.15$ (*Rb. sphaeroides*, R-26, **No carotene**)

$^1O_2 \uparrow \rightarrow \uparrow$ **Increase phototoxicity**

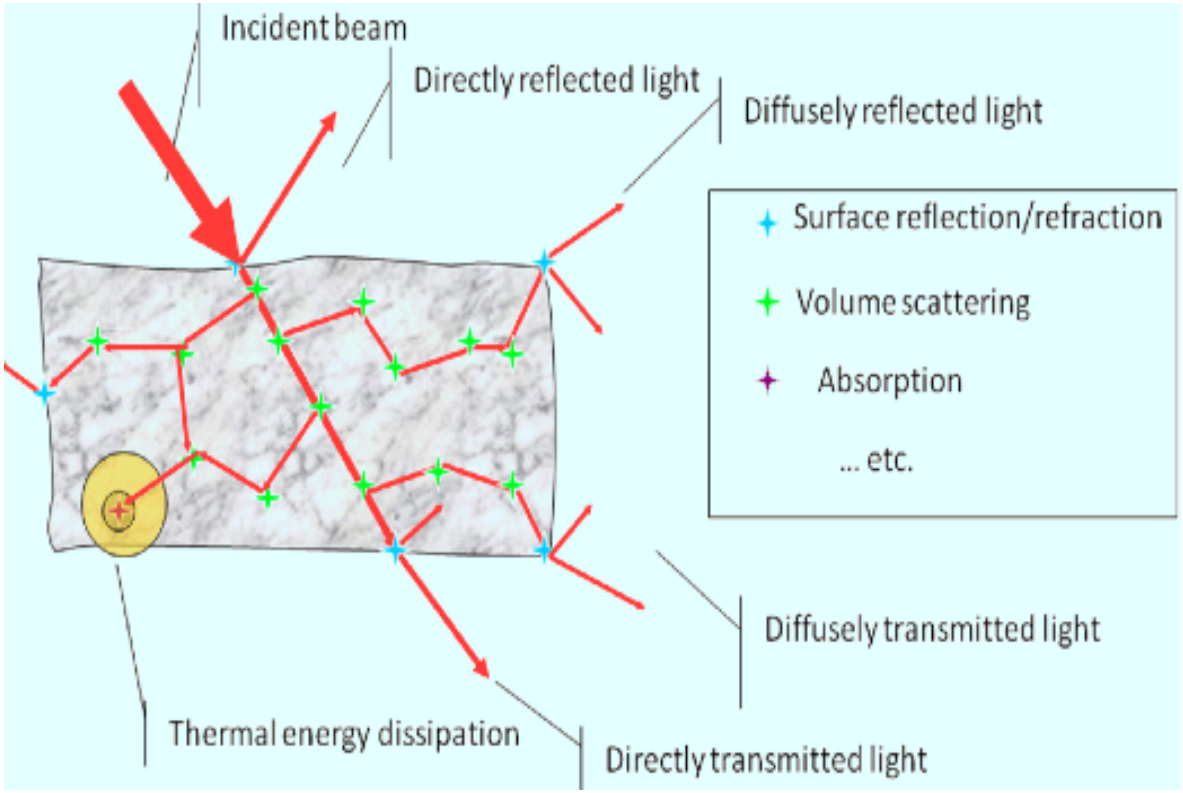
Uchoa et al *Eur J Biophys* **2008** 37, 843.
Knox et al *Biophysics* **2008**, 53, 291.
Uchoa AF, Tese de Doutorado IQUSP 2008.

→ **Carotenes are excellent suppressor of triplets and $^1O_2$**

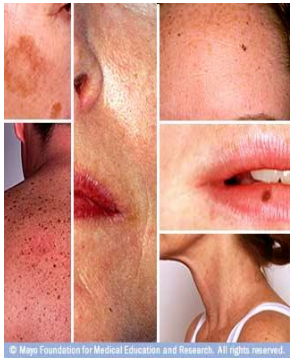
SKIN


hair
epidermis
dermis
hypodermis
sebaceous gland
hair follicle
blood vessels
sensory neuron
sweat gland
smooth muscle

Human Skin and light: in the search for a better sun protection!

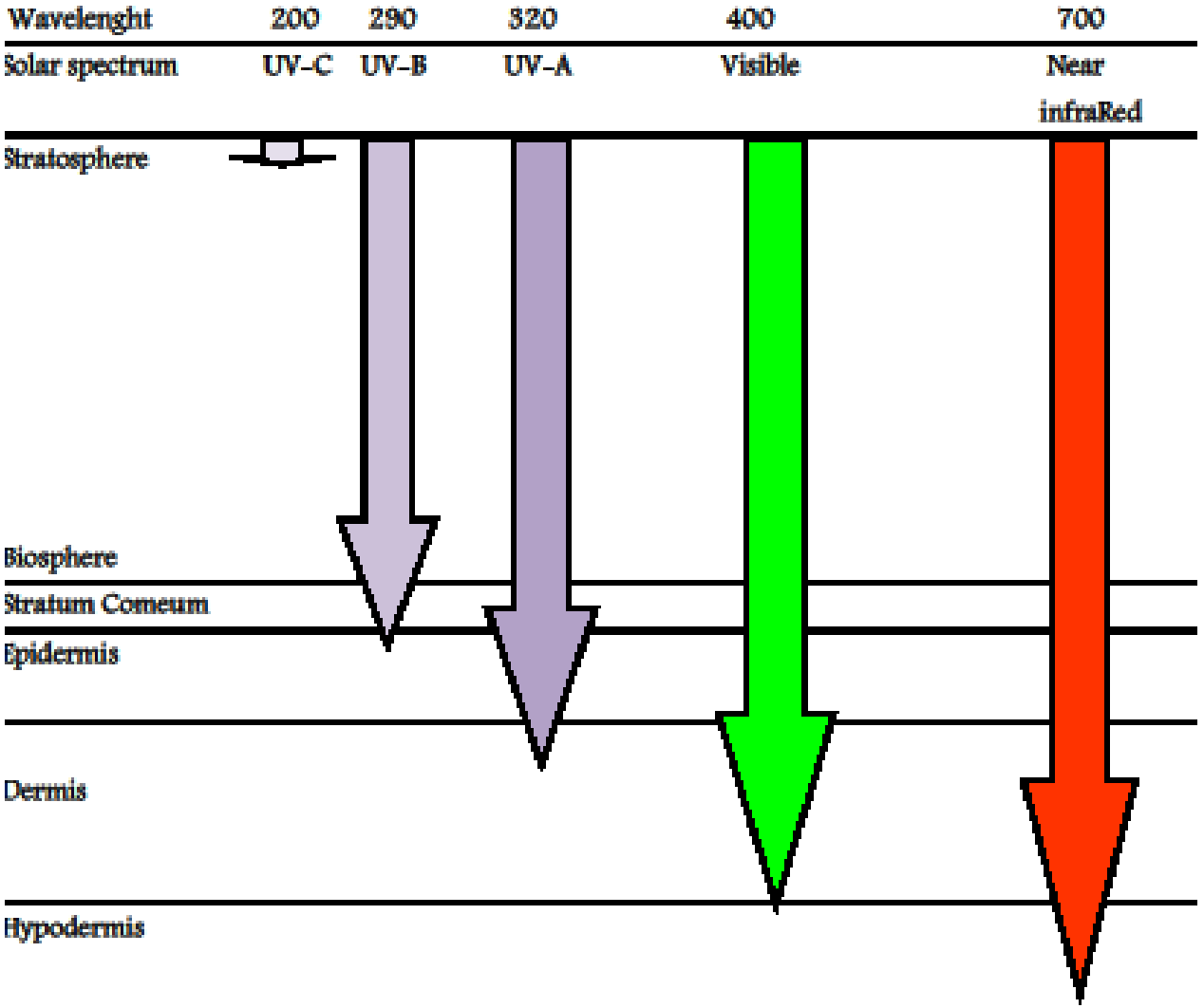


Do we understand the interaction of light with our skin or hair?



1. Which molecules absorb light?

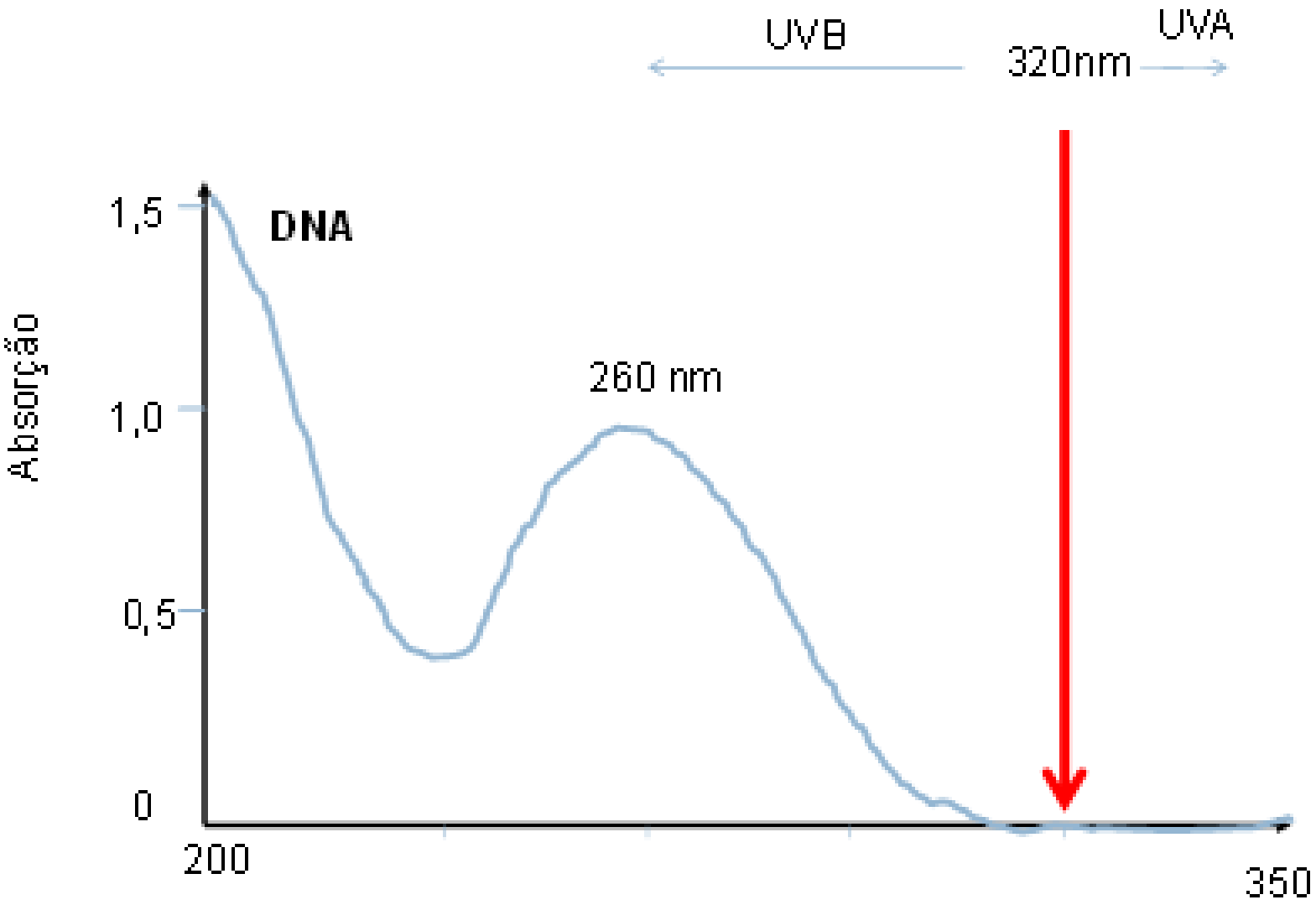
2. How much light is absorbed?



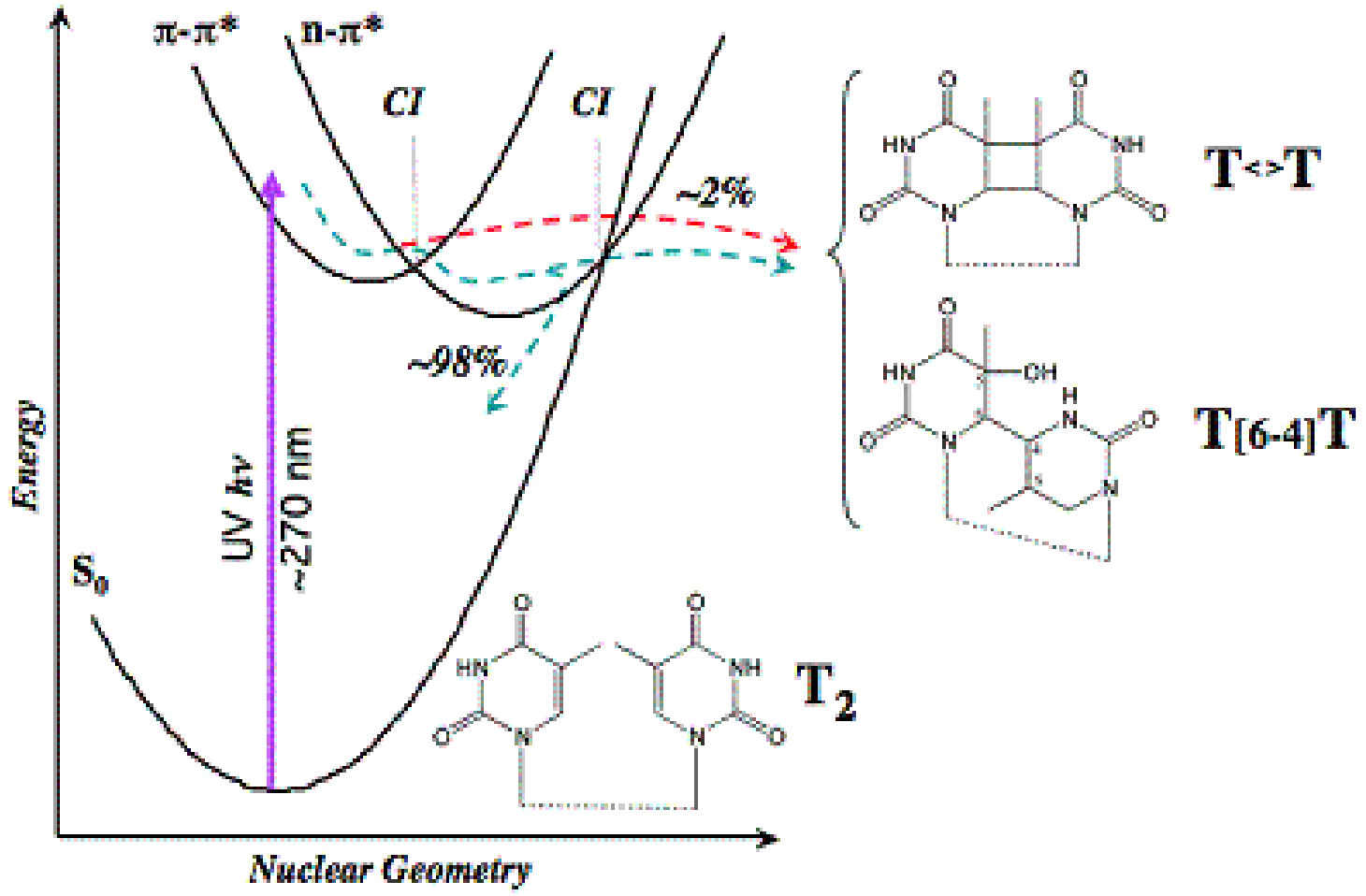
Baptista MS Photochemistry, Photobiology, and Redox Balance in Skin and Hair. Part I & II. *Cosmetiscope*, New York, 17(1, 3), p. 1-11; p. 1-10; 2011.

UVC (200-290nm)

- Very energetic (4.43-12.4 eV).
- All double bonds efficiently absorb UVC, usually getting bond breaks and ionization.
- Is used as sterilizing.
- Because da ozone layer, do not really reach our skin.


UVB
UVA
320nm
DNA
260 nm
Absorção
1,5
1,0
0,5
0
200
350

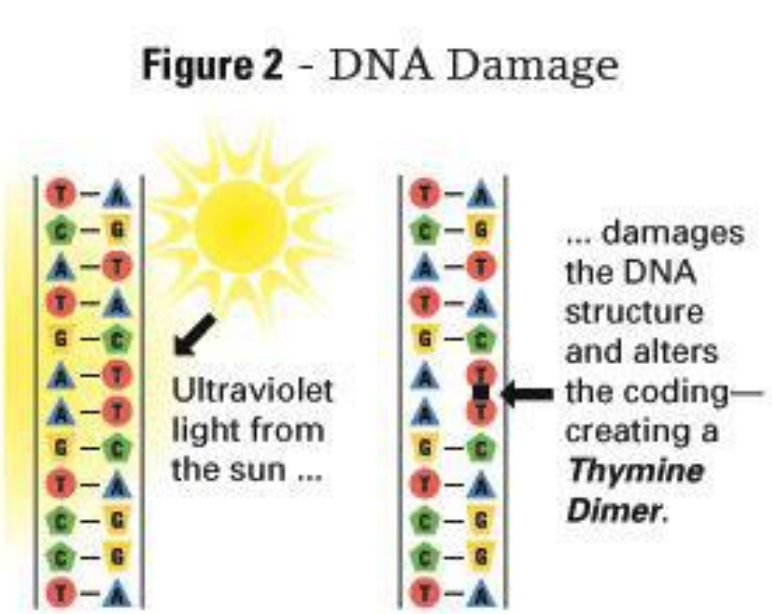
Reações no DNA induzidas pela absorção de luz

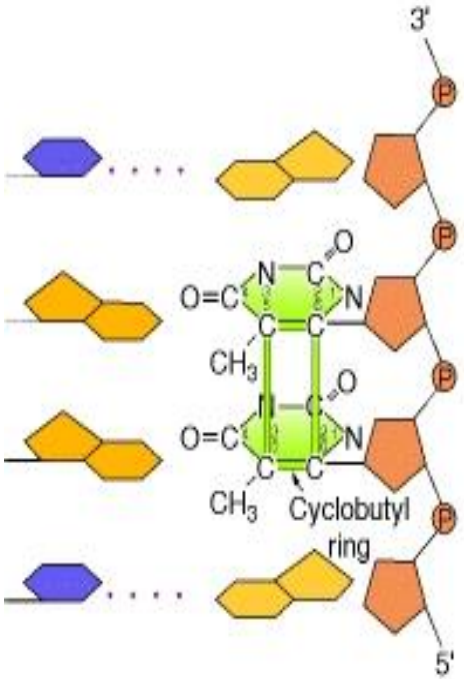


Direct DNA damage

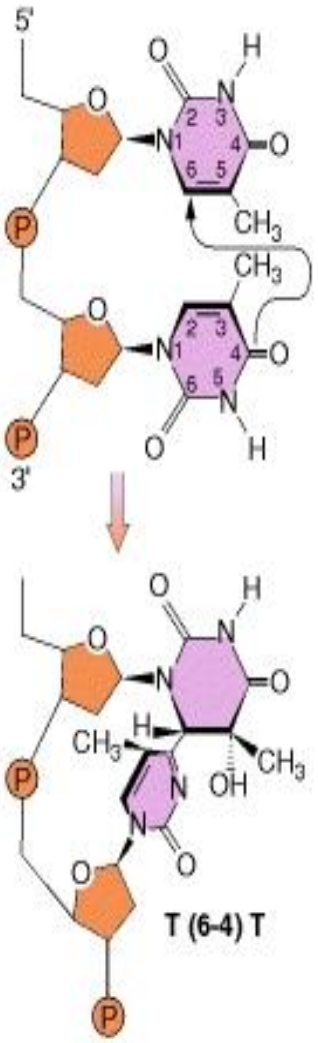
Dimers TT

Photoproduct 6-4



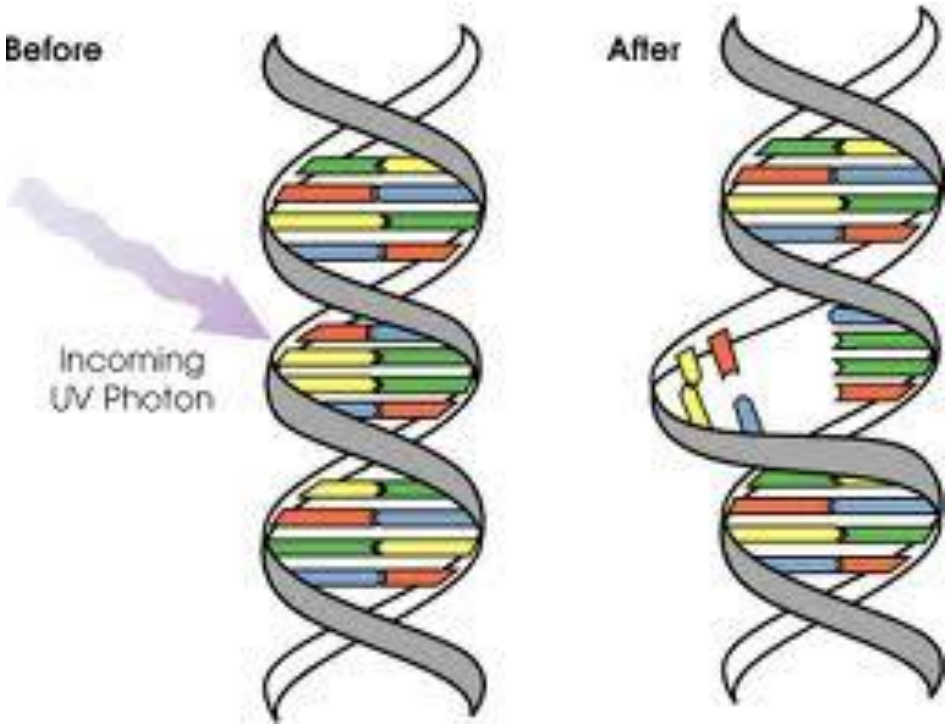


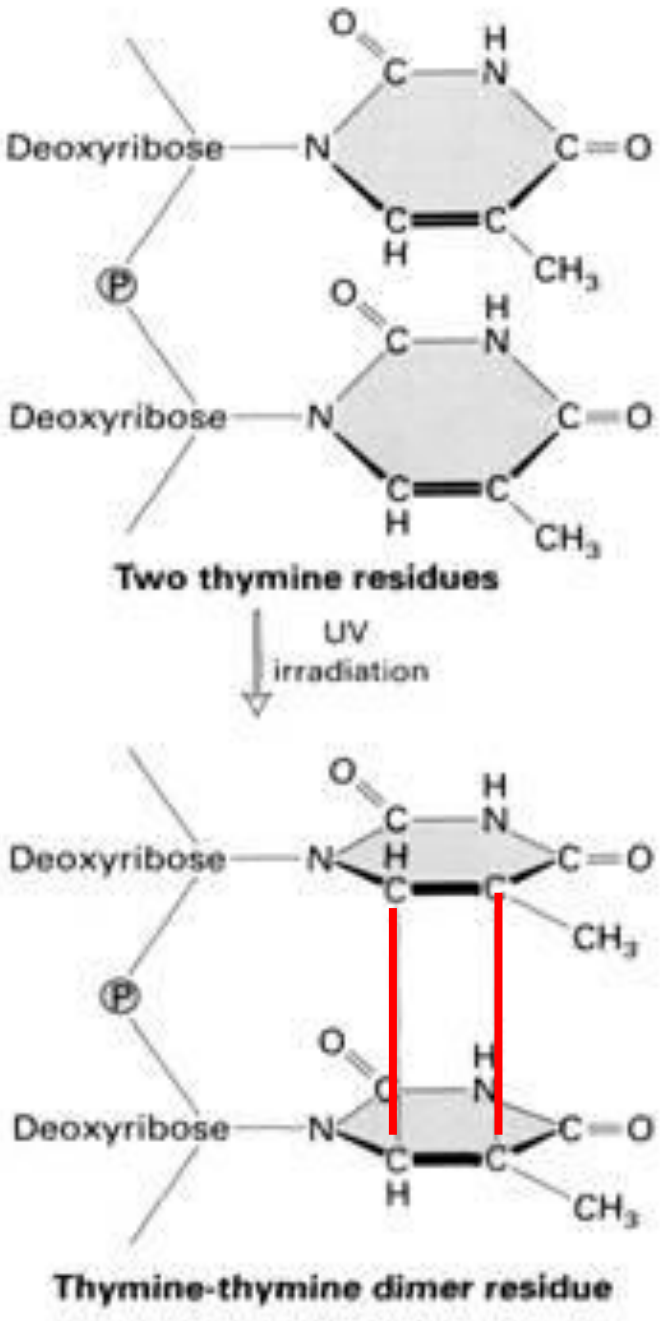
(a)



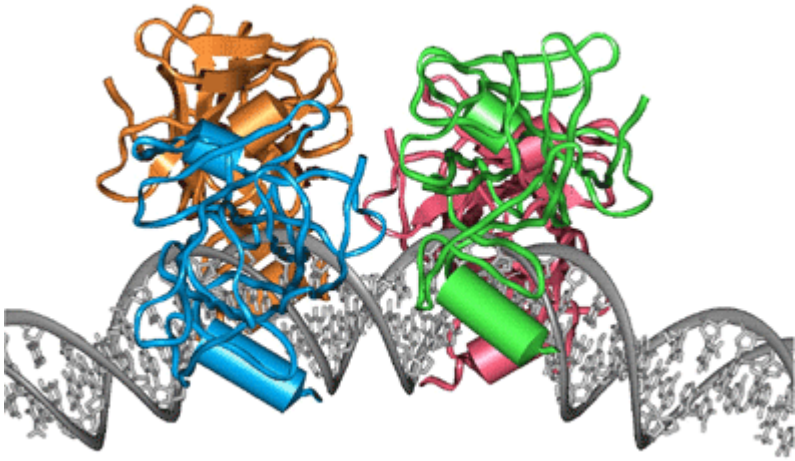
(b)

Distortion on the DNA



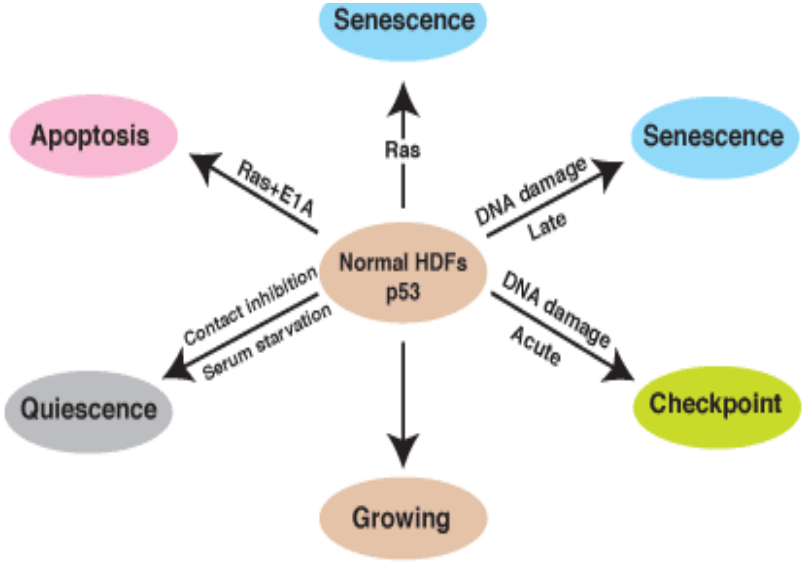


The p53 protein is the genome Guardiam, and leads potentially cancerous cells to suicide.

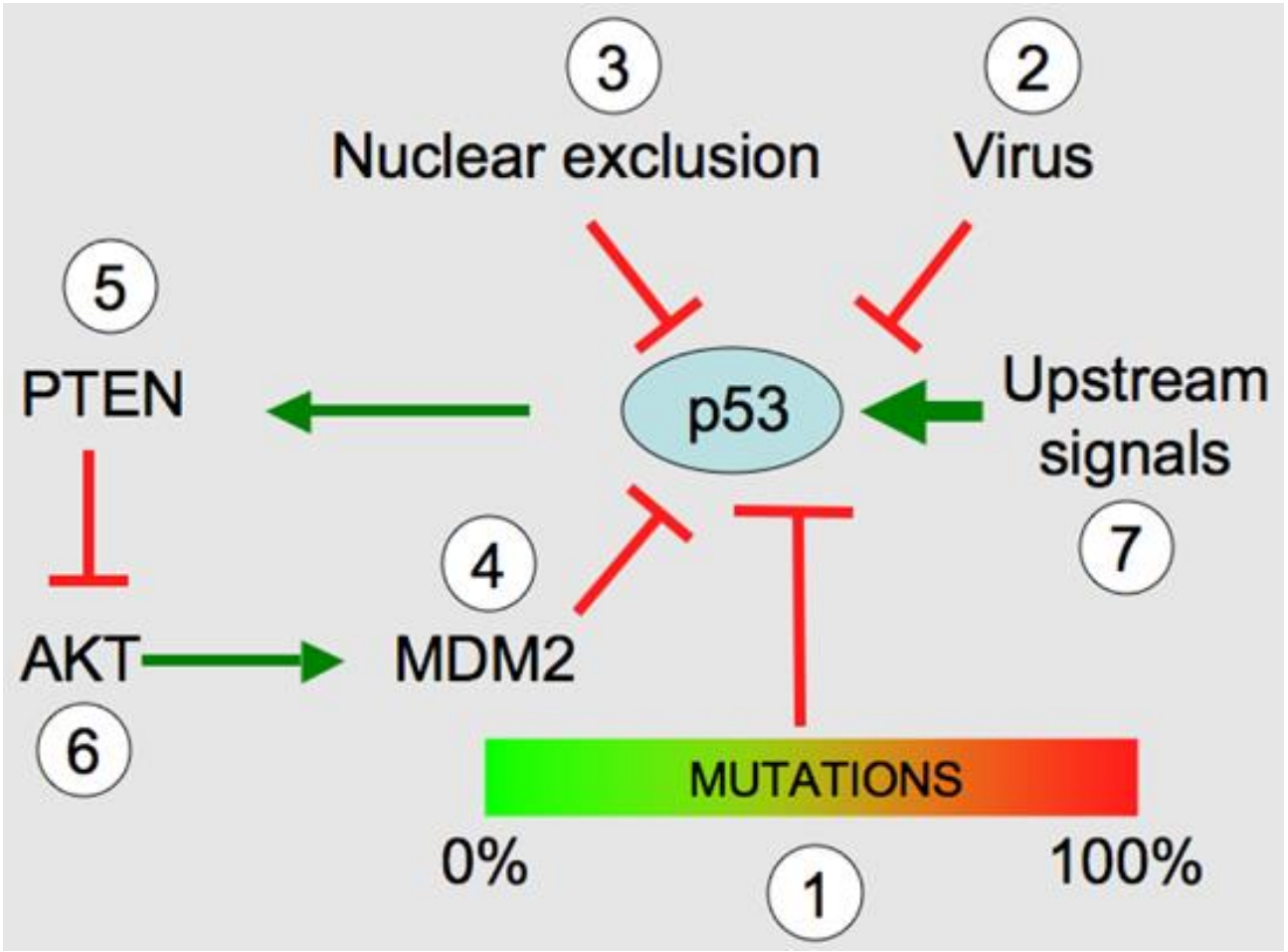


What happens if its gene is damaged
And the p53 protein can no longer
act properly?

Repair mechanisms!

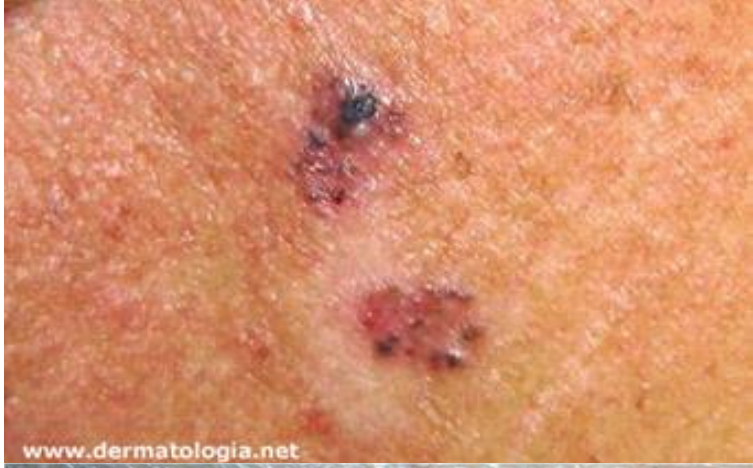


Most cancers involve mutations in the p53 or p53-related proteins

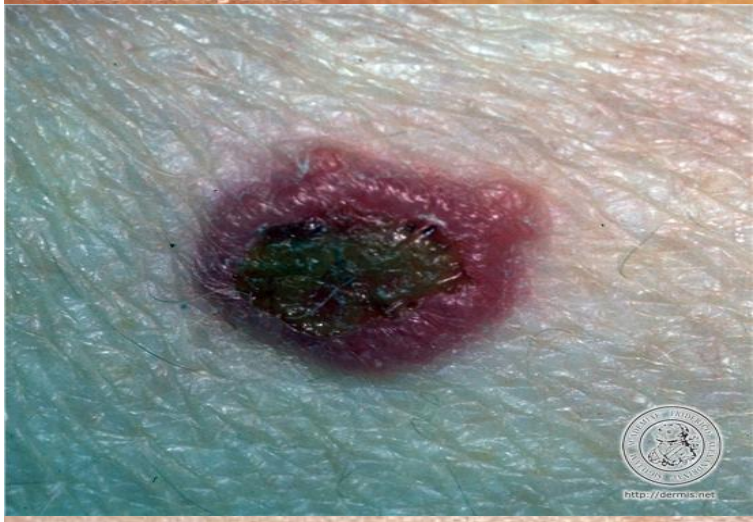


Super exposição ao sol

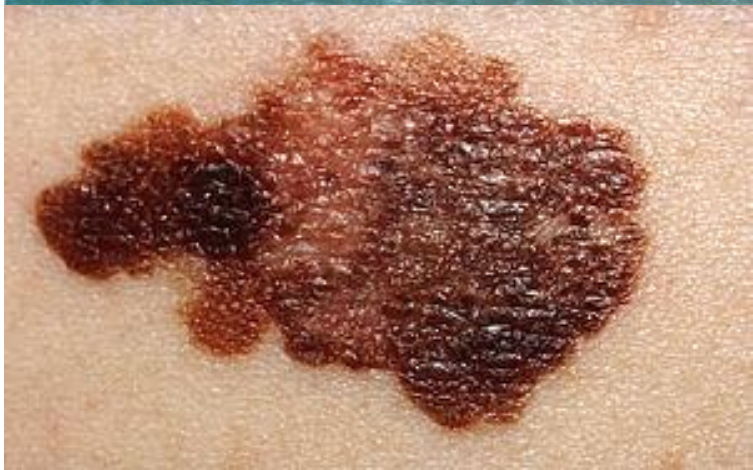
basocelular



espinocelular



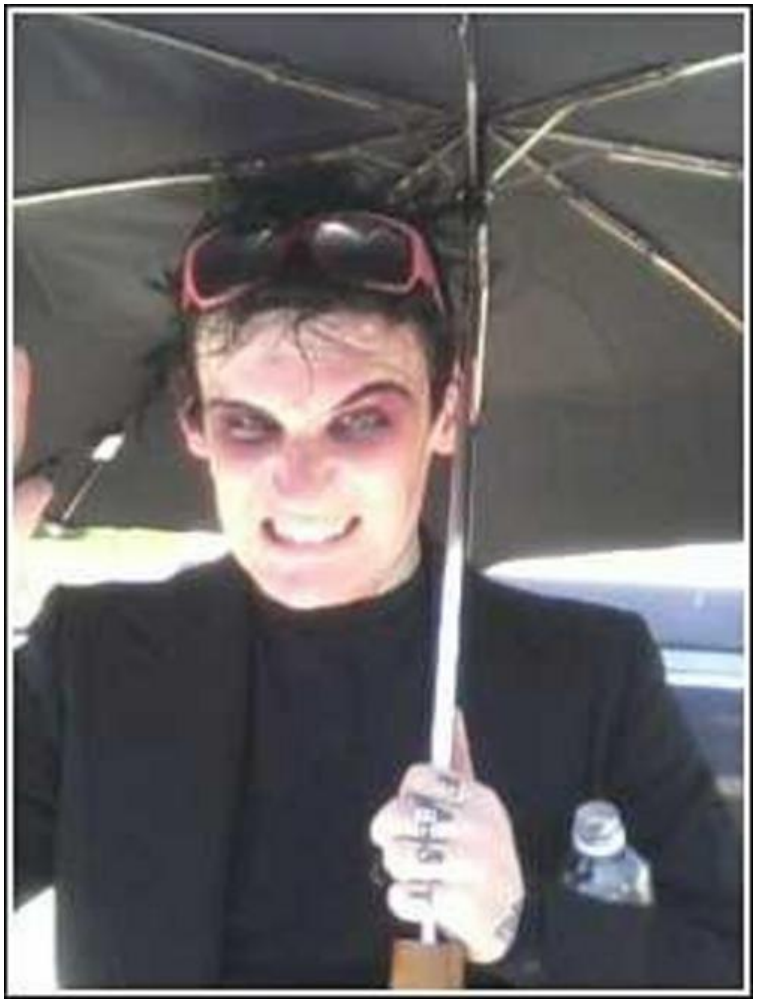
melanoma



UV level


IUV
11+
EXTREMO
(EXTREME)
10
9
MUITO
ALTO
(VERY HIGH)
8
7
ALTO
(HIGH)
6
5
4
MÉDIO
(MODERATE)
3
2
BAIXO
(LOW)
1
PROTEJA-SE DO SOL
USE CAMISETA, ÓCULOS
CHAPÉU E FILTRO SOLAR,
MESMO EM DIAS NUBLADOS
24/1/2011

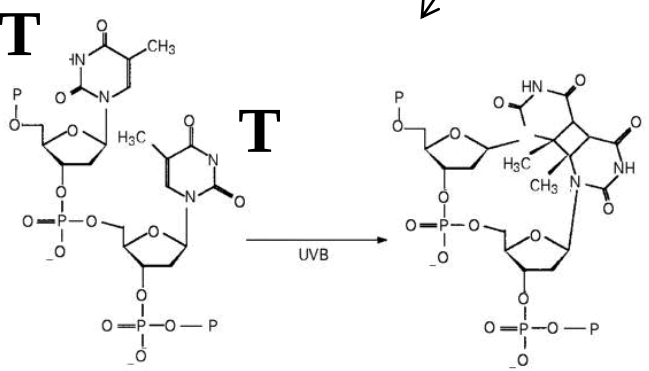
Can we totally avoid sun exposition?



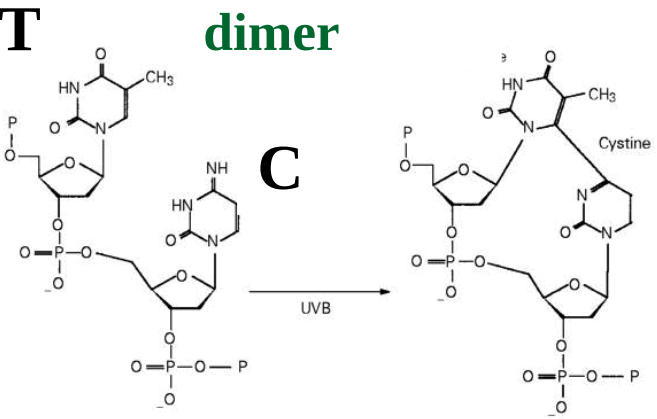
UVB (290-320nm)

Cycloaddition x **electrocyclic conrotatory** 6π

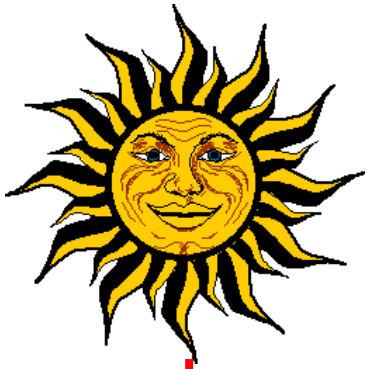
The contradiction in photobiology

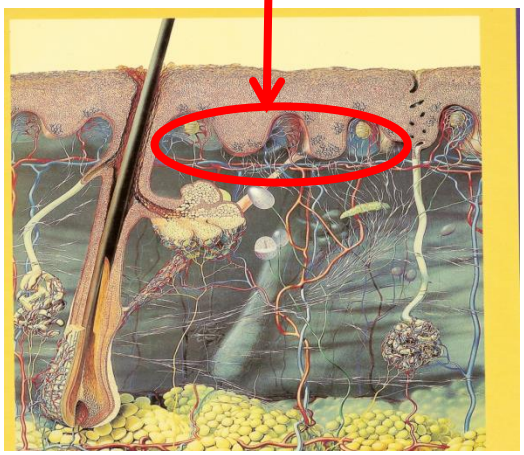


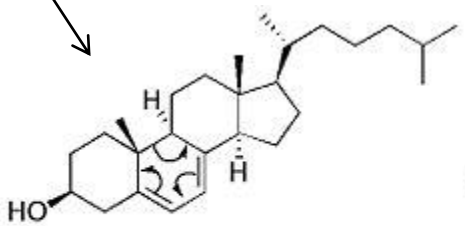
Cyclobutyl-pyrimidine dimer



Photoproduct 6-4

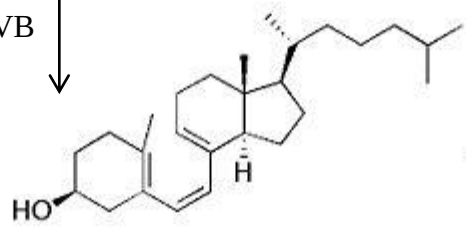






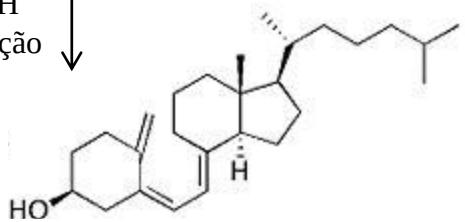
7-dehidrocolesterol

UVB



Pre-vitamin D3

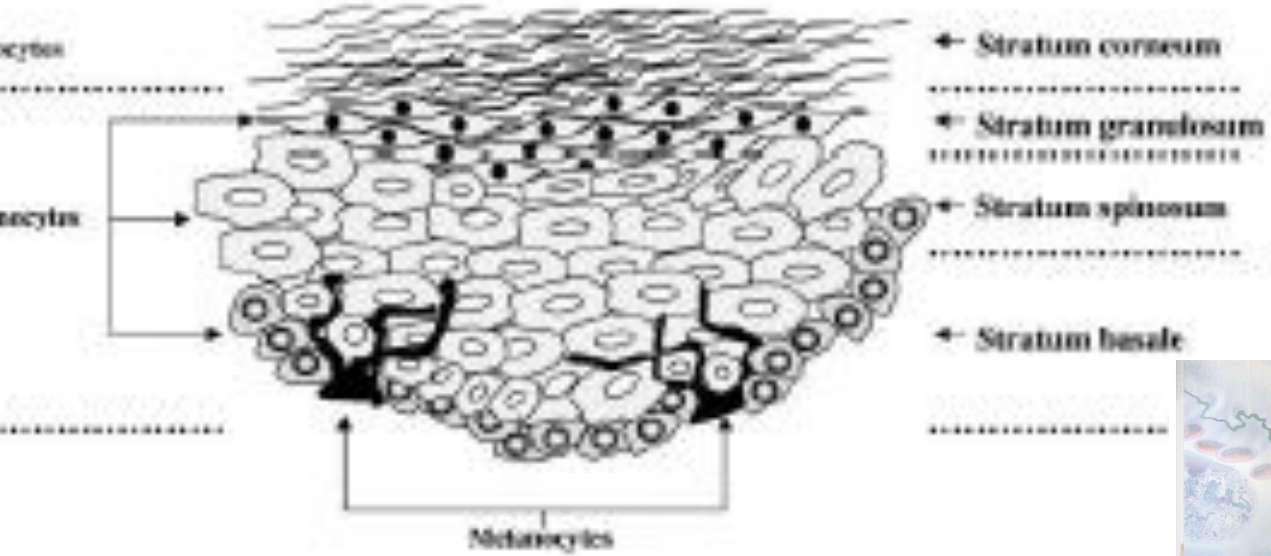
[1,7]-H migração

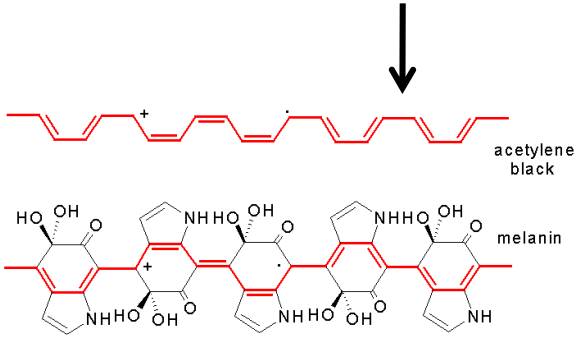


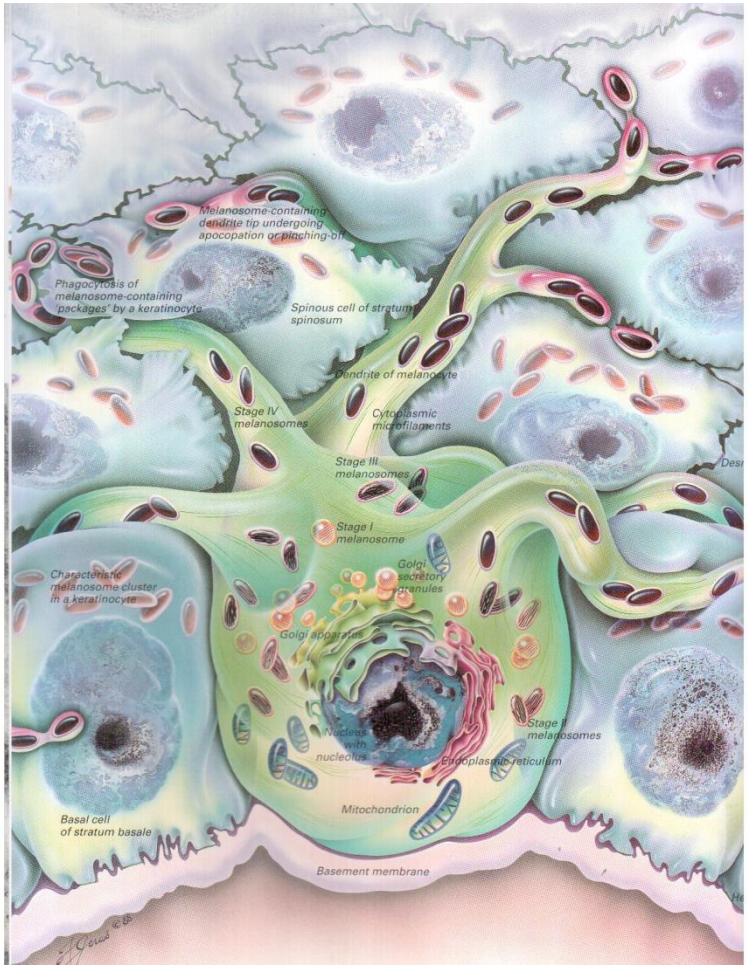
Vitamin D3

Niida & Nakanishi *Mutagenesis* **2006**, 21, 3–9.
Lehmann and Meurer *Dermatol Ther* **2010**, 23, 2.
Baeke et al *Curr Opin Pharmacol* **2010**, 10, 482.

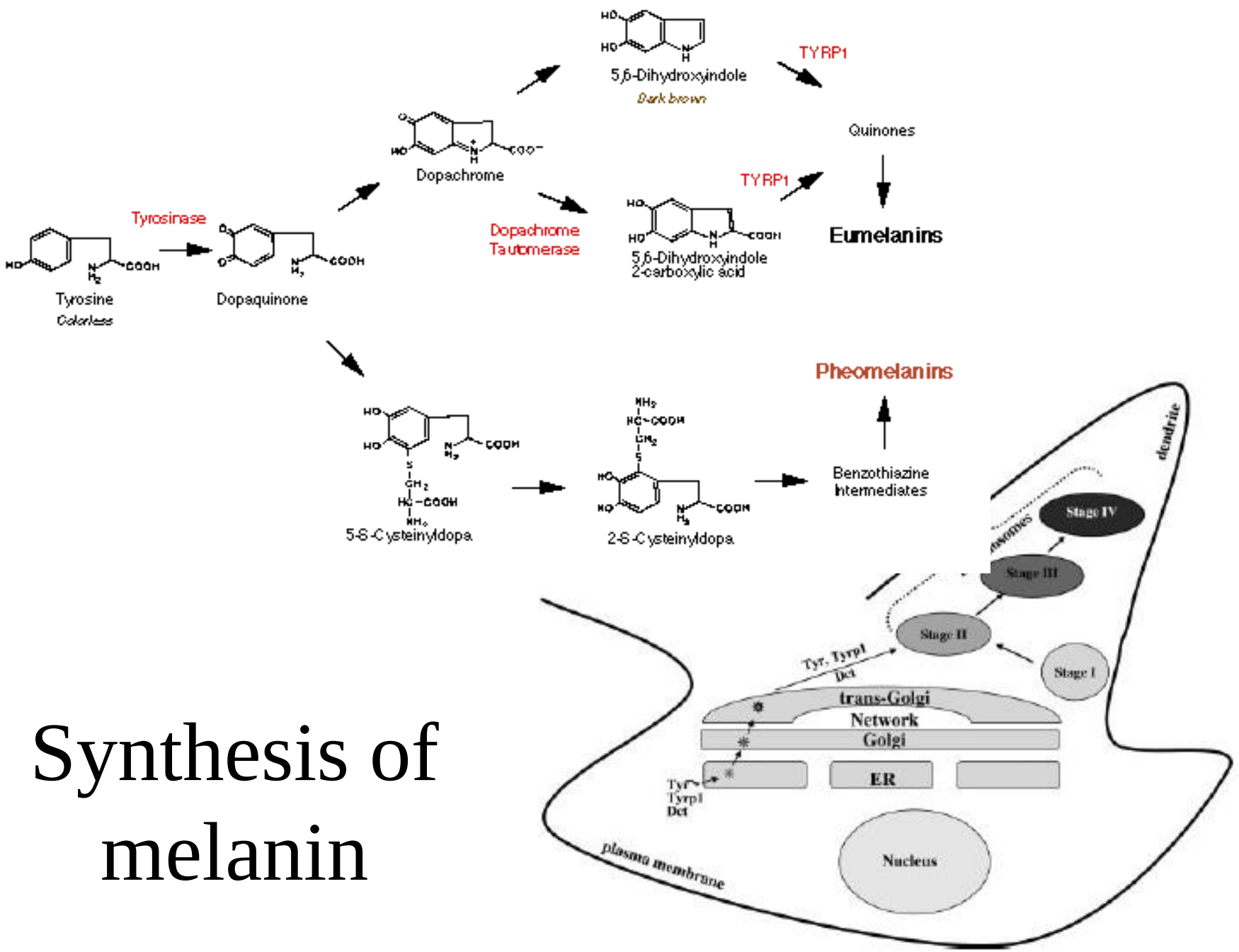
Melanocytes produce melanin



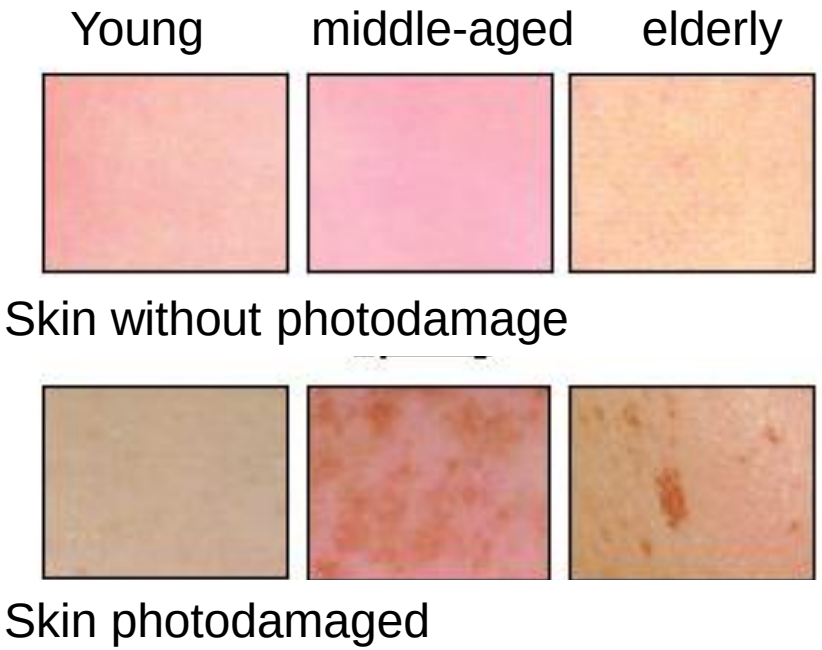


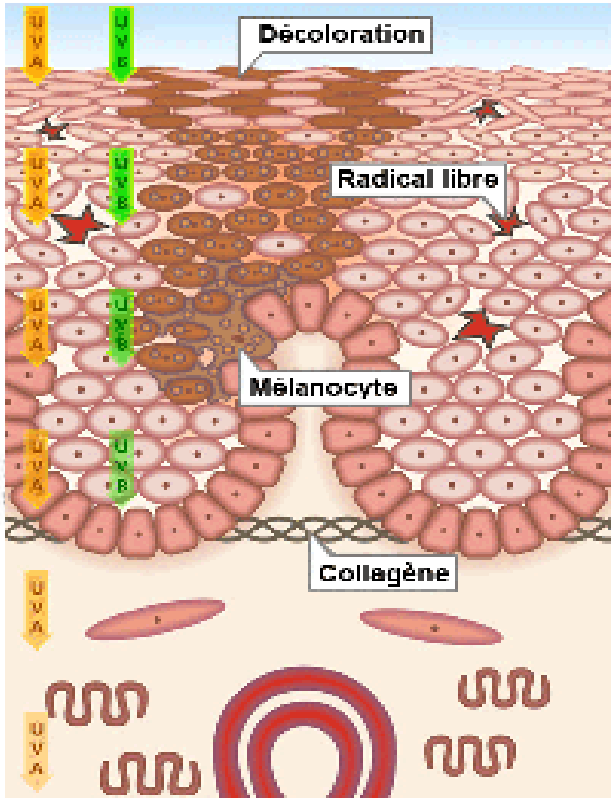


Synthesis of melanin

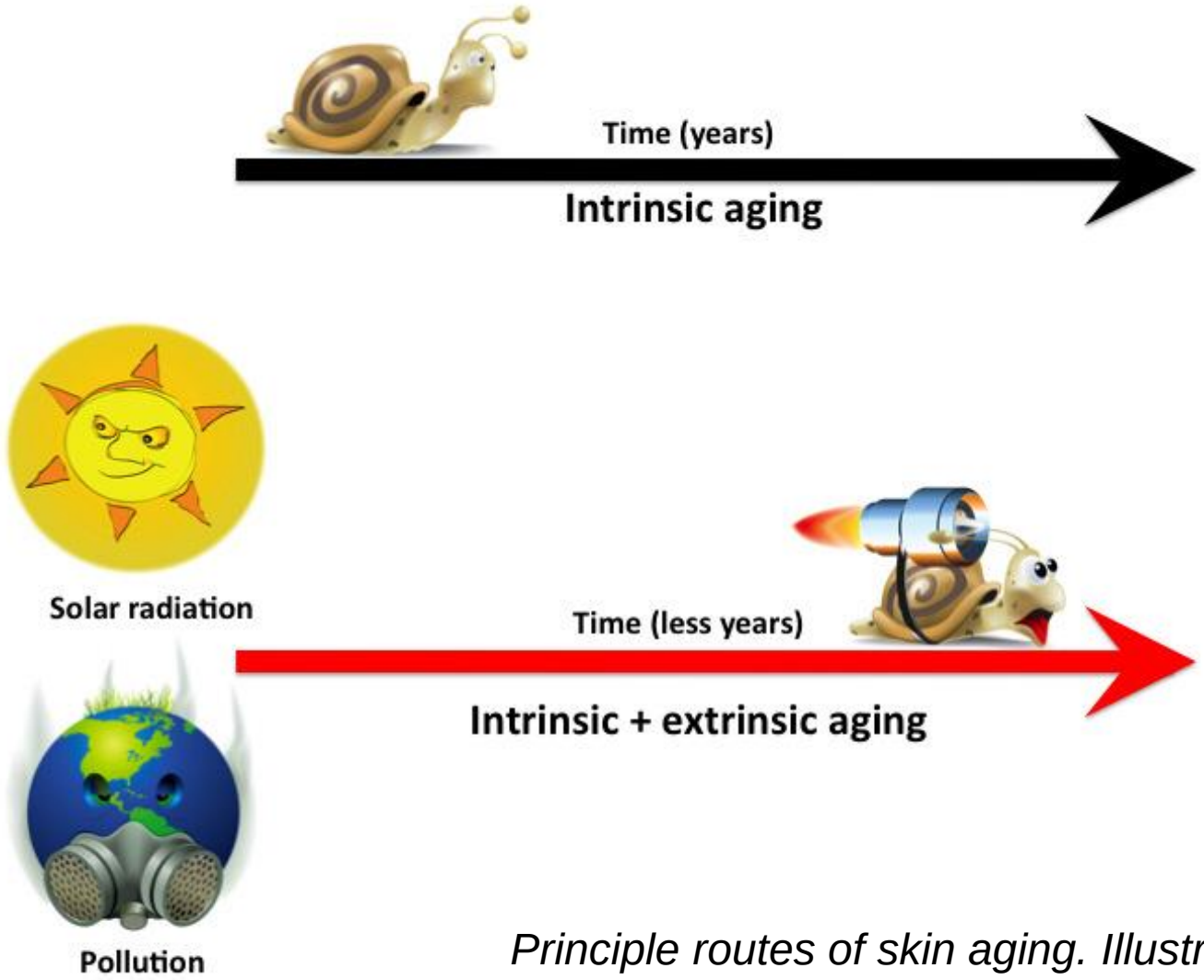


Increased activity of melanocytes after many years exposed to UV





Costin & Hearing, The FASEB Journal 2007, 21, 962.



Principle routes of skin aging. Illustration courtesy of Roger L. McMullen

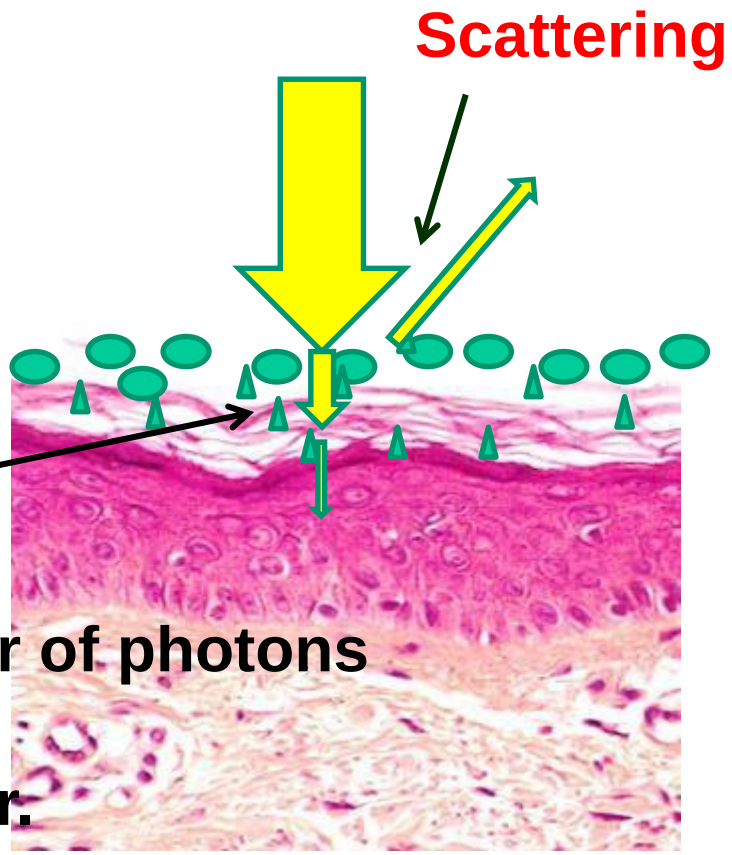
Baptista MS Photochemistry, Photobiology, and Redox Balance in Skin and Hair. Part I & II. *Cosmetiscope*, New York, 17(1, 3), p. 1-11; p. 1-10.

uv filters sunscreen

Avoids burning, photoaging.

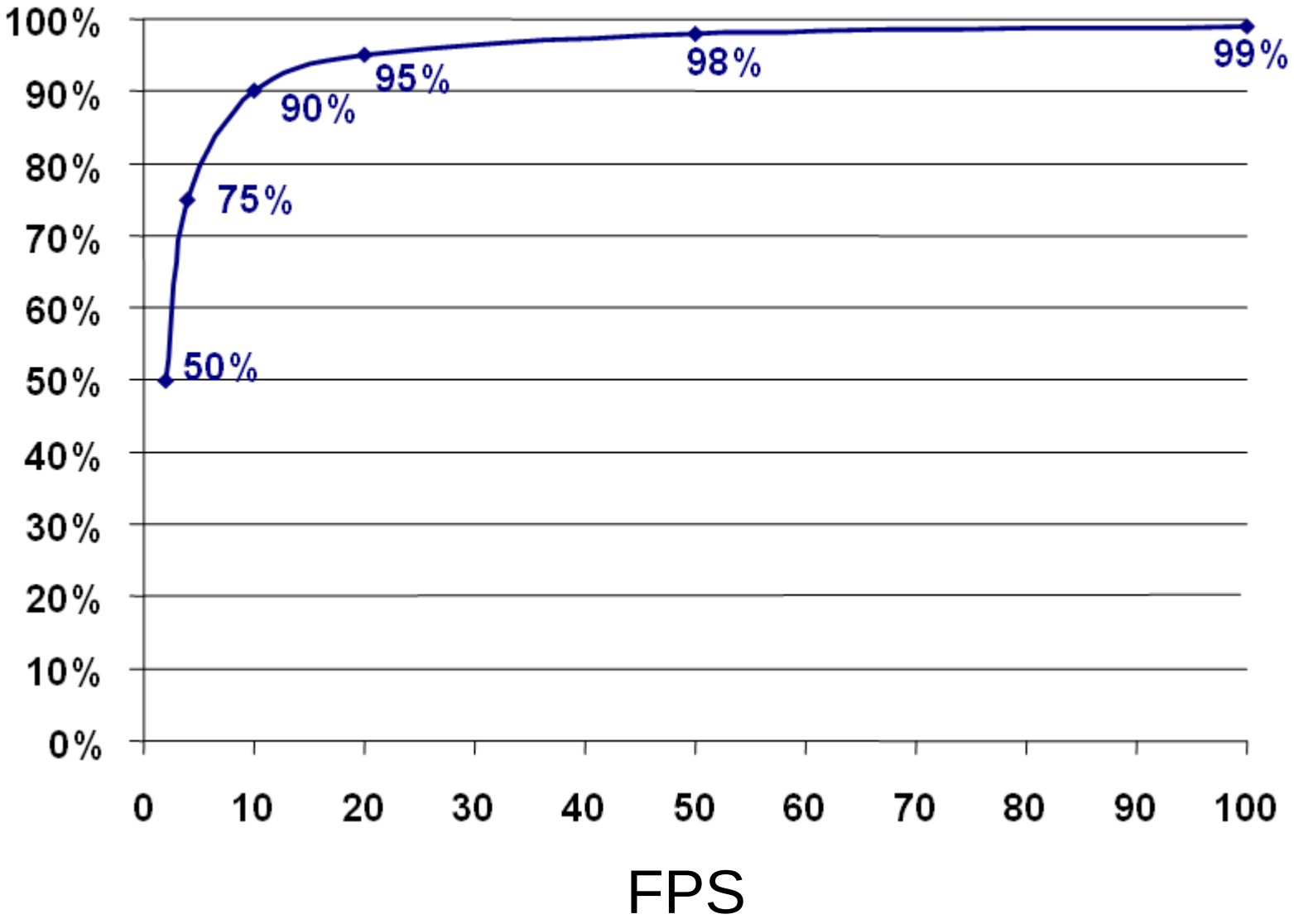
Filtros solares "orgânicos":
Unstable and can cause photosensitivity

Inorganic (zinc e titanium):
Photo-stable
Superior on the UVA
Low penetration and lower health hazards



Sun screens: Decrease the number of photons that penetrate :
FPS 10: From 100 photons 10 enter.
FPS 50: From 100 photons 2 enter.

SPF and light absorption



SPF measure: in-vivo

Principle: erythema induction Measure 20 ± 4 h after irradiation

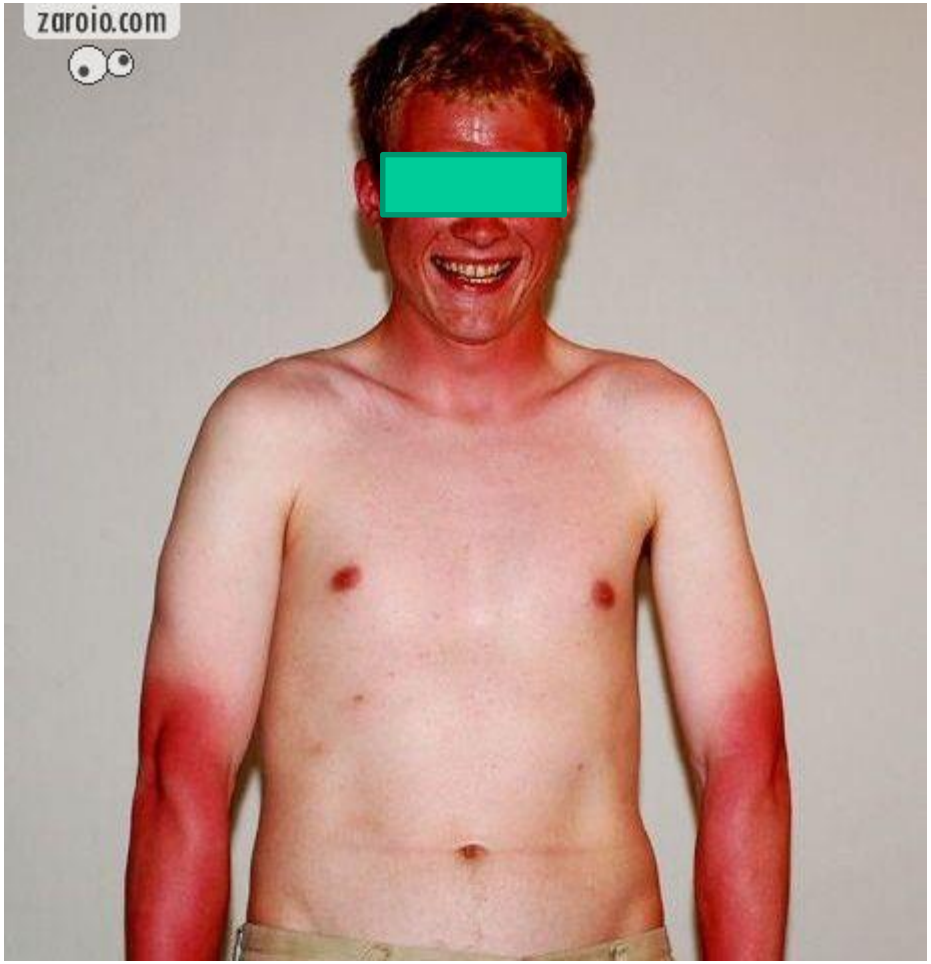
Irradiation: xenon lamp that mimics the sun

Application: 10-20 2mg / cm2 individual skin type I, II and III

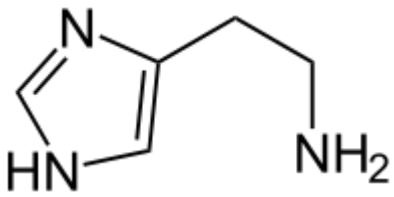
Pre-test: Determination of the minimal erythema dose (DME)

SPF = DME (protected) / DME (unprotected)

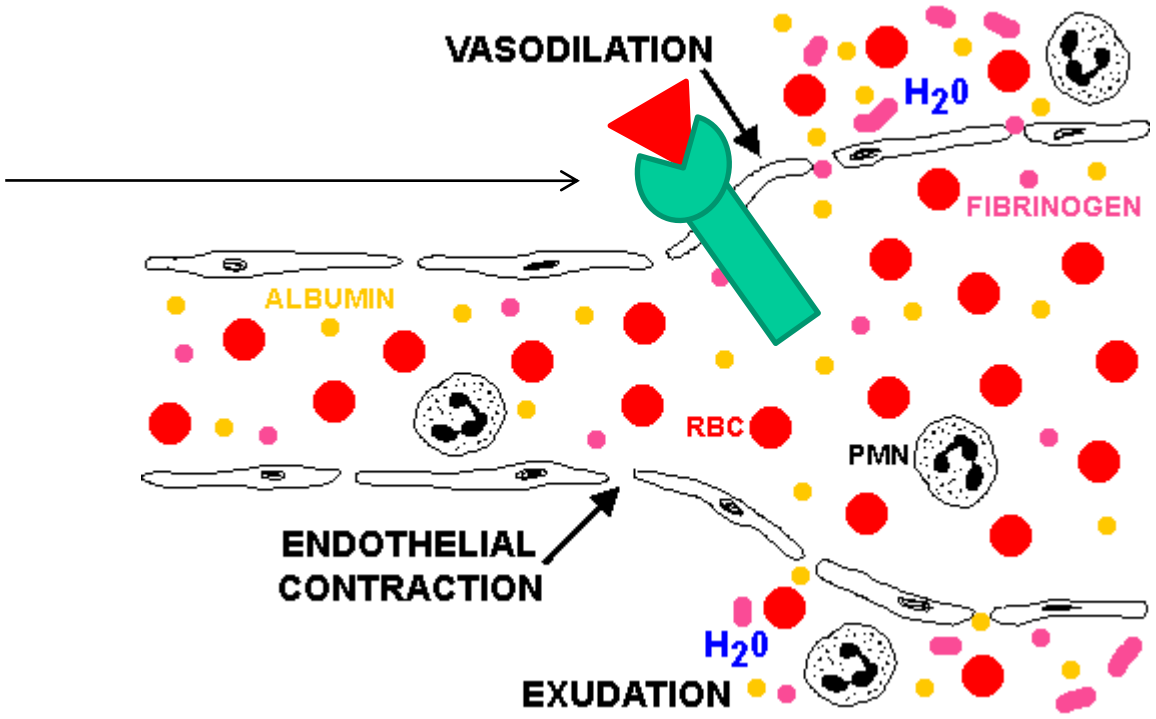
Erythema: only UVB



Erythema



Histamine Receptor



How about UVA, does it affect the skin?

Why in certain situations, the scientific knowledge takes so long to bring benefits to the population?

UVA and human skin

J Photochem Photobiol B: Biology **1989,** *4*, 227 .

Division of Photobiology, Department of Dermatology I, University of Vienna

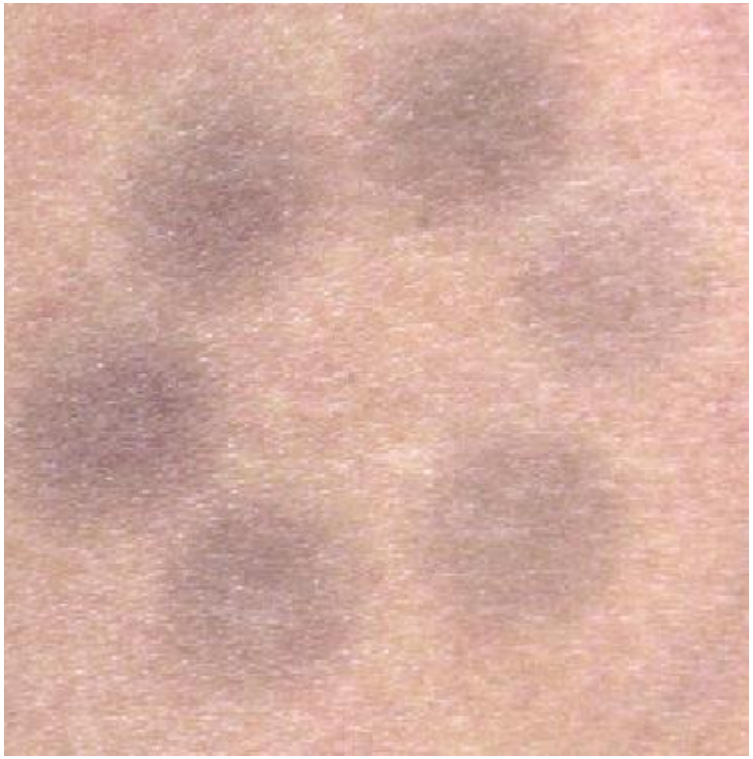
…The potential risks of UVA exposure of human skin should be made much more widely known to the public. In this context I would like to raise an additional problem that has not had much attention so far. The introduction of sunscreens with high sun protection factors (SPF) may lead sunbathers to feel safe to stay in the sun much longer than previously. **The protection against sunburn by a high SPF preparation obviously abrogates the skin's own warning signal of redness and smarting. However, most sunscreens absorb in the UVB region only and provide no substantial protection against UVA. Even if the UVA irradiance is only about 20 times that of UVB the cumulative dose of UVA penetrating the skin during a summer holiday can be of significant biological importance.** Presently, no regulations on UVA protection exist, and perhaps sunscreen manufacturers will be disinterested in UVA protection because filtering UVA would stop the skin from tanning. From the dermatological point of view an appropriate UVA filter has to be incorporated at least into sunscreens with UVB protection factors above 10. The UVA-SPF should be determined separately and should be not lower than 3. Unfortunately, none of the existing SPF regulations (FDA, DIN, Australian Standard) address this issue.

"…recent experiments using laser capture microdissection of human skin lesions from actinic keratosis and squamous cell carcinoma (SCC) have demonstrated that in both conditions the basal epidermal layer harbors more UVA than UVB fingerprint mutations as revealed by p53 mutational analysis."

G. M. Halliday, N. S. Agar, R. S. Barnetson, H. N. Ananthaswamy and A. M. Jones, UV-A fingerprint mutations in human skin cancer, *Photochem. Photobiol.*, 2005, **81**(1), 3–8

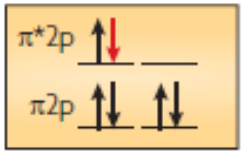
UVA-protection is characterized according pigmentation

Method known as PPD (Persistent Pigment Darkening) - Persistent Pigment Darkening
Radiation: UVA I & II (320-400 nm)
Energy: (8 - 25J / cm2)
Volunteers: Caucasians - skin type II V
Answer: after 2pm
Analyse: chromic



Mechanisms of photosensitized oxidations

Type II



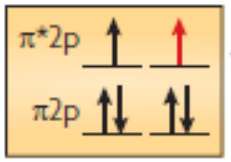
$^1O_2$*

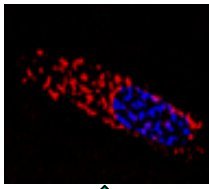
SUBSTRATE

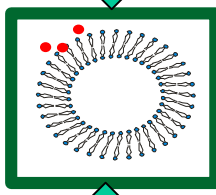
PRODUCT

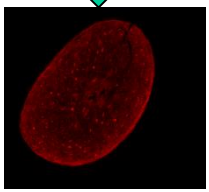
React with DNA, proteins and lipids

O_2









FS → ^{1}FS* → ^{3}FS*

BM

RADICALS

BM

$e^- + 2H^+$

H_2O_2 ← $O_2^{\bullet -}$

O_2

O_2

Fe^{2+}

Fe^{3+}

OH^-

$OH^{\bullet}$

PRODUCT

PRODUCT

Type I

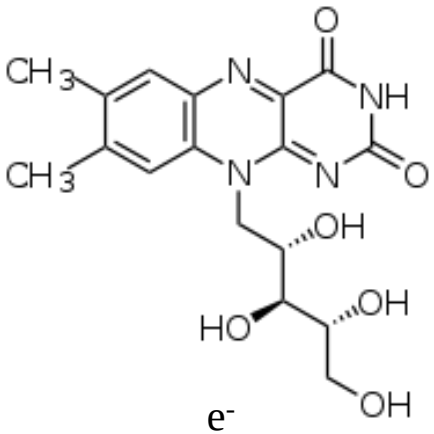
Foote, C.S. *Science* **1968**, *162*, 963.

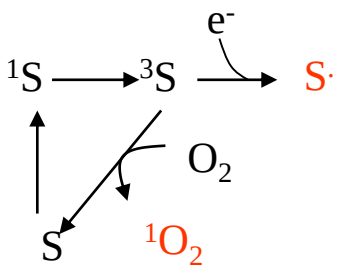
UVA-photosensitization

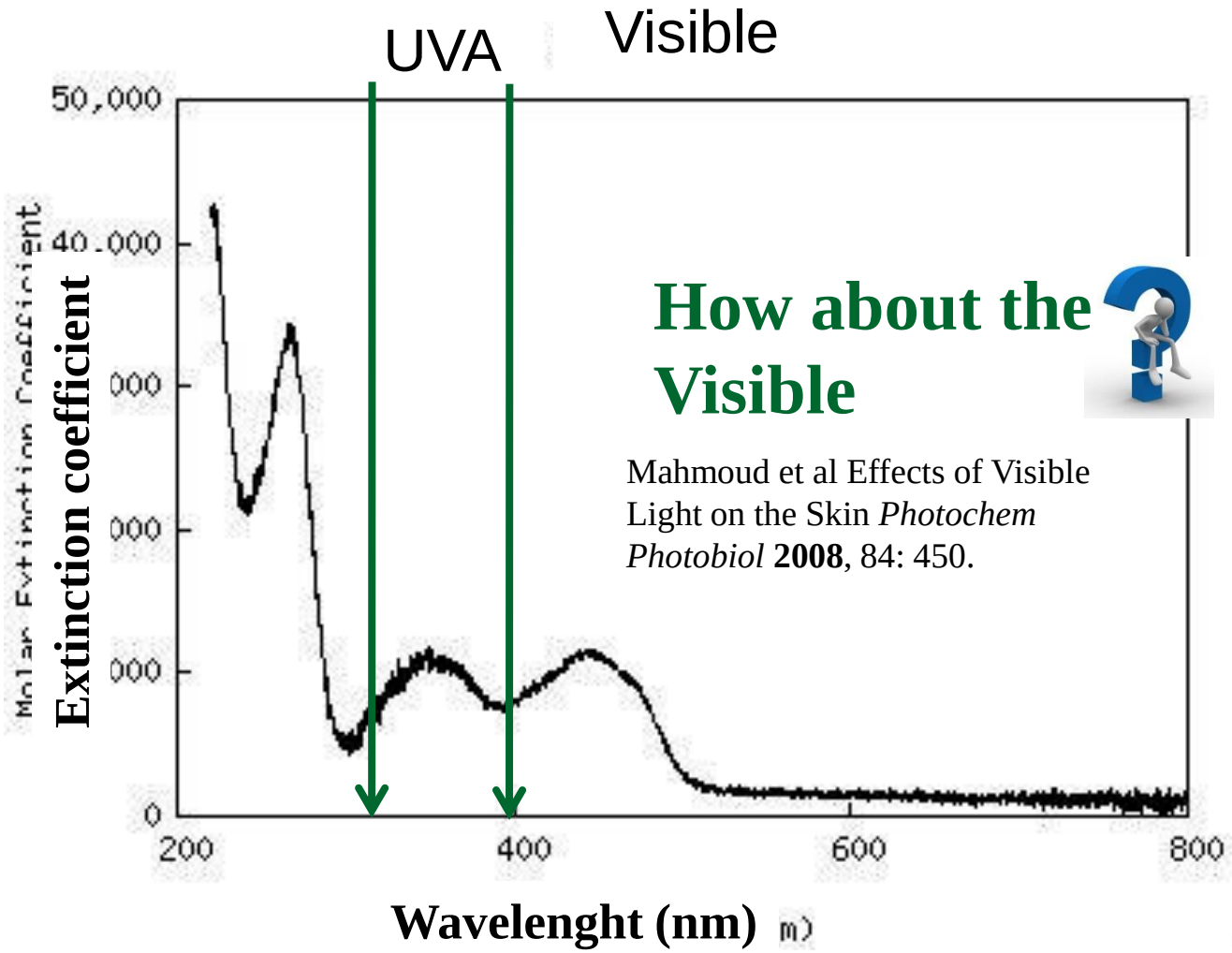
- S Mouret et al, *PNAS* **2006**, 103, 13765.
- Bäumler et al *Biophys J* **2006**, 91, 1452;

AV Silva et al *Tetrahedron* **2015**, 71, 457.

Flavins





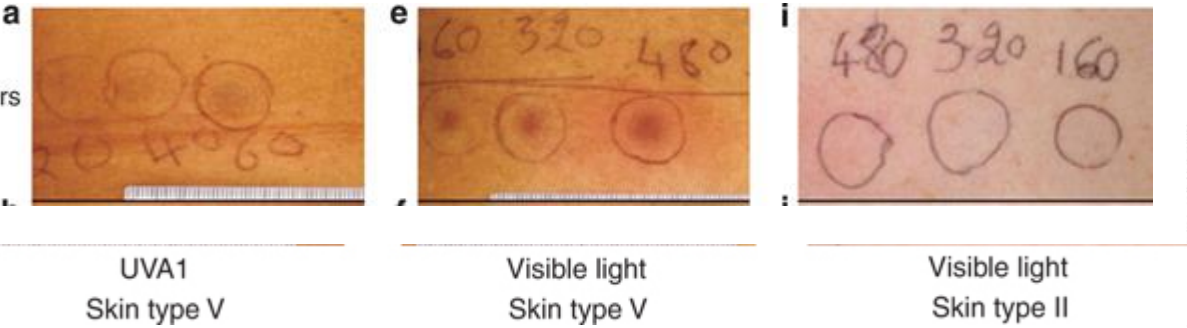


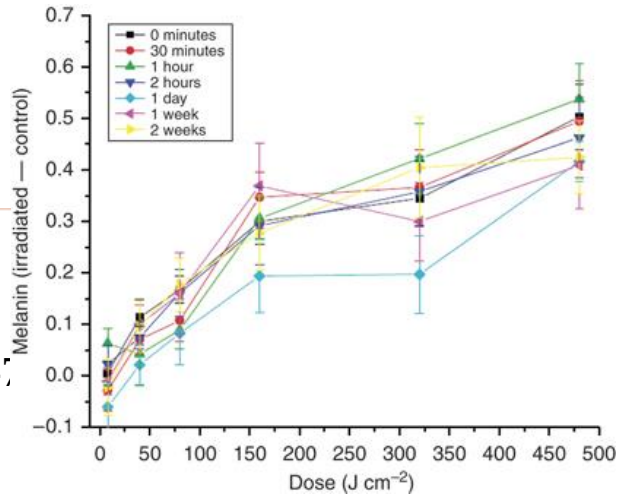
How about the Visible

Mahmoud et al Effects of Visible Light on the Skin *Photochem Photobiol* **2008**, 84: 450.

Pigmentation induced by visible light was darker and more sustained than pigmentation induced by UVA in melano competent individuals

Type II skin does not pigment well, neither with UVA nor with visible!!!

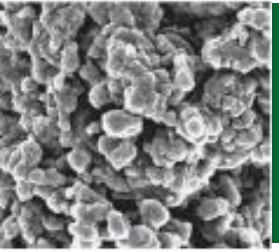




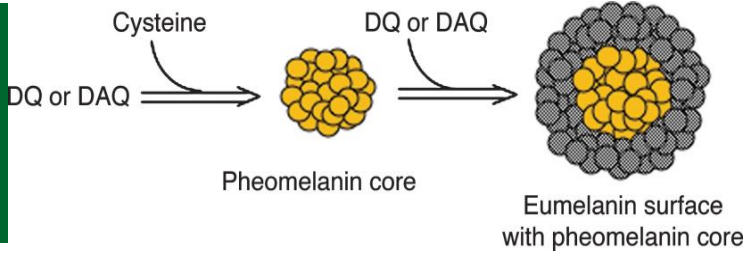
Mahmoud…H.W. Lim et al *J Invest Dermatol* **2010**, 130(8):2092-7
J&J: NJ USA; Dermatology Detroit,Michigan USA

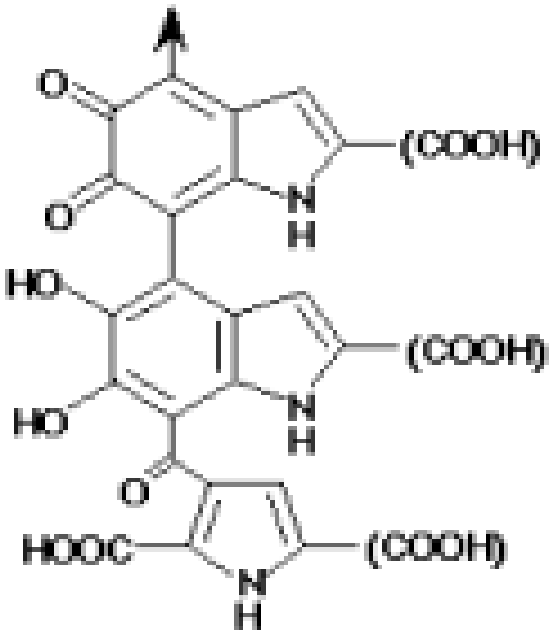
Does melanin cause any harm in the skin that would favor the synthesis of more melanin?

Nofsinger J, Liu Y, Simon JD *Free Rad. Biol. Med.* **2002**, 32, 720.

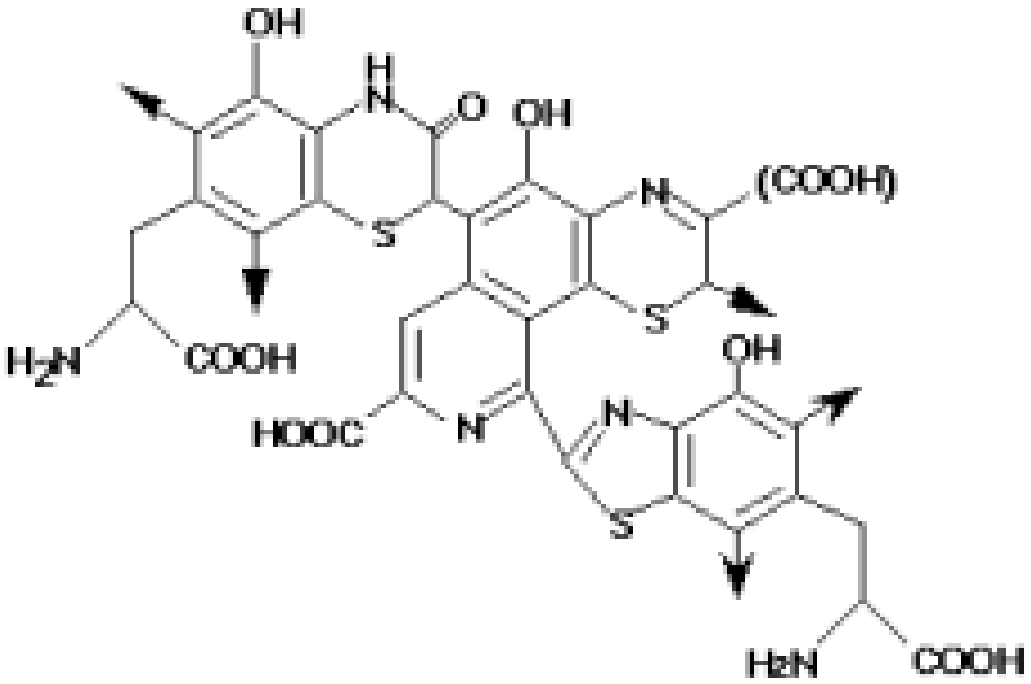


Melanin





Eumelanin

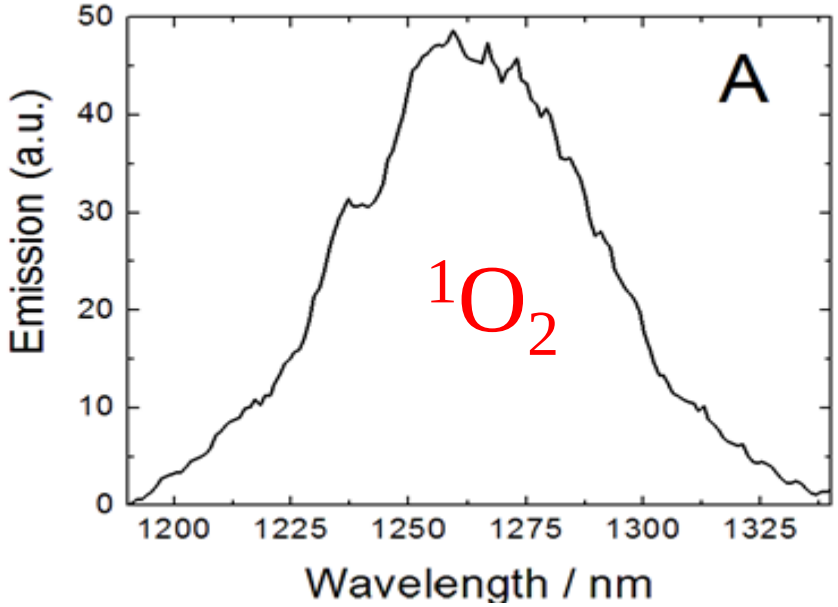


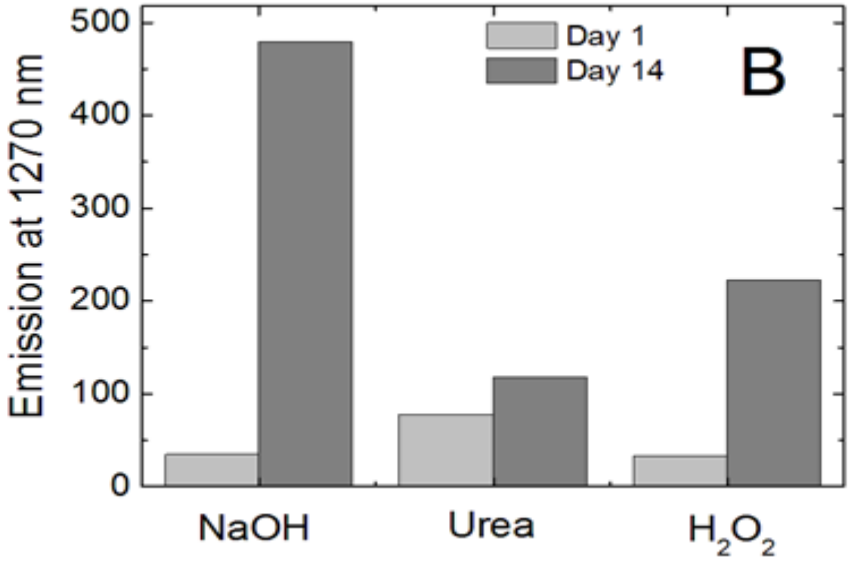
Pheomelanin

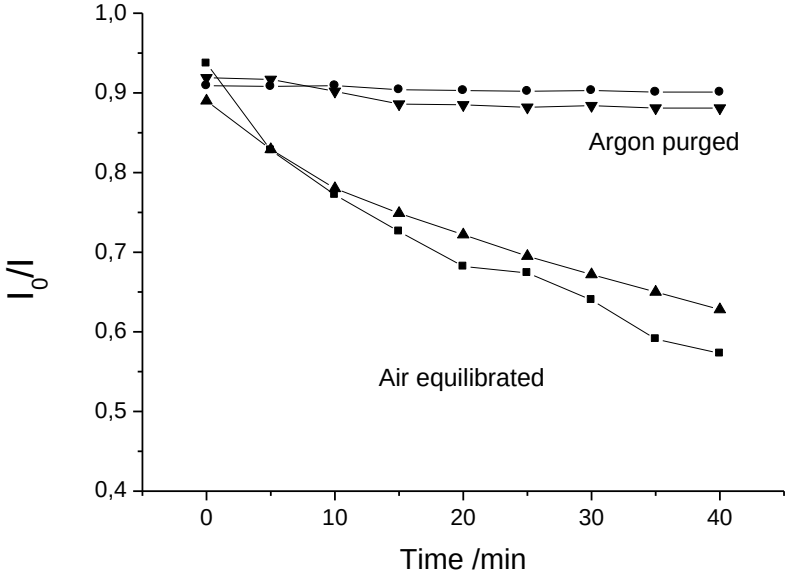
K Wakamatsu and S Ito *Pigment Cell Res* 2002, 15.

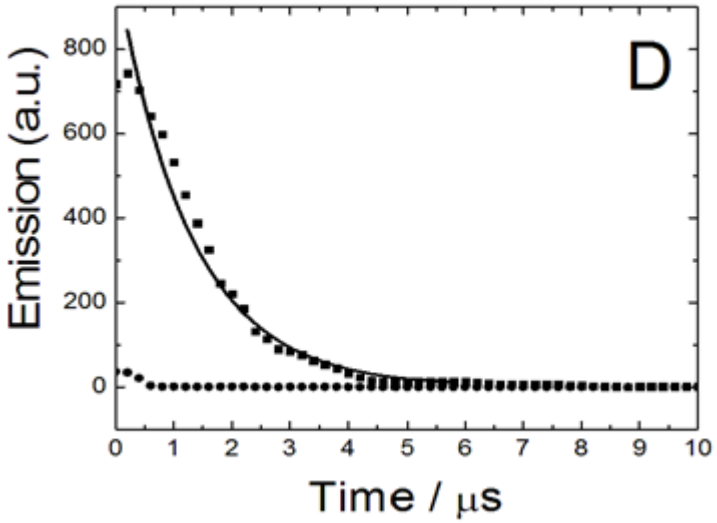
Does melanin generate $^1O_2$

Melanin generates singlet oxygen with irradiation in VISIBLE (532nm)

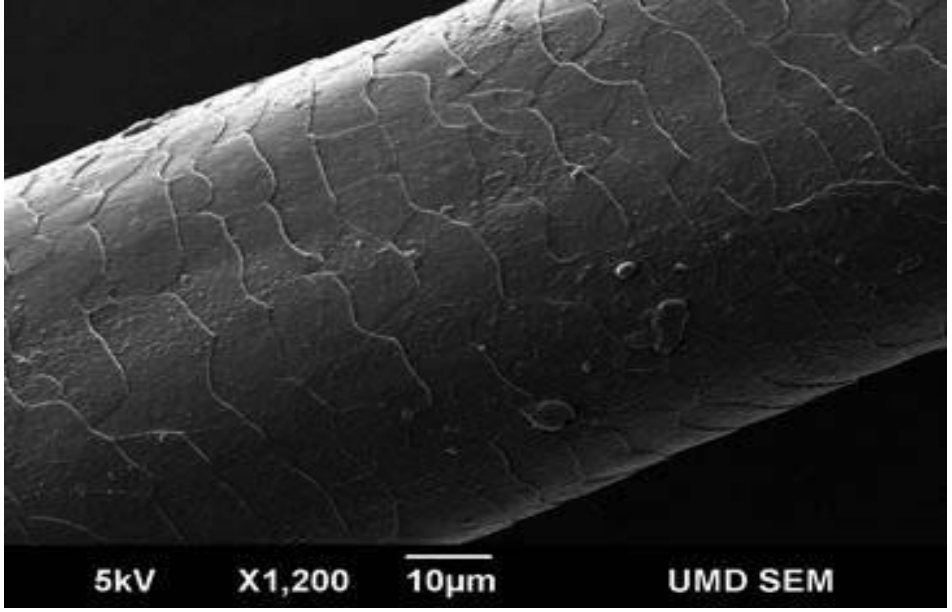


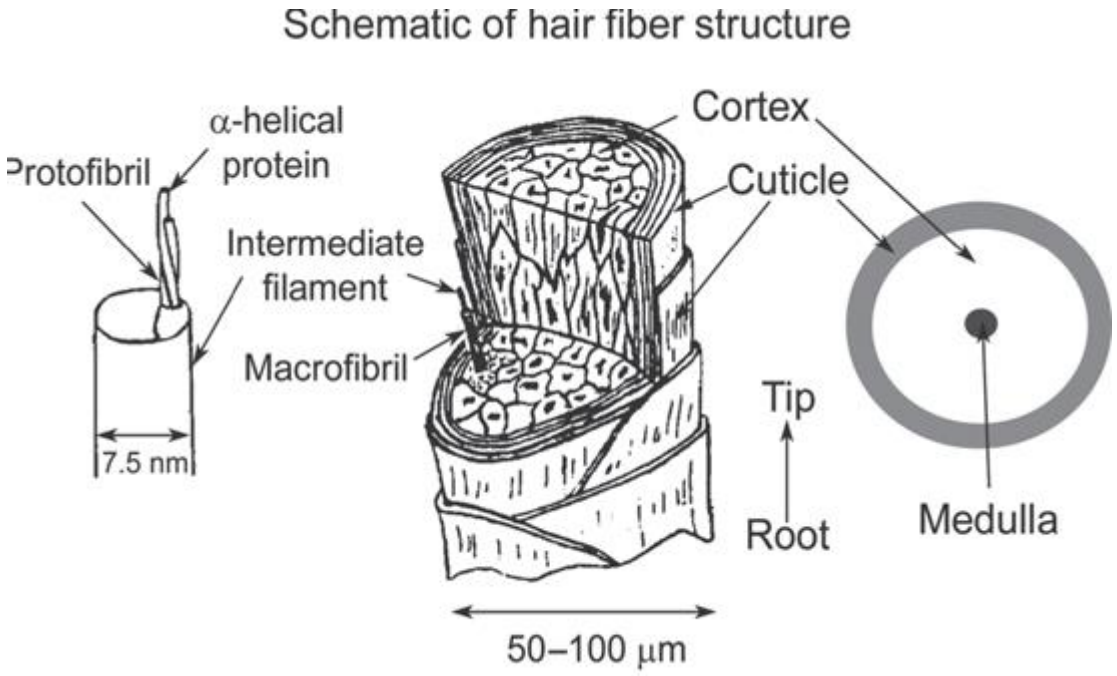




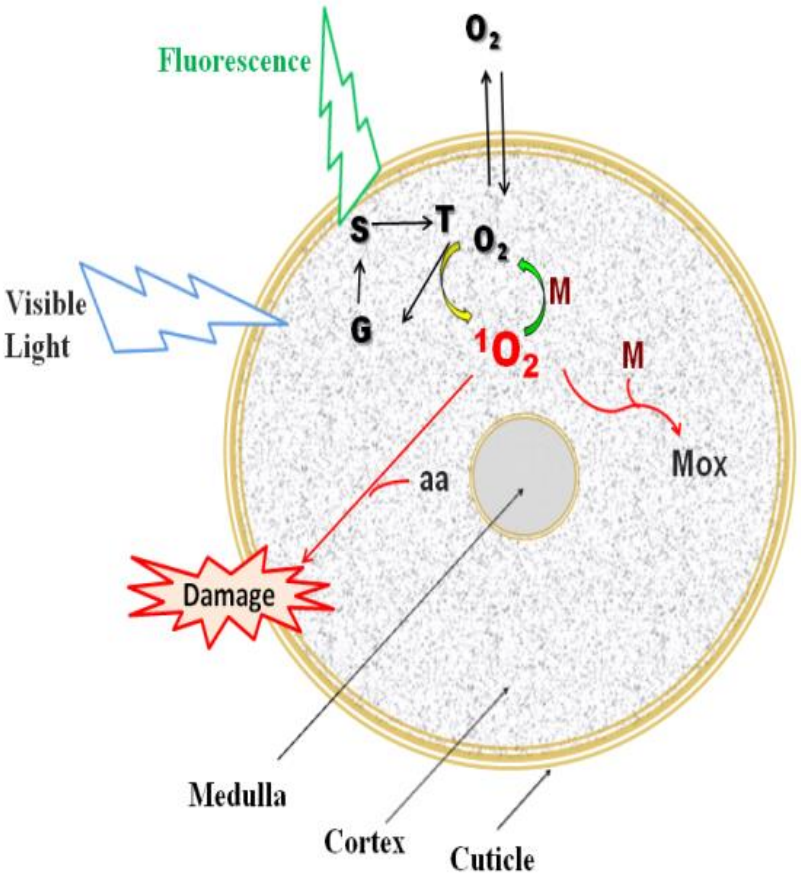


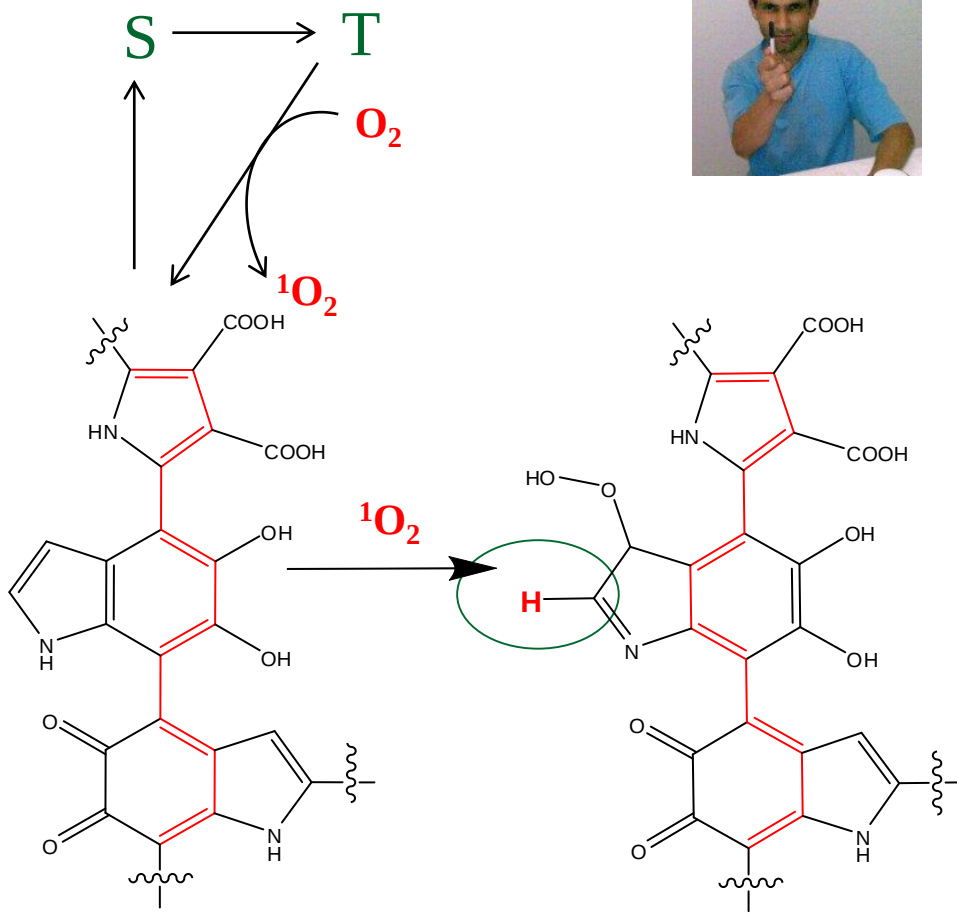
Will melanin photosensitization of singlet oxygen affect hair?





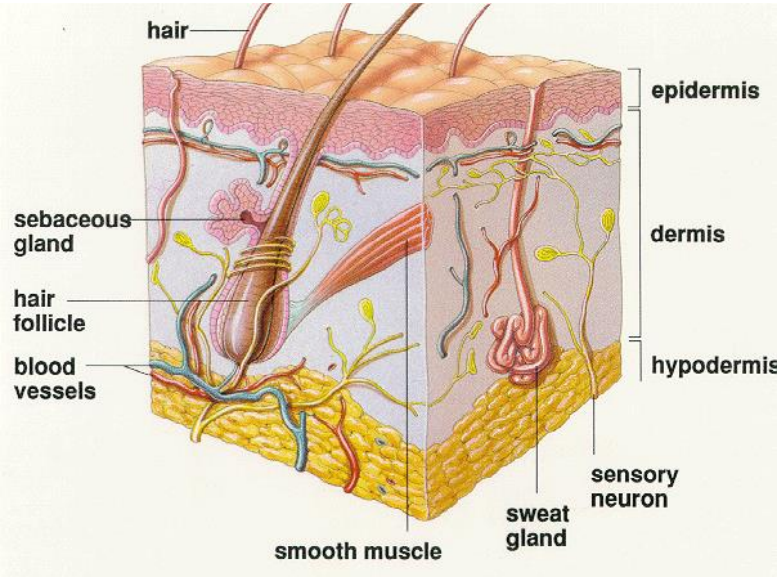
Photolysis of melanin with visible light (400-700nm) is much faster in the presence of oxygen. Photolysis product showed the presence of a hydroperoxide at C3 of indol





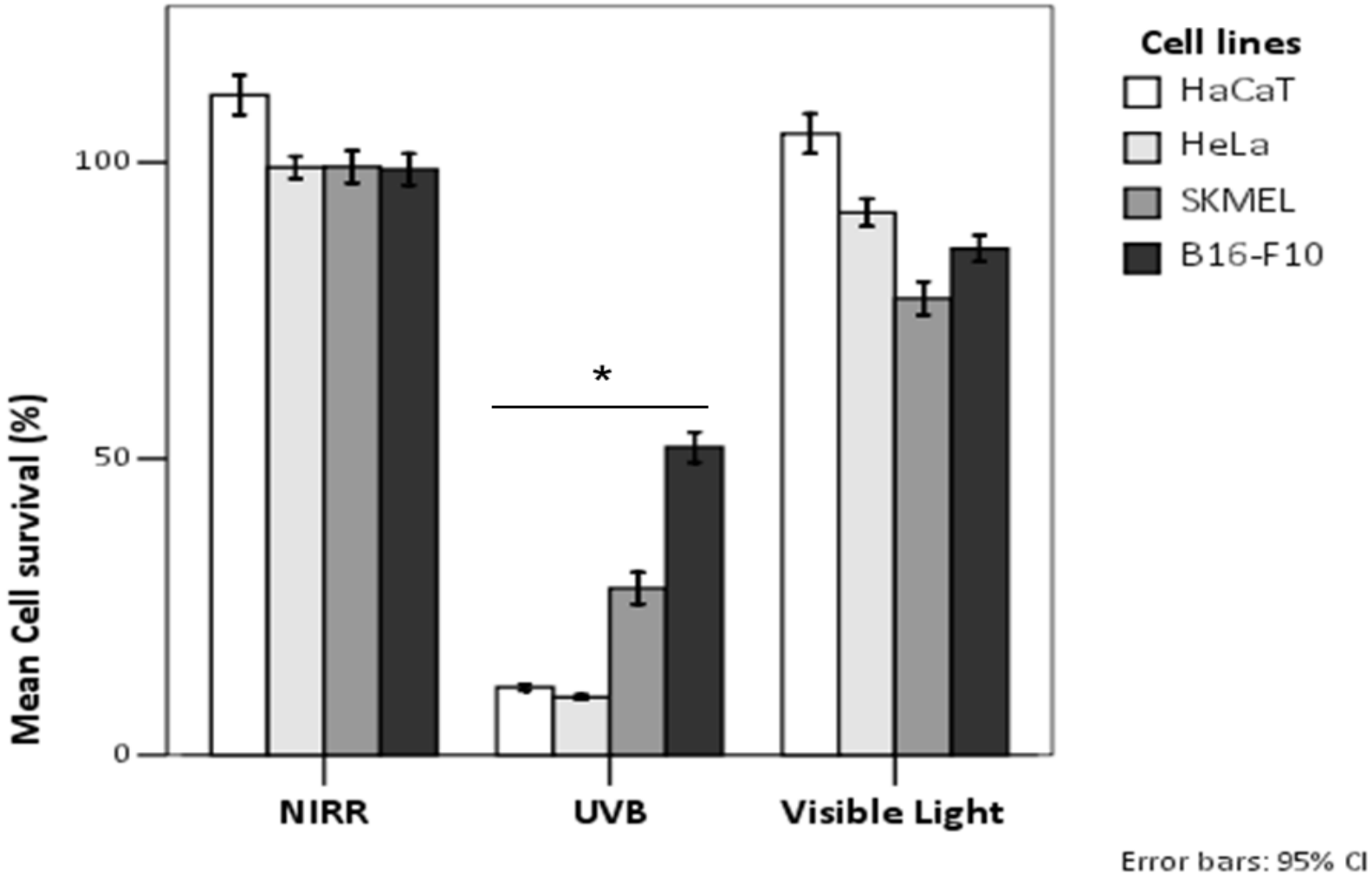
Chiarelli-Neto et al *Free Radic Biol Med* **2011**, 51, 1195

Will the photosensitization of melanin affect the viability of melanocompetent cells



Chiarelli-Neto O et al. (2014) Melanin Photosensitization and the Effect of Visible Light on Epithelial Cells. PLoS ONE 9(11): e113266

Melanin protects against UVB



PROTOCOL OF MELANIN OVER-STIMULATION IN MELANO-COMPETENT CELLS

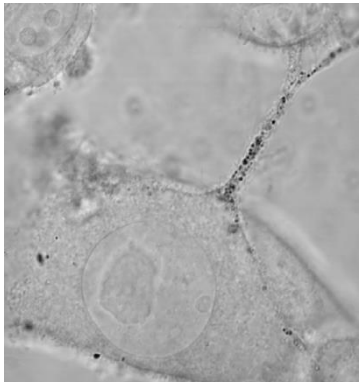
METHODS

B16F10 Cells

Tyr +NH_4Cl

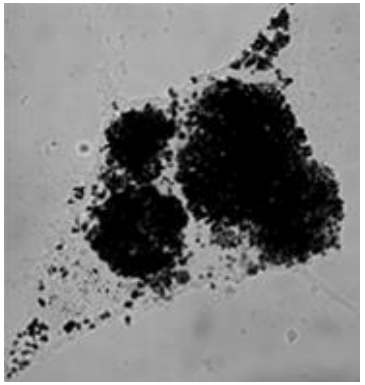
48h

$(Melanin)_n$



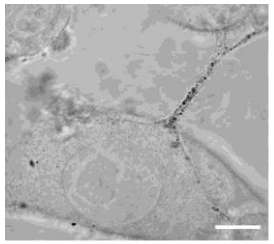
B16F10

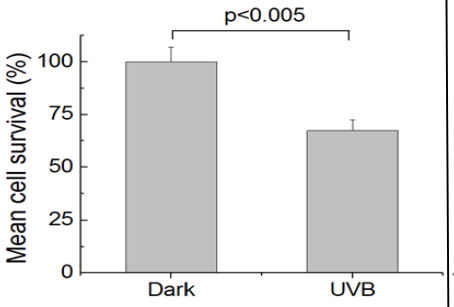
48h

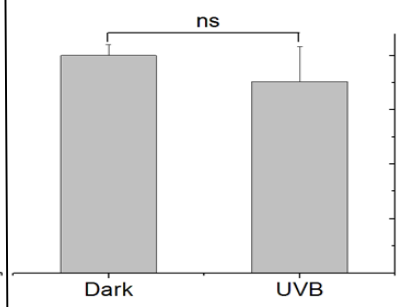


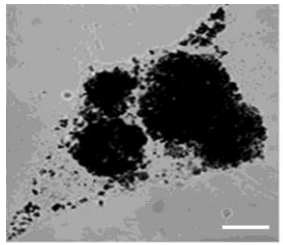
B16F10

A

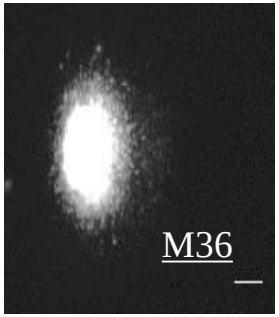


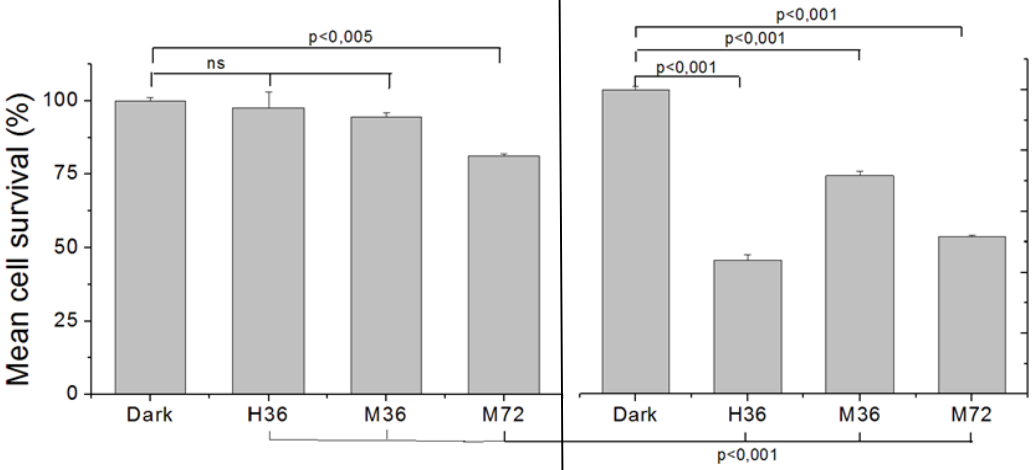

p<0.005
Mean cell survival (%)
100
75
50
25
0
Dark
UVB

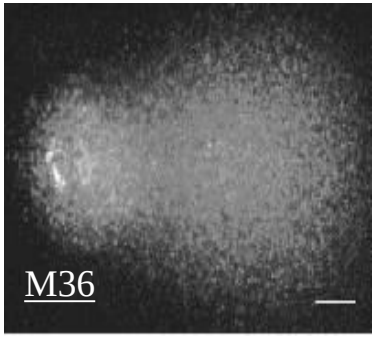

ns
Dark
UVB



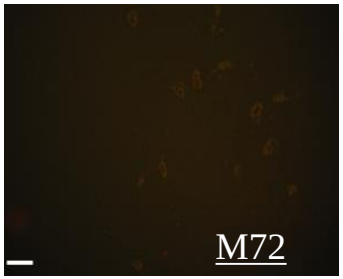
B

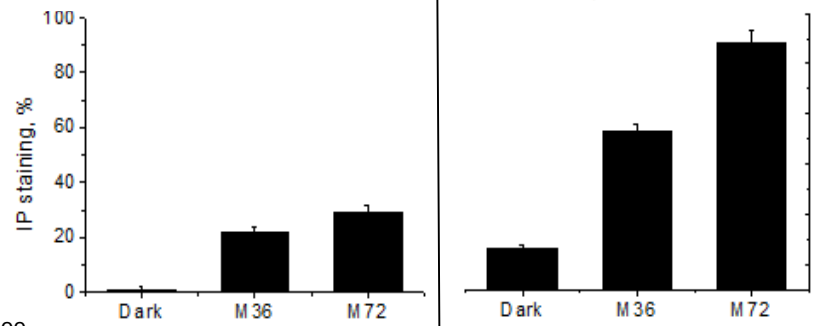

M36

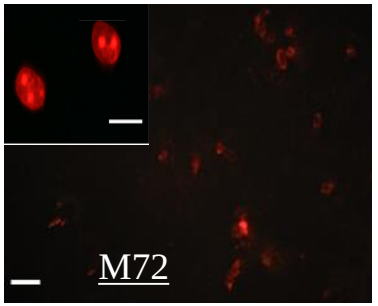

p<0,005
ns
Mean cell survival (%)
100
75
50
25
0
Dark
H36
M36
M72
p<0,001
p<0,001
p<0,001
p<0,001
Dark
H36
M36
M72


M36

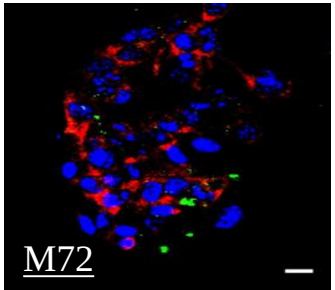
C

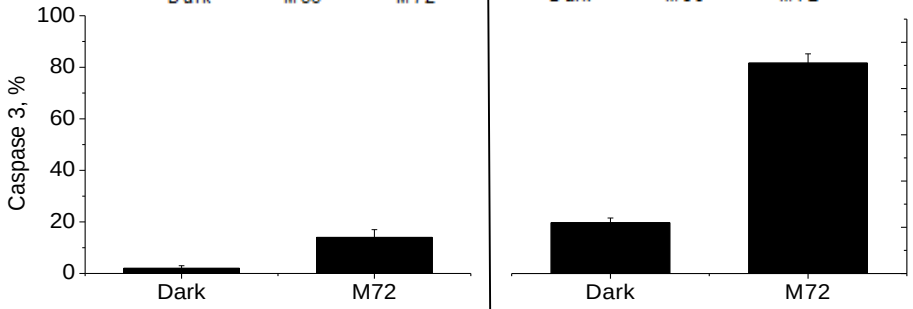

M72

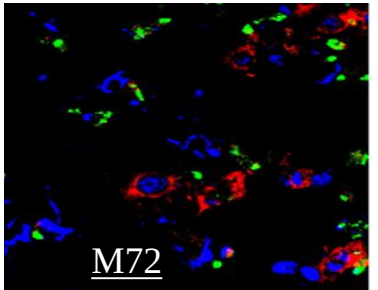

IP staining, %
100
80
60
40
20
0
Dark
M36
M72
Dark
M36
M72


M72

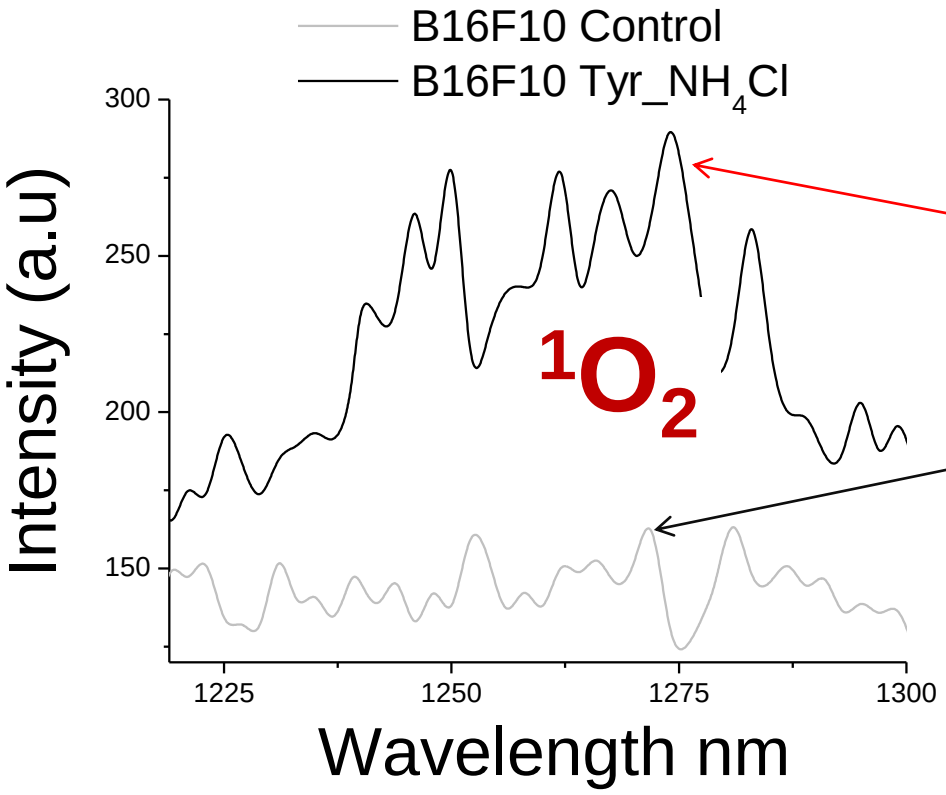
D

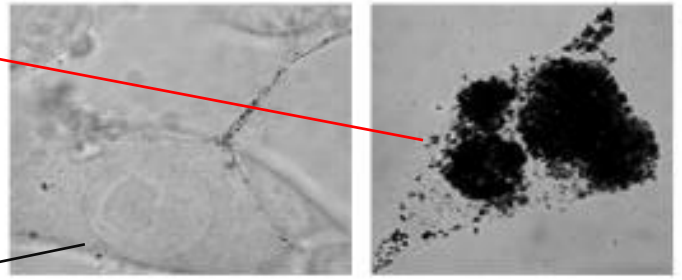

M72


Caspase 3, %
100
80
60
40
20
0
Dark
M72
Dark
M72


M72

$^1O_2$ IN OVER PIGMENTATED B16F10 CELLS



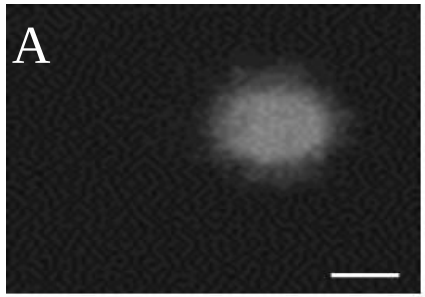


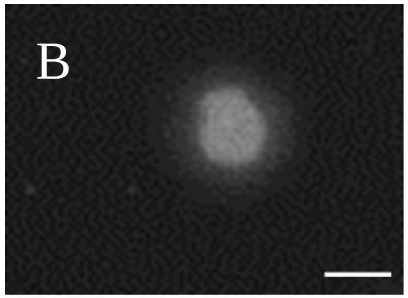
B16F10 B16F10

Tyr (0,5 mM)+NH_4Cl (10mM)

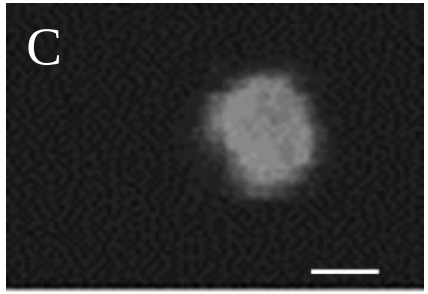
There is $^1O_2$ generation under visible light in cells over pigmentated. The over pigmentation causes damages in cells.

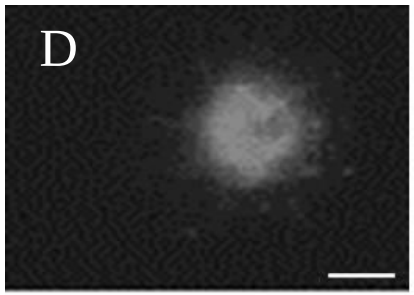
Melanin **CT** +++++



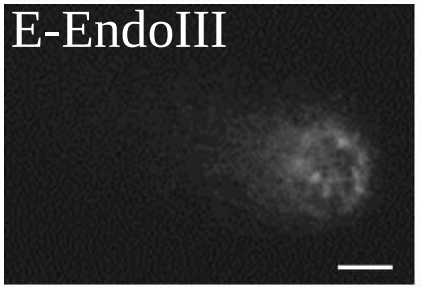


0

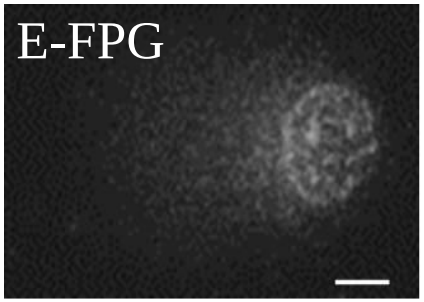




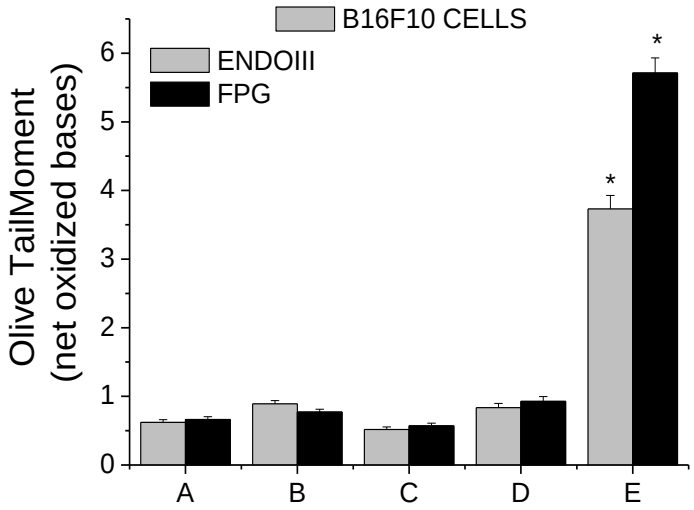
6J



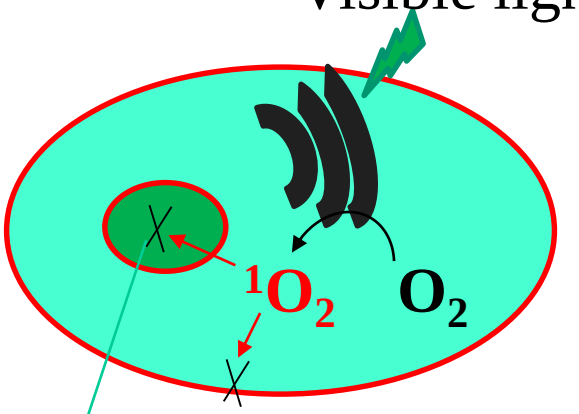
6J

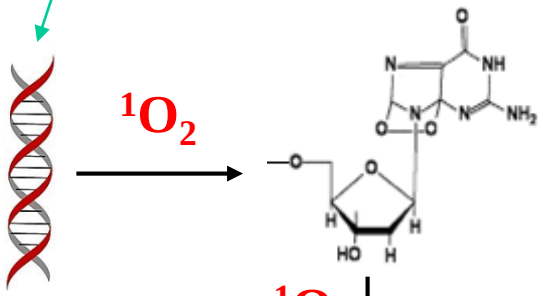


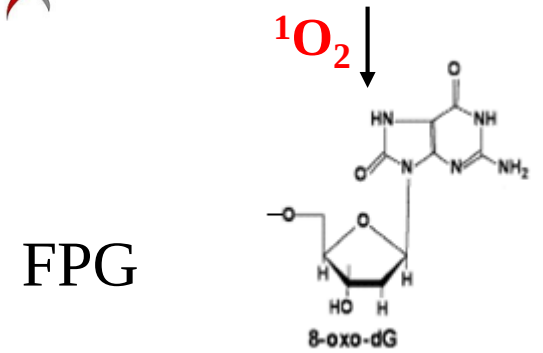
6J



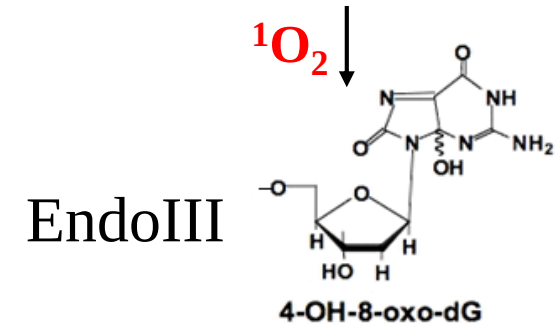
Visible light





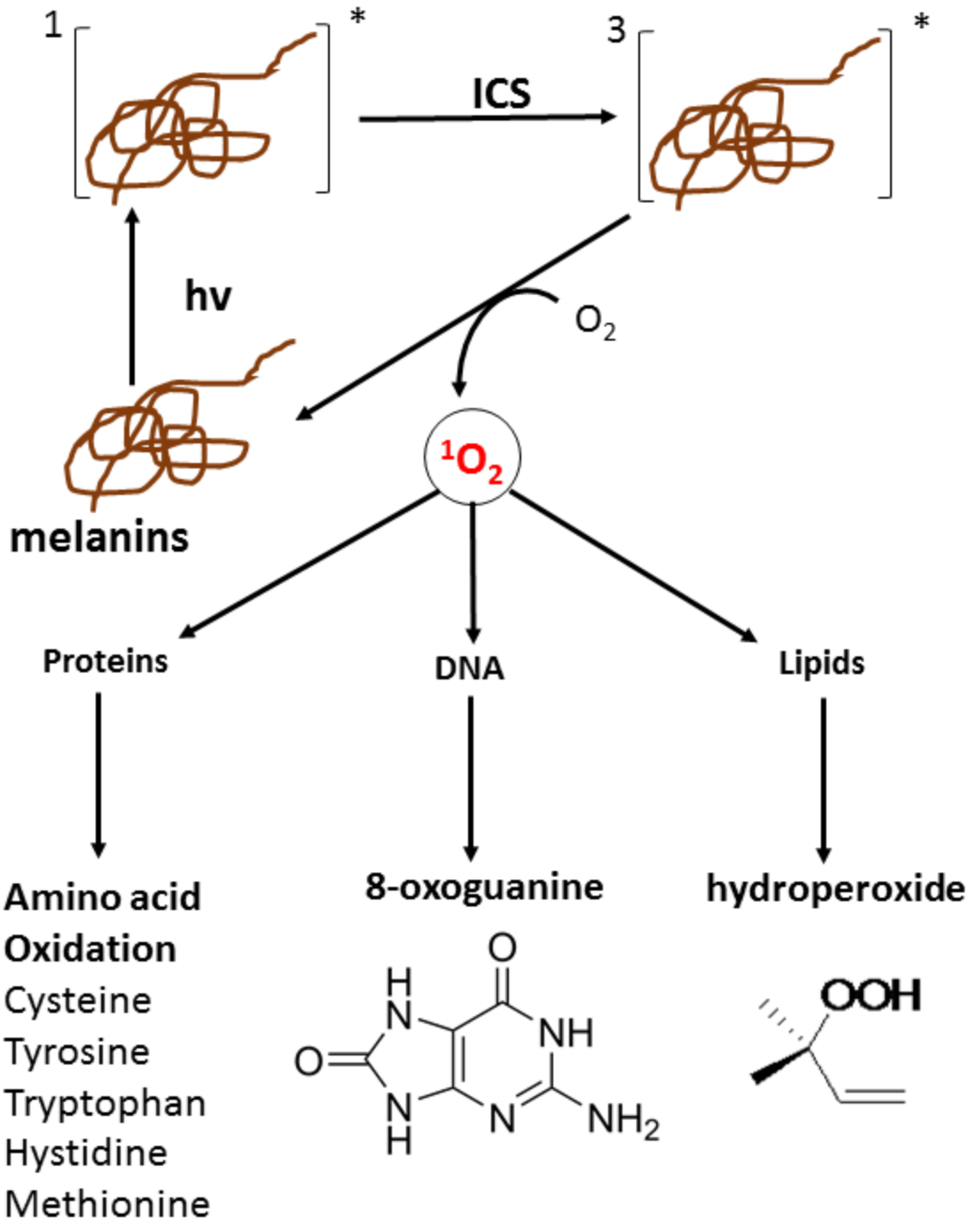


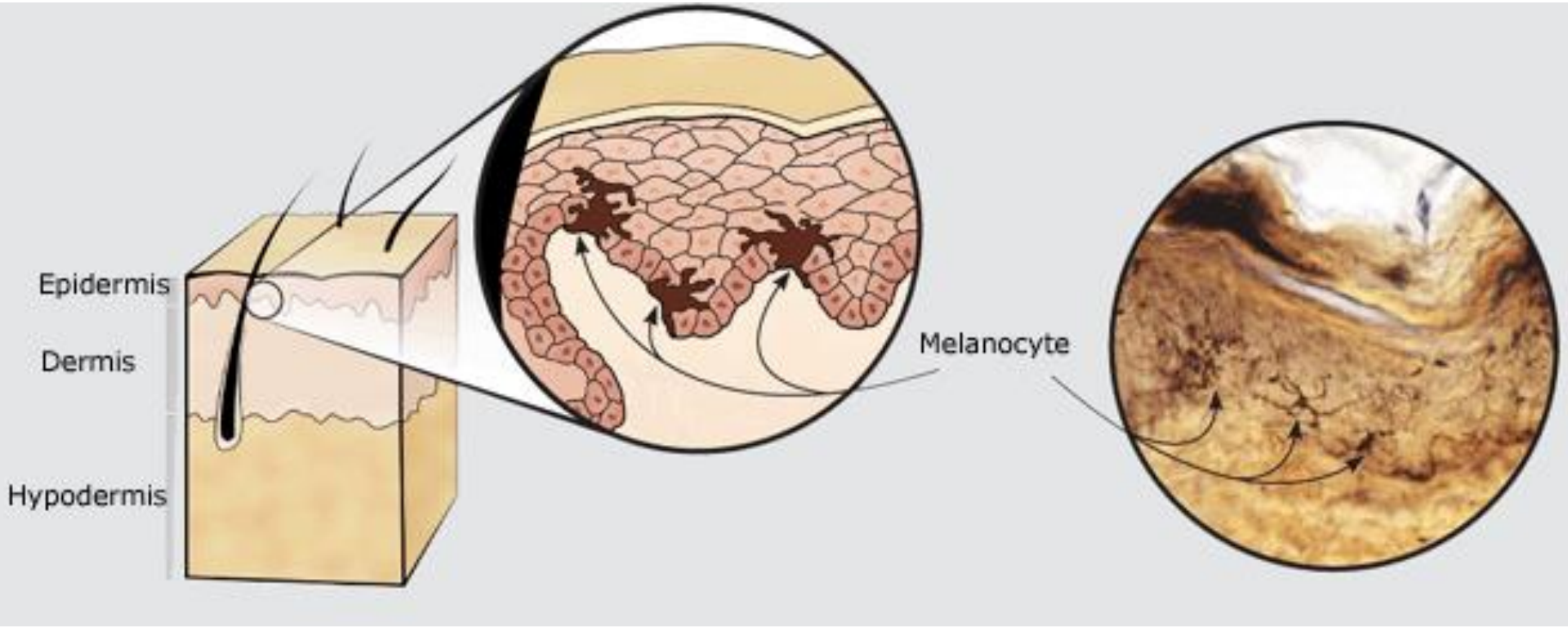
FPG



EndoIII

Visible light--singlet oxygen--DNA damage

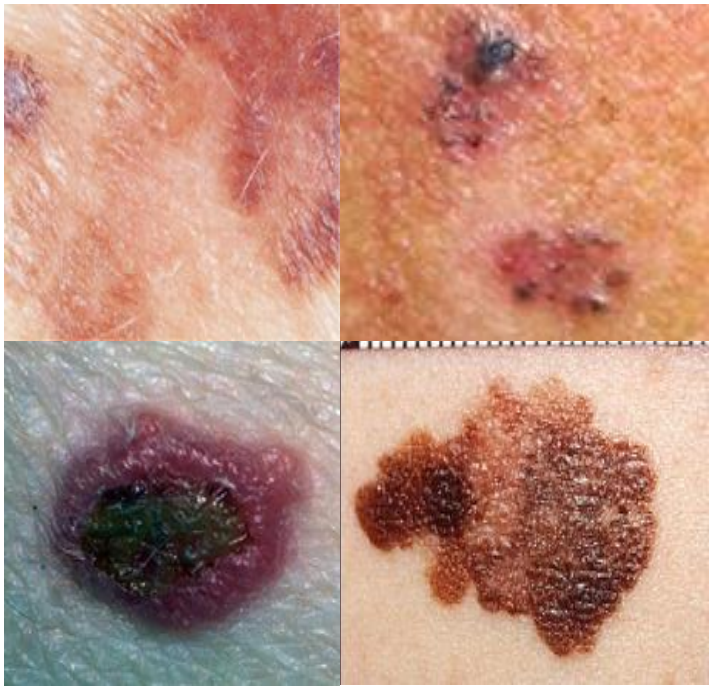

1 *
ICS
3 *
hv
O_2
melanins
$^1O_2$
Proteins
DNA
Lipids
Amino acid
Oxidation
Cysteine
Tyrosine
Tryptophan
Hystidine
Methionine
8-oxoguanine
hydroperoxide
OOH

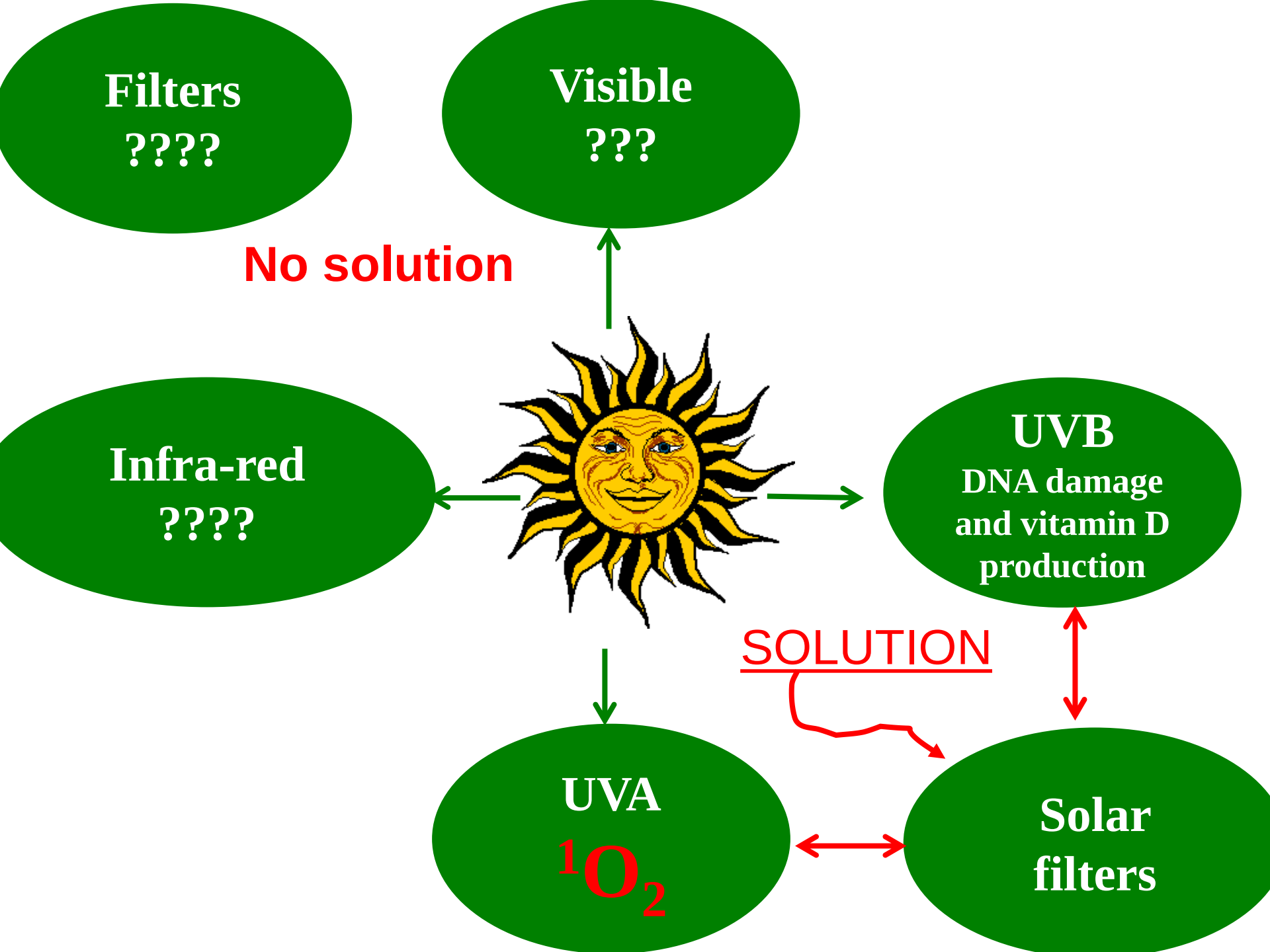

Epidermis
Dermis
Hypodermis
Melanocyte

Conclusion

Visible light should also be considered in protecting the skin from photoinduced damage. It is certainly involved in the photoaging and other consequences in the skin.

How the melanin photosensitization affects the evolution of melanome and/or of other pigmented cancerous lesions?




Filters
????
Visible
???
No solution
Infra-red
????
UVB
DNA damage
and vitamin D
production
SOLUTION
UVA
$^1O_2$
Solar
filters

Visible
???

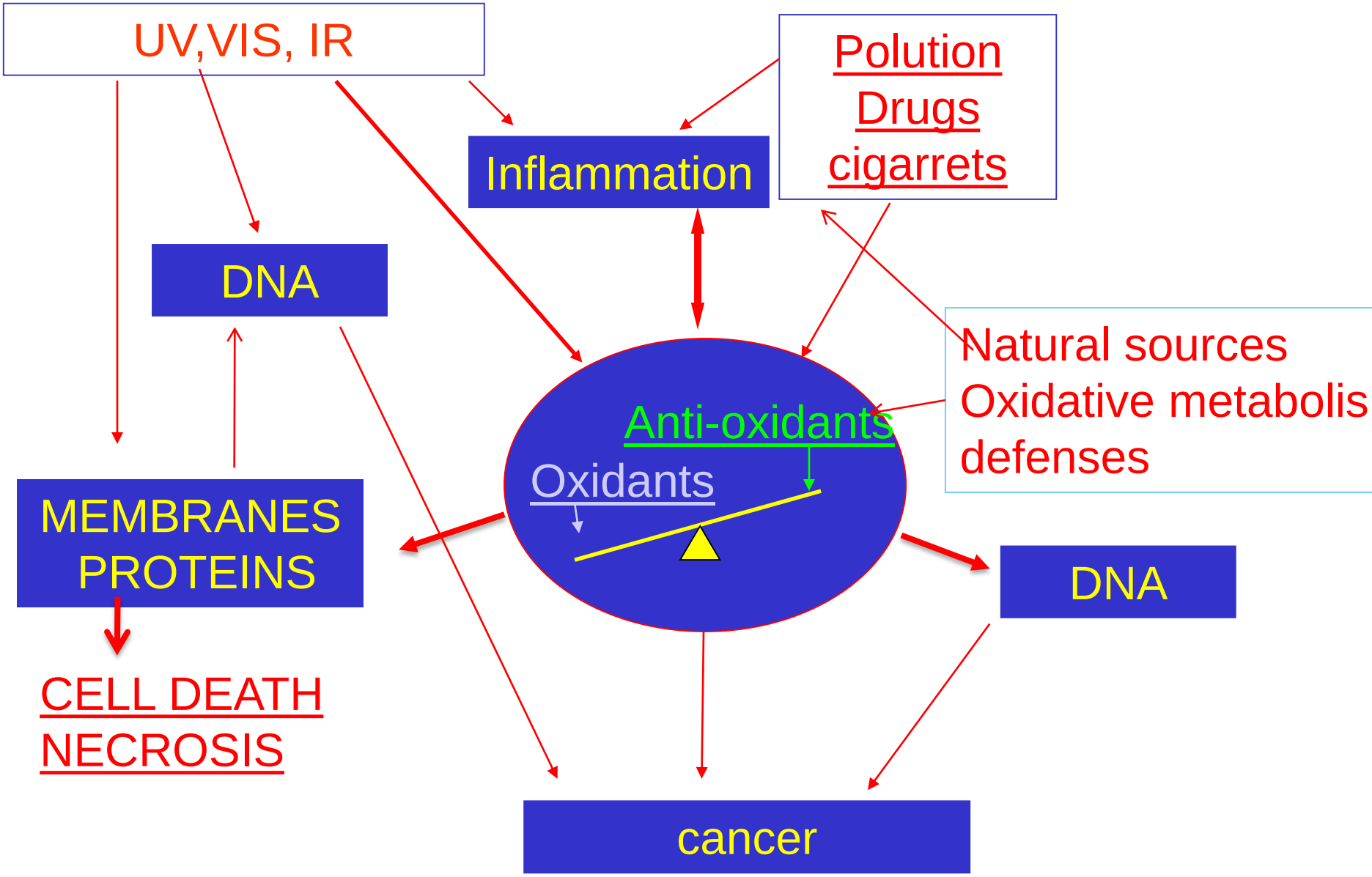
Filters
????

Anti-oxidant

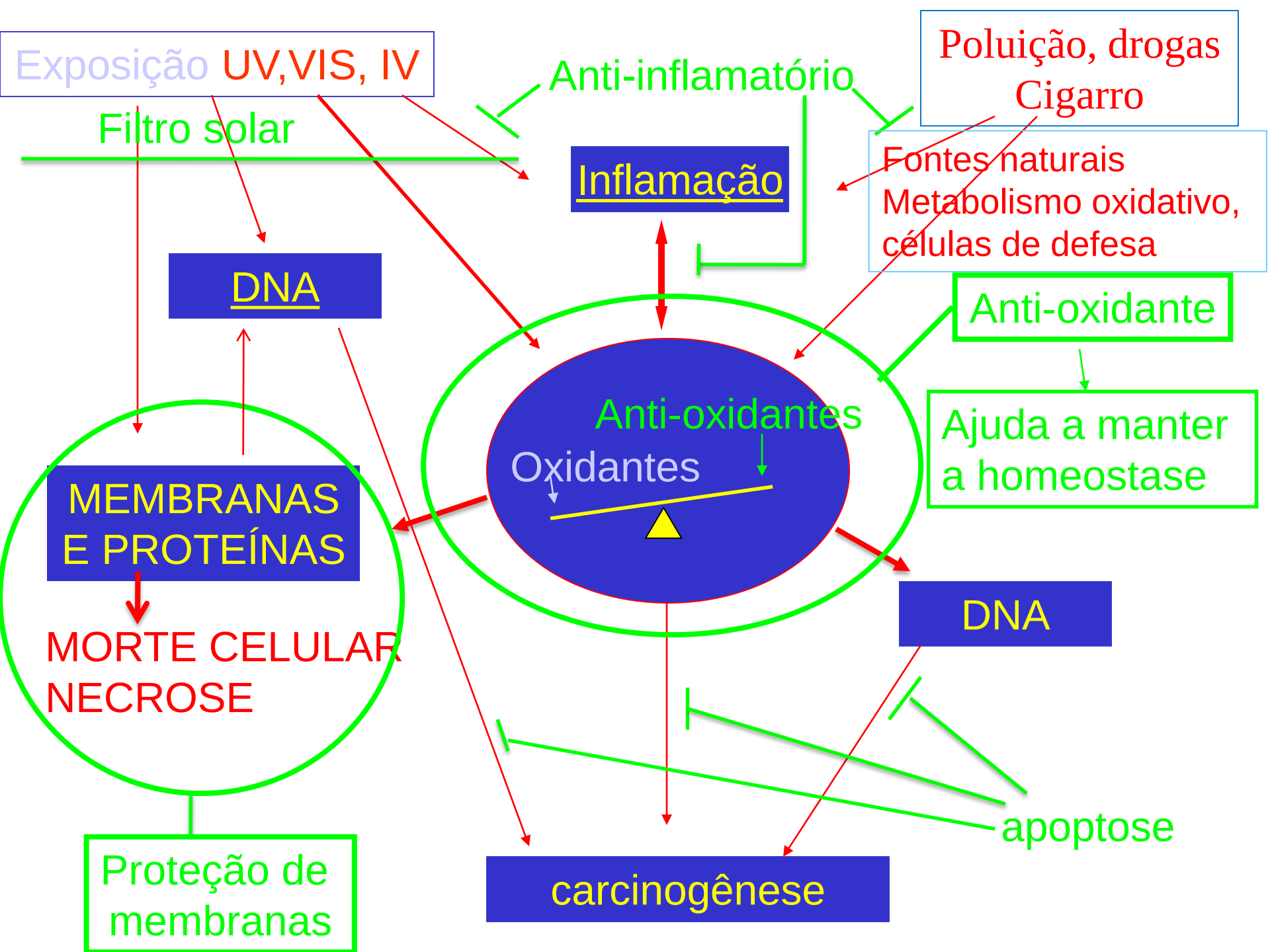
Membrane
protection

Visible
???

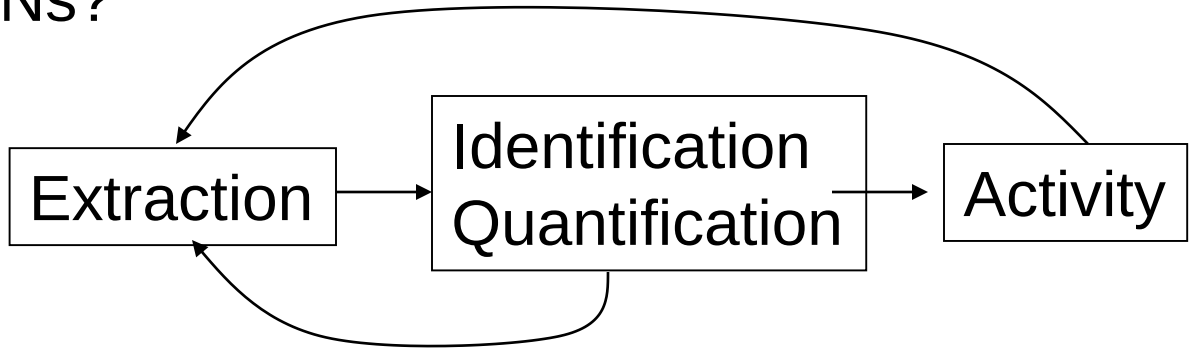
Anti-oxidant



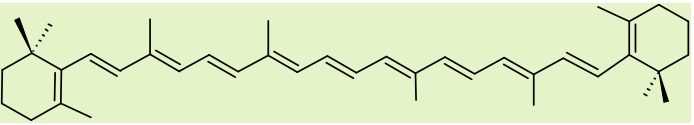
Baptista MS Photochemistry, Photobiology, and Redox Balance in Skin and Hair. Part I & II. *Cosmetiscope*, New York, 17(1, 3), p. 1-11; p. 1-10.


Exposição UV,VIS, IV
Filtro solar
Anti-inflamatório
Poluição, drogas
Cigarro
Fontes naturais
Metabolismo oxidativo,
células de defesa
Inflamação
DNA
Anti-oxidante
Anti-oxidantes
Oxidantes
Ajuda a manter
a homeostase
MEMBRANAS
E PROTEÍNAS
MORTE CELULAR
NECROSE
DNA
apoptose
Proteção de
membranas
carcinogênese

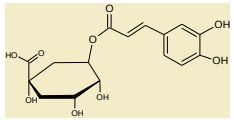
Which structures nature have developed to protect against EROs e ERNs?



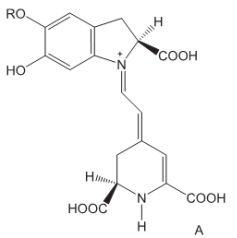
β-carotene



Flavonoids



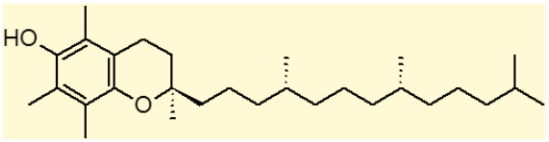
Ructin

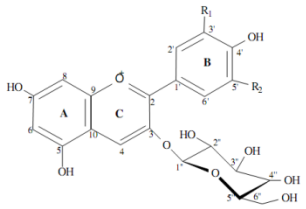


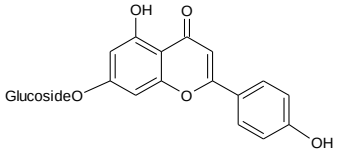
Ác. chlorogenic

Anthocyanines

α-tocopherol



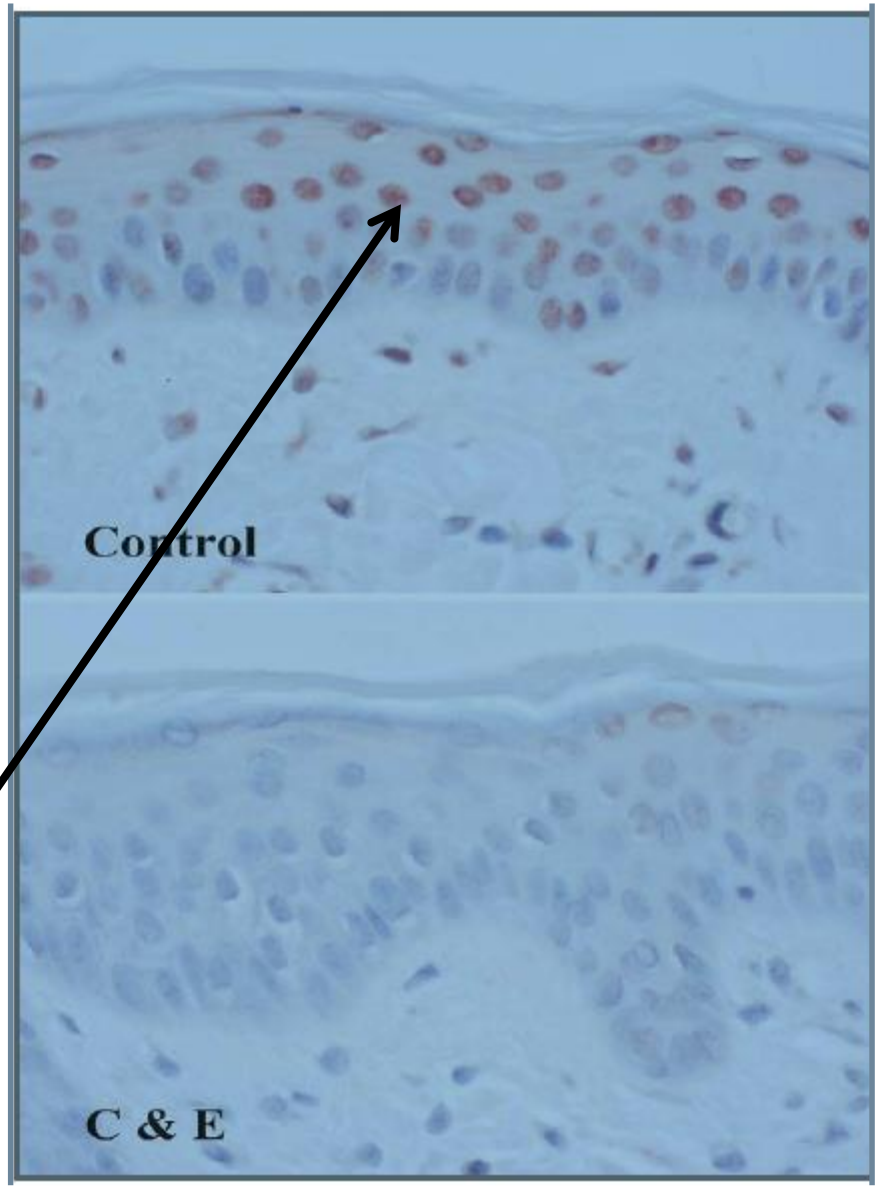




Apigenin

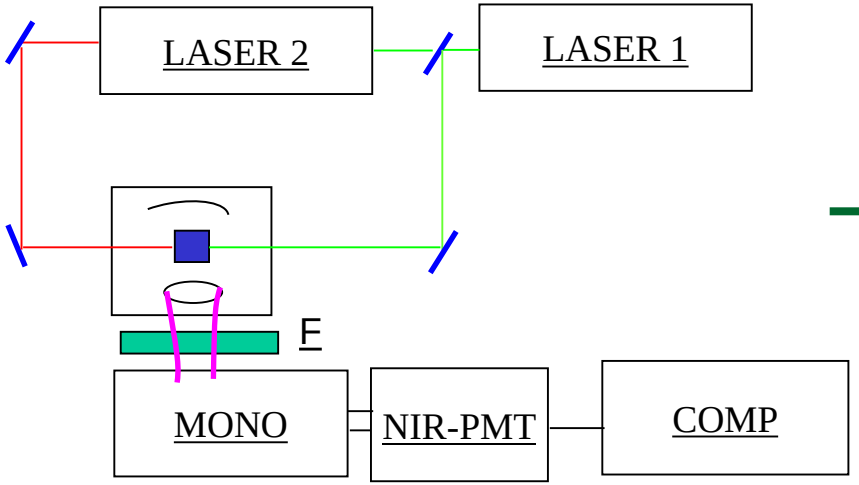
contra danos ocasionados por UV

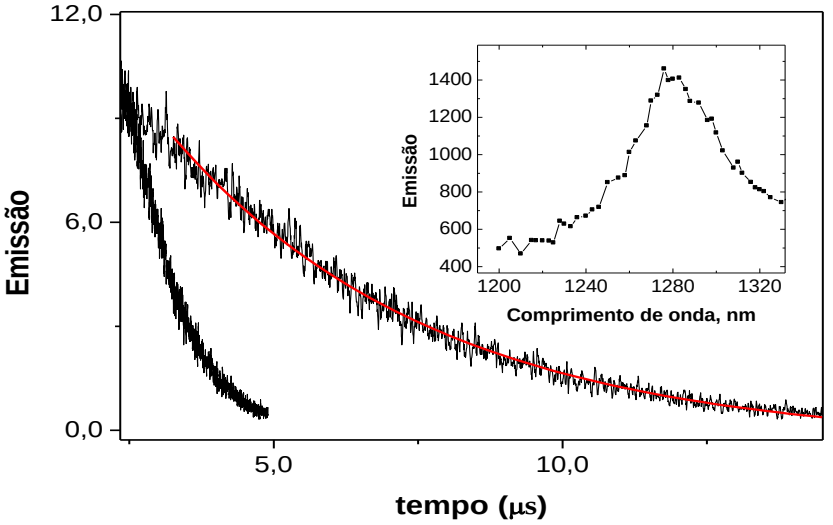
Combination of vitamins C and E was applied to the pigskin for 4 days. Control and treated skin were irradiated with simulated solar minimal erythema doses of UV radiation for 4 days. One day later thymine dimers were determined by immunohistochemistry.



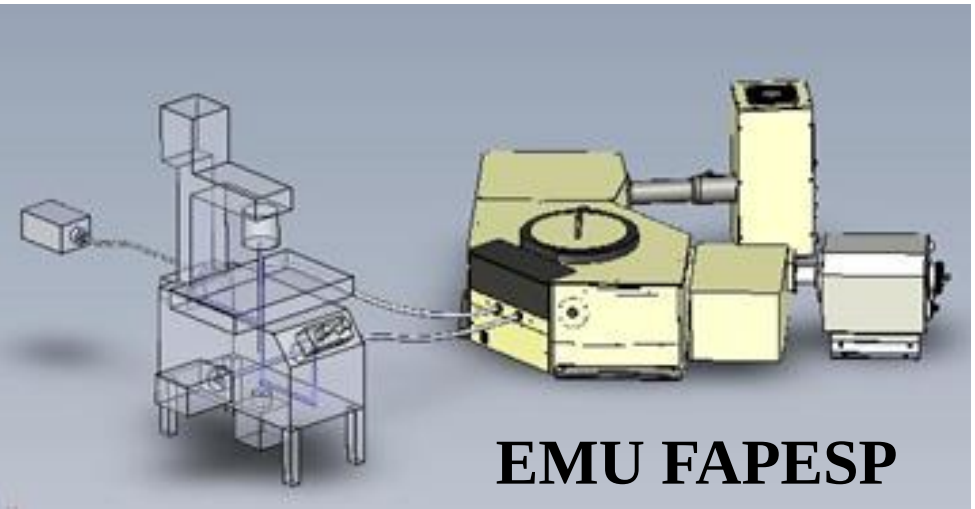
Jing-Yi Lin et al J Am Acad Dermatol 2003;48:866-74.

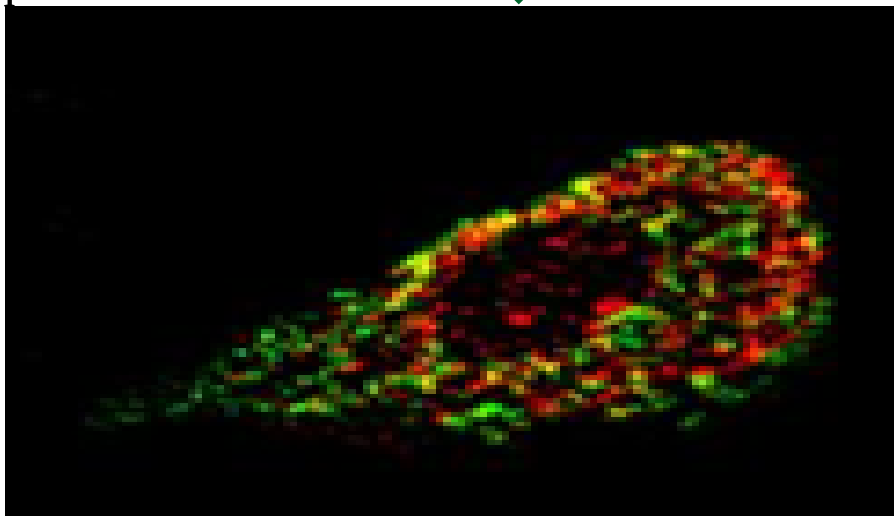
Instrumentation development do characterize and quantify $^1O_2$





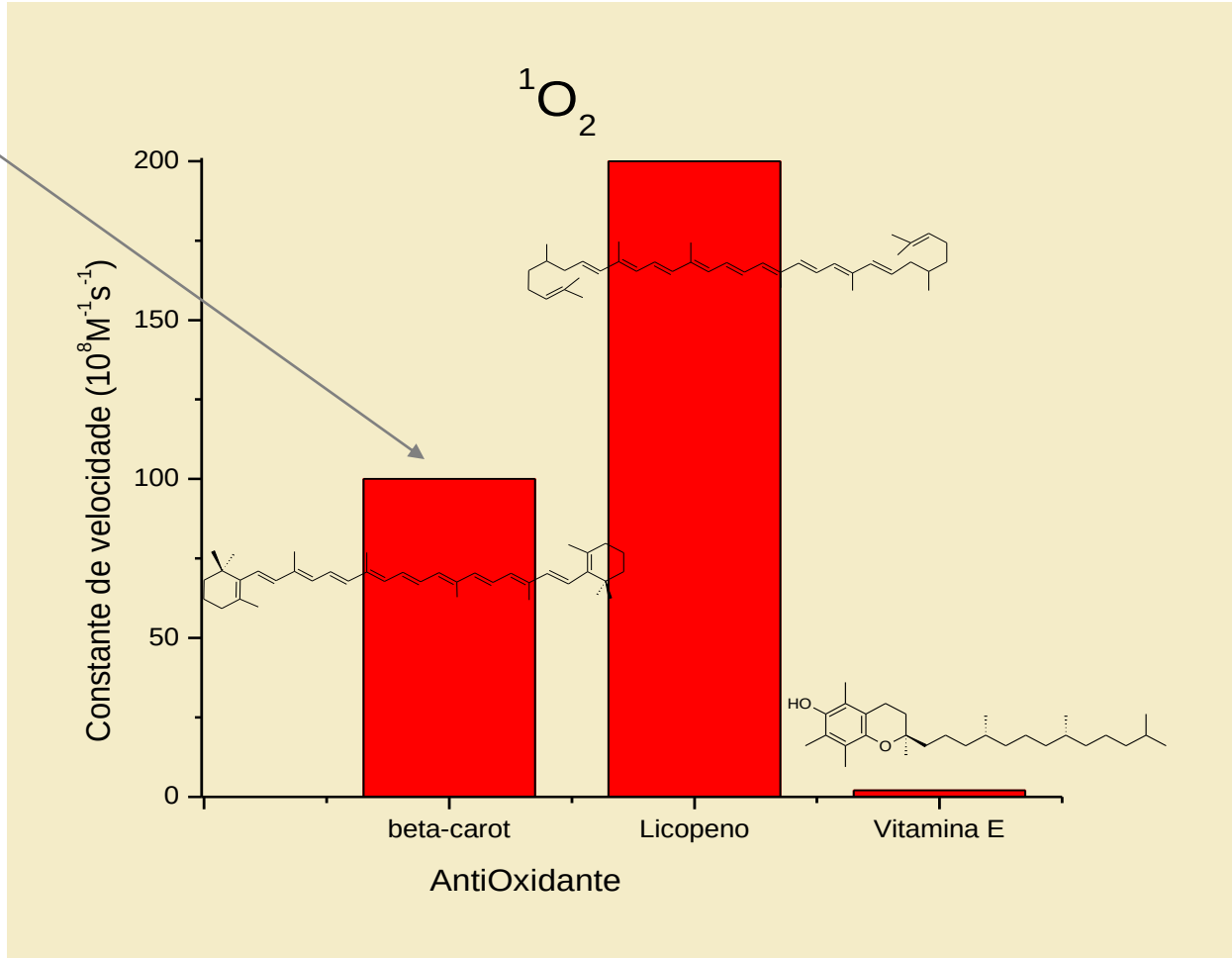
P. Di Mascio, M. Madeiros, S. Miyamoto
+ 20 pesquisadores

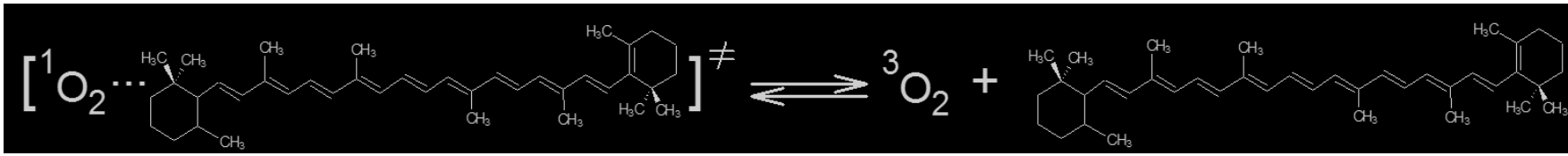




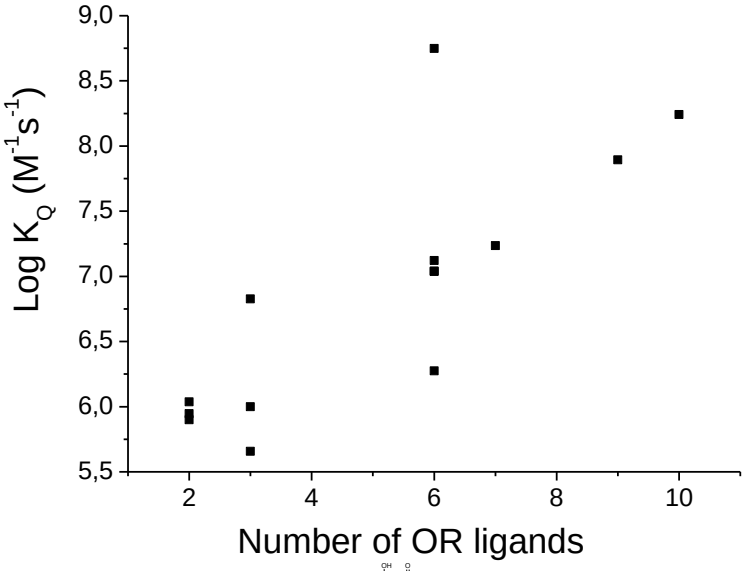
Efficiency and mechanism of $^1O_2$ supression

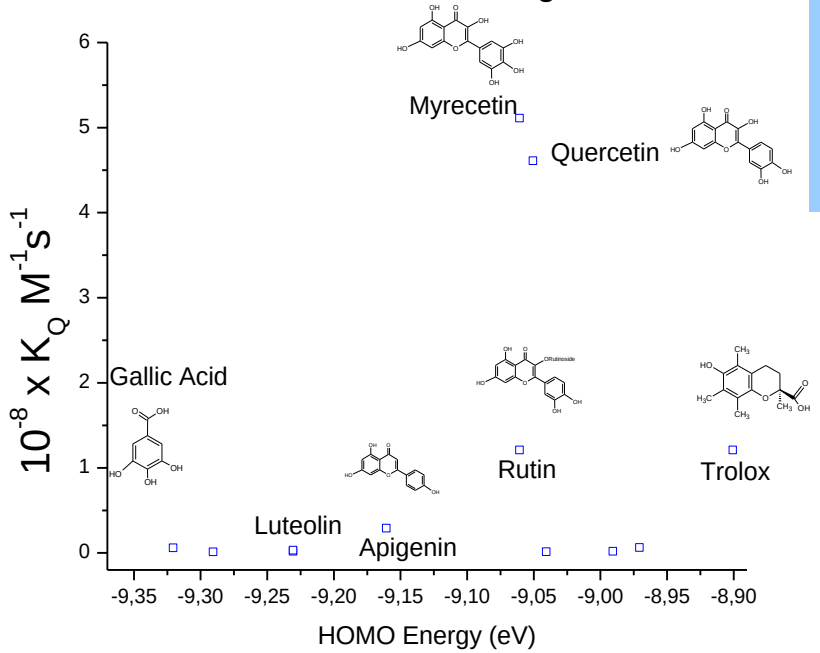
Efficiency depends on conjugated double bonds

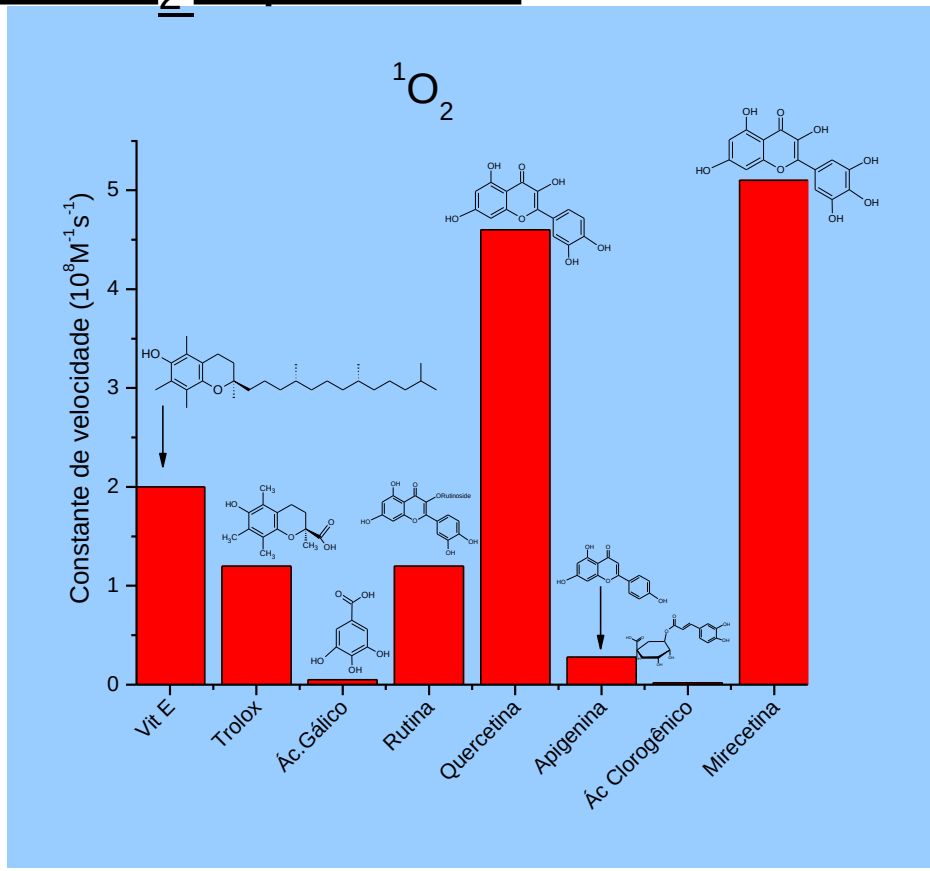


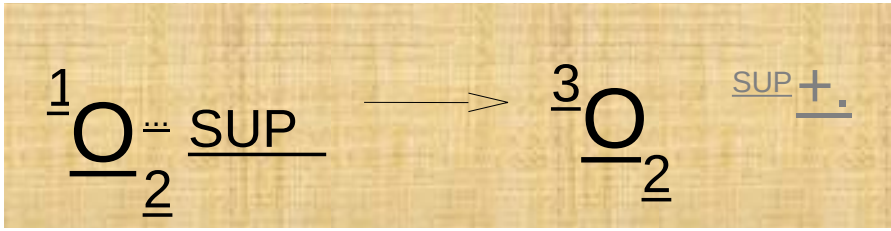


Efficiency and mechanism of $^1O_2$ supression





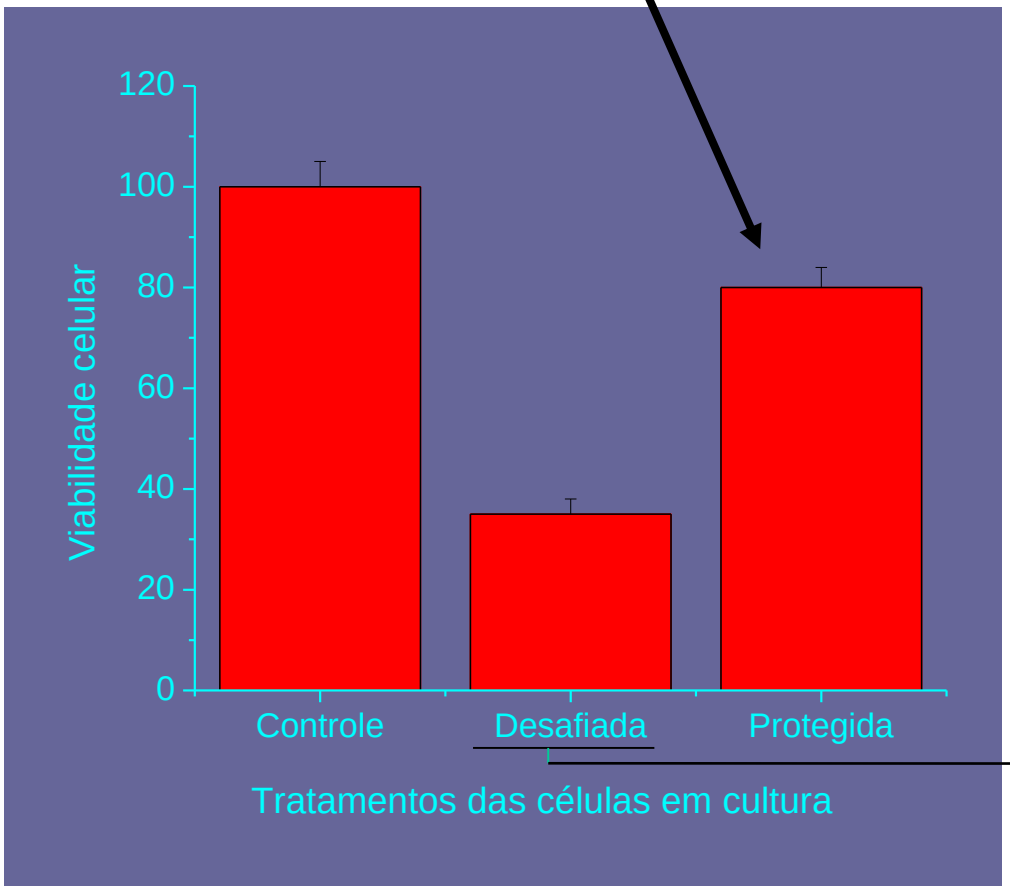




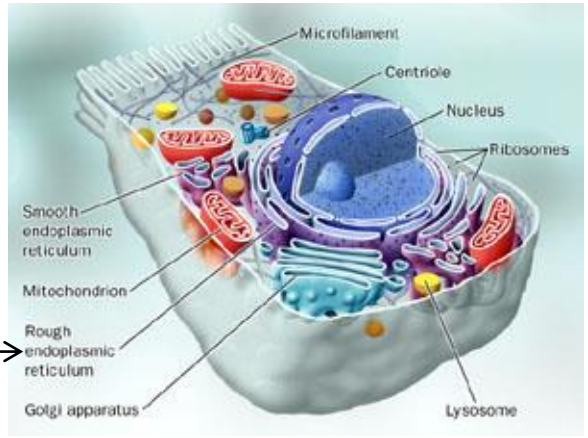
Uchoa, Severino, Baptista, Anti-Oxidant properties of Singlet Oxygen Suppressors, book chapter 2011
Mukai *et al*, *Free Radical Biology & Medicine*, 39, 752-761 2005

Can we protect eukaryotic cells with anti-oxidant action?

1% chamomile extract increases ~ 3 x viability of cells challenged with singlet oxygen

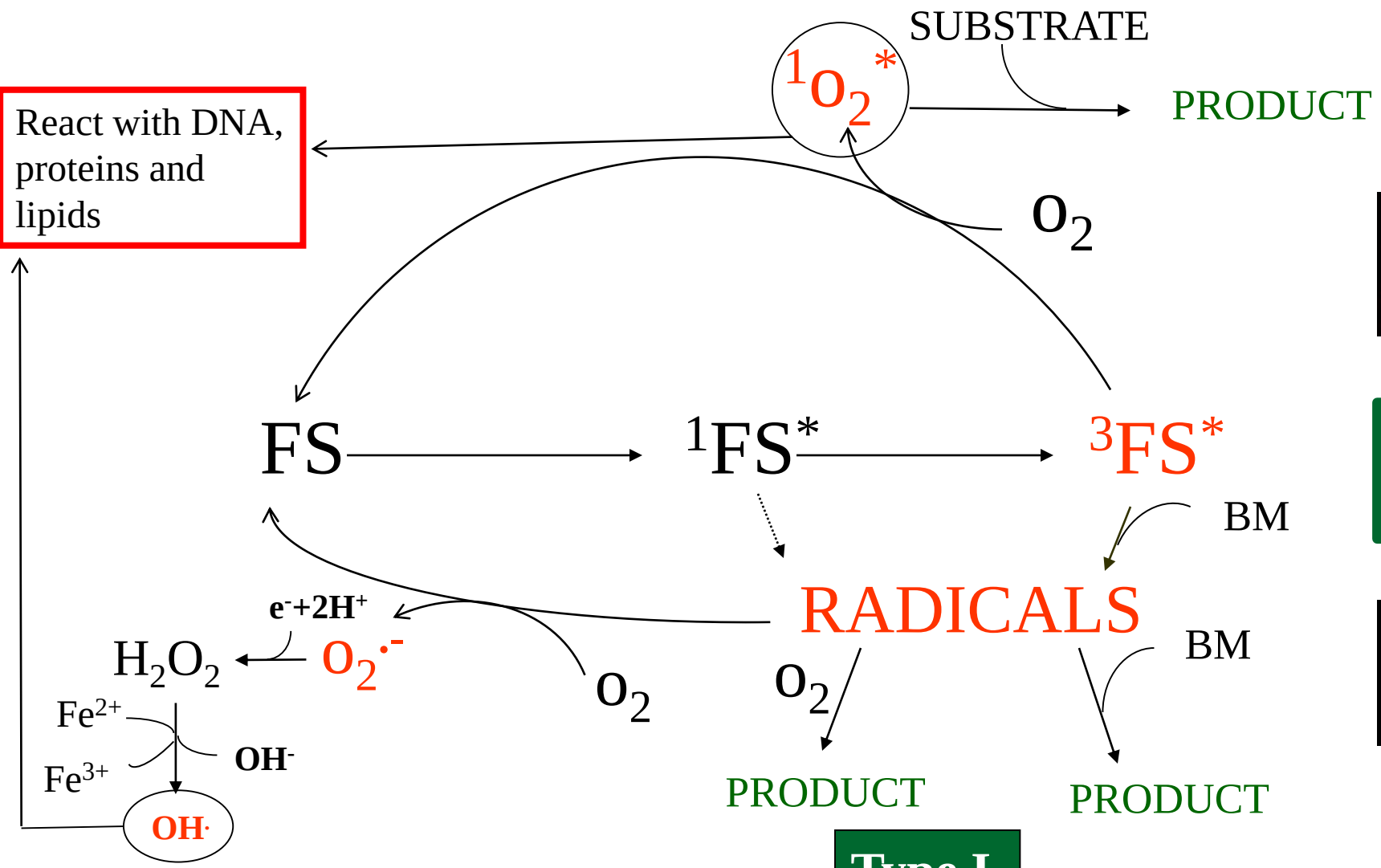


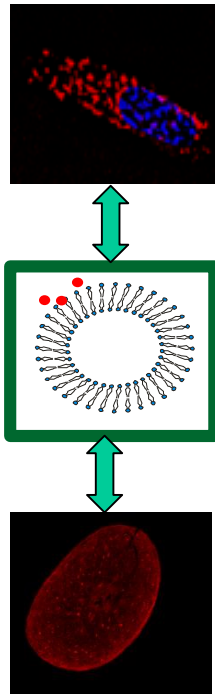
Challenged cells with oxidative imbalance induced by singlet oxygen viability falls to 30%



Mechanisms of photosensitized oxidations

Type II





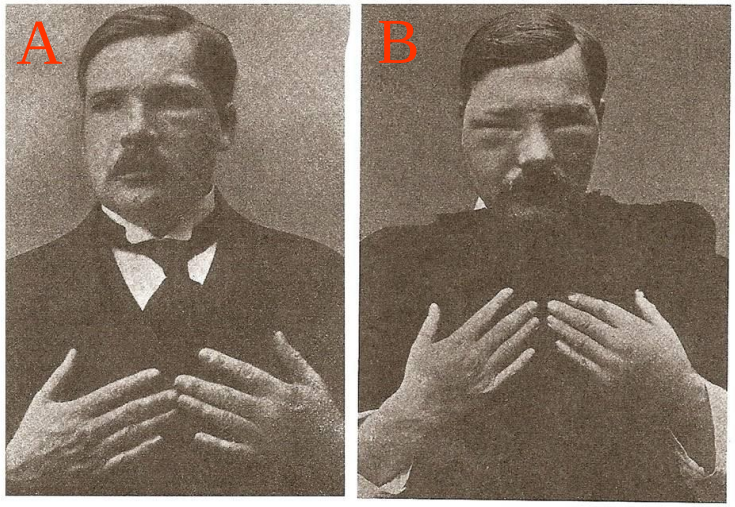
Type I

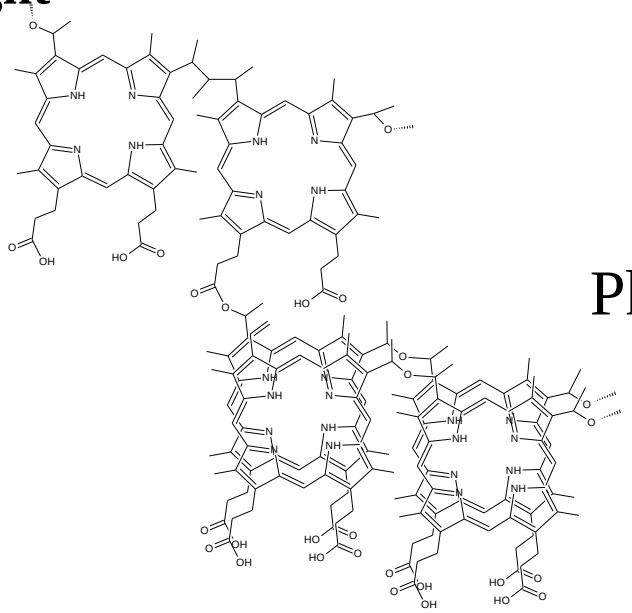
Foote, C.S. *Science* **1968**, *162*, 963.

PHOTODYNAMIC EFFECT: Historical perspective

1. Raab, O. *Zeitschrift fuer Biologie* (1900) 39, 524-546

Paramecium getting killed by eosine + light





Photofrin

Friedrich Betz Meyer before and after application of Hematoporfirine IX (A)
And after sun exposition (B)**, 1912.**

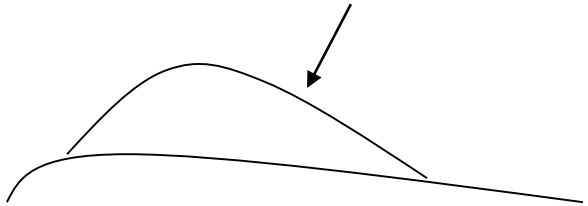
2. T.J. Dougherty et al Photoradiation Therapy for the Treatment of Malignant Tumors. *Cancer Research, 1978,* 38, 2628.

T. J. Dougherty et al Photodynamic Therapy. *Journal of the National Cancer Institute*, 1998, 90.

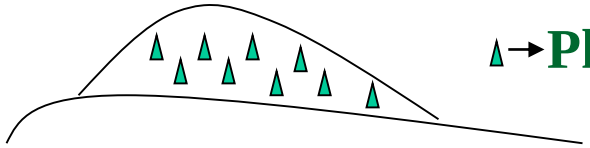
3. Efficient drugs, cheaper light sources. Expand the type of diseases treated with PDT. Several pharmaceutical industries are involved.

Use of photo-induced reactions to treat diseases: Photodynamic Therapy

Diseased tissue

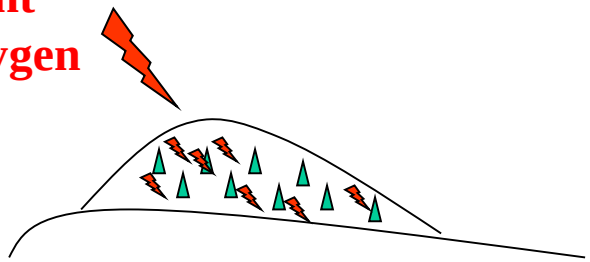


1. Incorporation/ Localization



2. Photodynamic effect

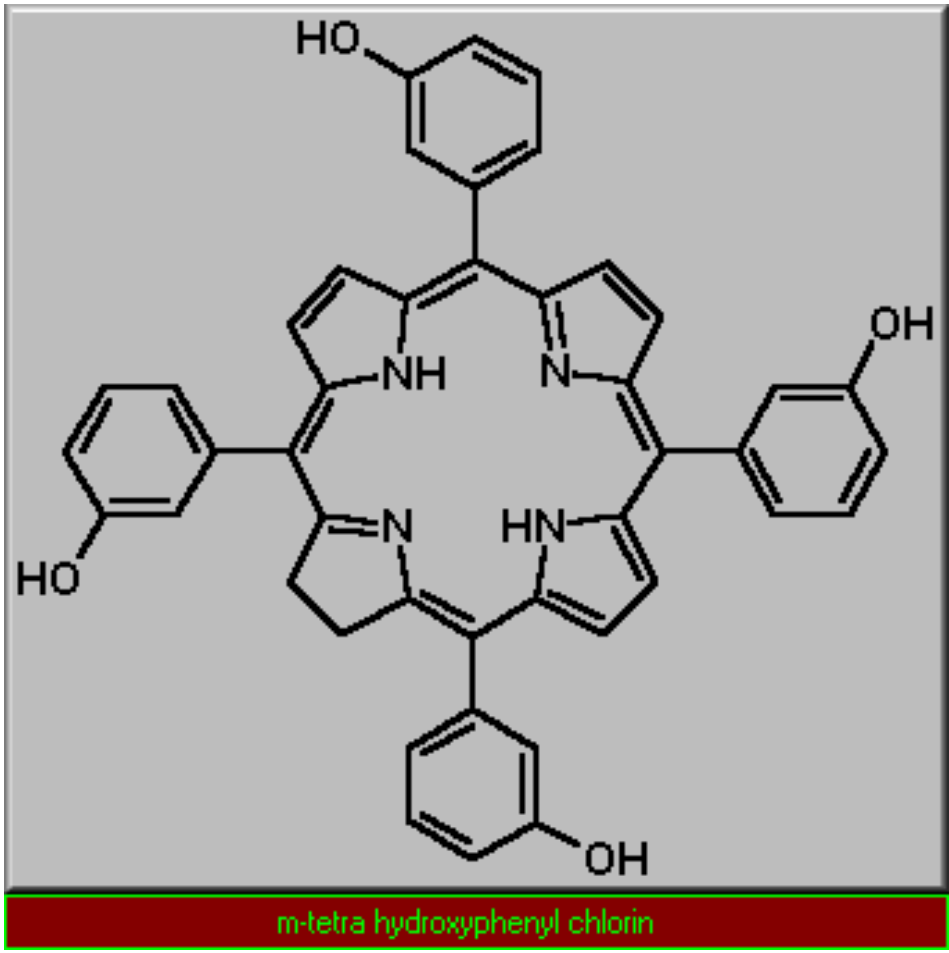
Photosensitizer
Light
Oxygen



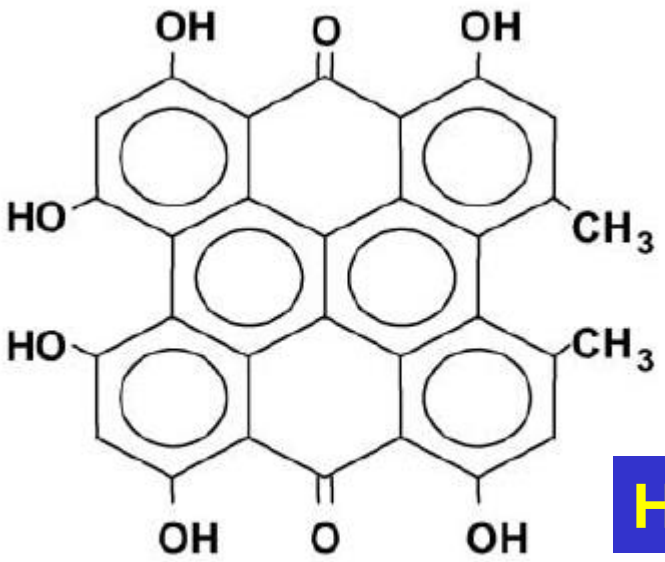
Light: 600 nm < λ <1000 nm

3. Biological responses:
Cell death
Stimulus of Immune system

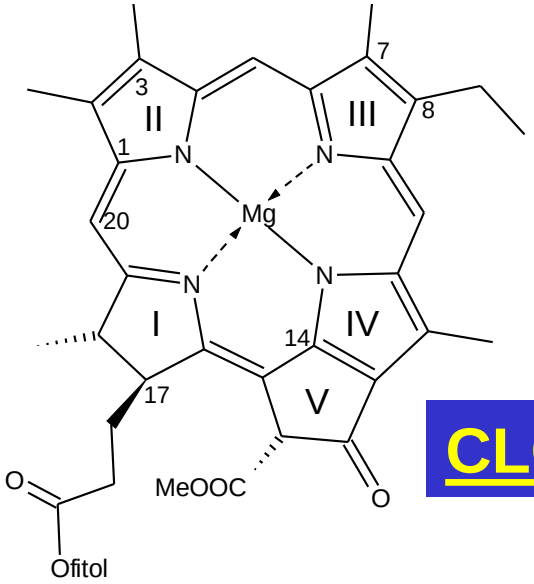
Hydroxiphenil Meta-tetra clorin (m-THPC) **FOSCAN** is a second generation photosensitizer 200 times more efficient than PHOTOFRIN.



Hypericum perforatum (*HP*, Saint Jones herba)



HYPERICIN

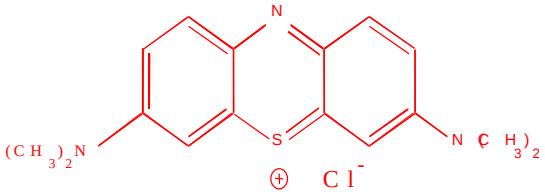


CLOROPHYL a

Usual protocols of PDT are still very expensive:
A dose of Foscan costs 4000.00 Euros

Low cost PDT

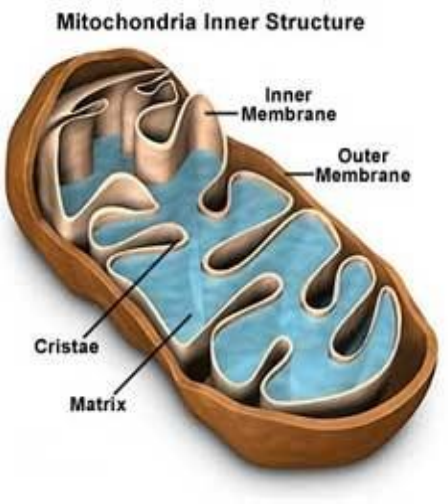
Light sources and efficient drugs
at low cost!

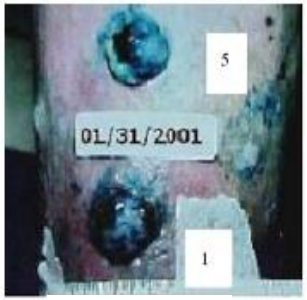


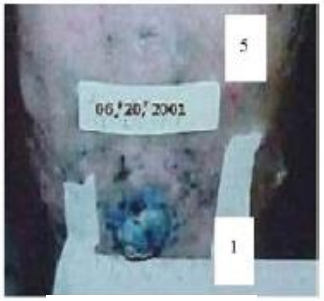
-Phenothiazines
-Hipericum extract

- efficient absorption> 650nm
- Efficient generation of triplet
- Mechanisms Type I and Type II
-Mitochondrial target

-AFFORDABLE COST



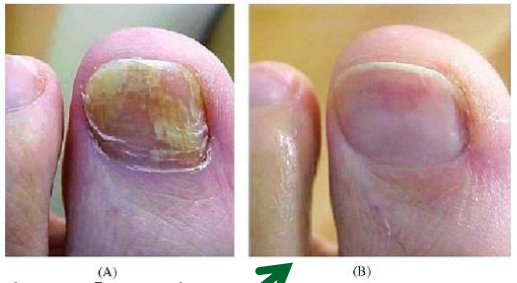




05/11/2003 09/24/2004

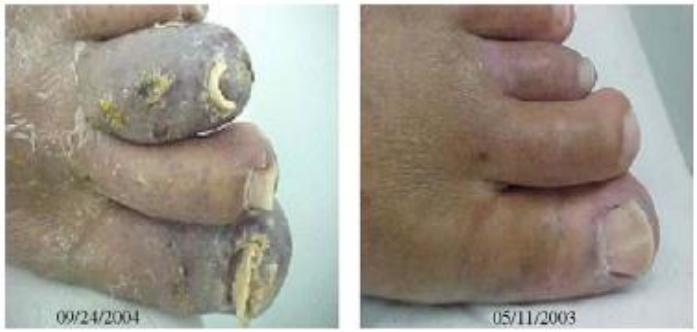
Melanoma *Photodyag Photodyn Ther* **2004**, 1, 345.

Dra. Belotto (Pérola Byington)
Dr. Lindoso (FM-USP)
Dr. Tardivo (FM-ABC)



Onychomycosis and Review

Photodyag Photodyn Ther **2005**, 2/3, 175.



Sarcoma de Kaposi *Photomed Laser Surg* **2006**, 24 (4), 528.

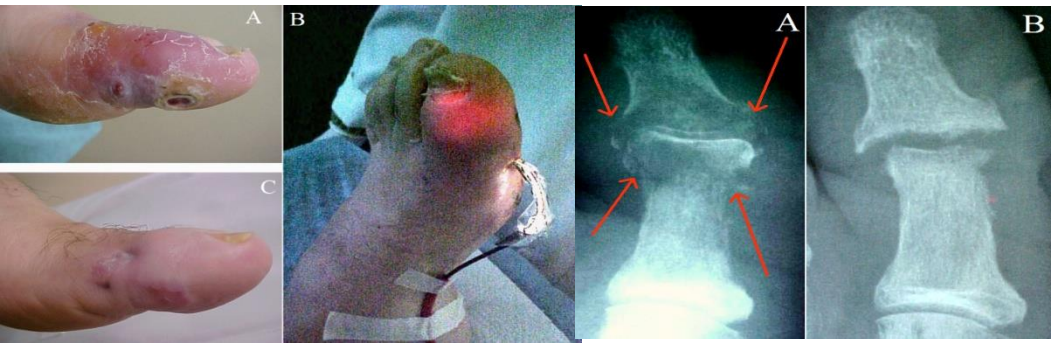
Low cost PDT

Decontamination of blood

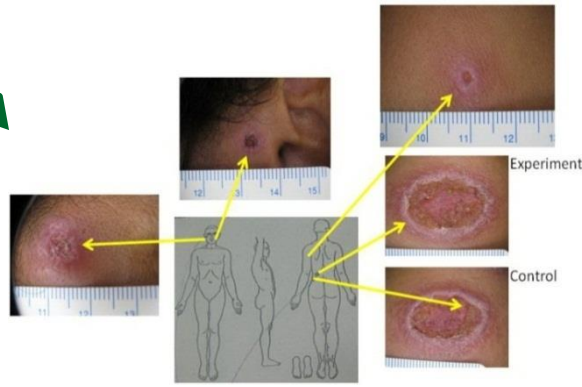
Wainwrigth & Baptista
Photodyagn Photodyn Ther **2011**, 8, 240.

Herpes and viruses

Tardivo, Wainwright, Baptista
Photodyagn Photodyn Ther **2012, 9, 118**.



Osteomielite *Photomed Laser Surg* **2009**, *27*, 145.



Leishmaniose tropical diseases

- **Song** et al *Photomed Laser Surg* **2011,** 29, 711.
- Baptista & Wainwrigth *Braz. J. Med. Biol. Res.* **2011**, 44, 10.

Conclusion

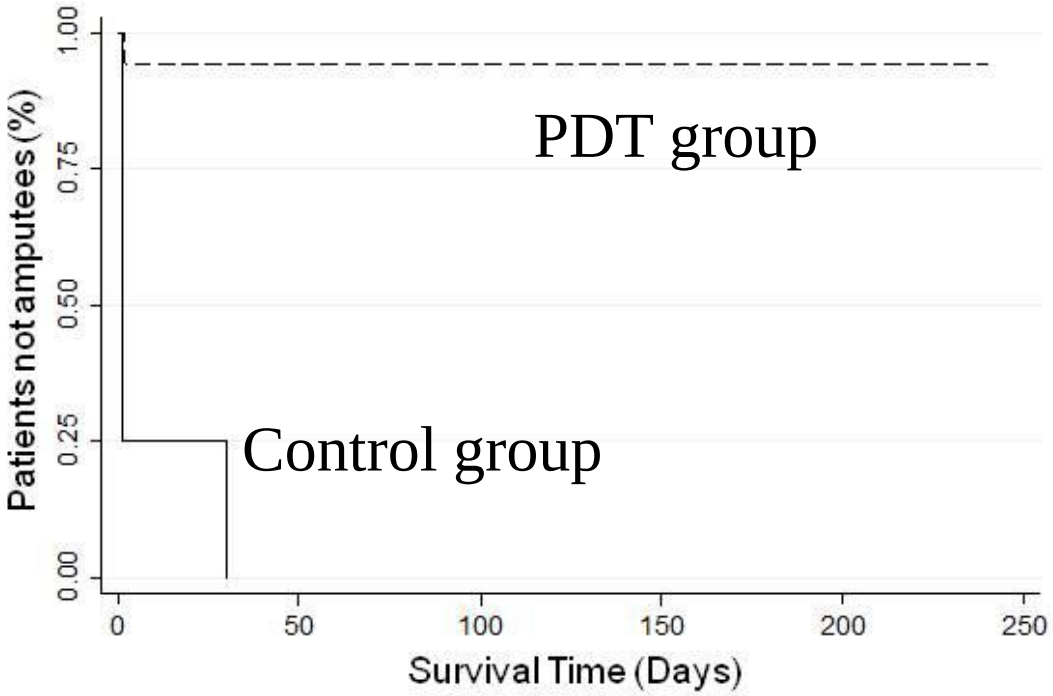
PDT may be useful to health

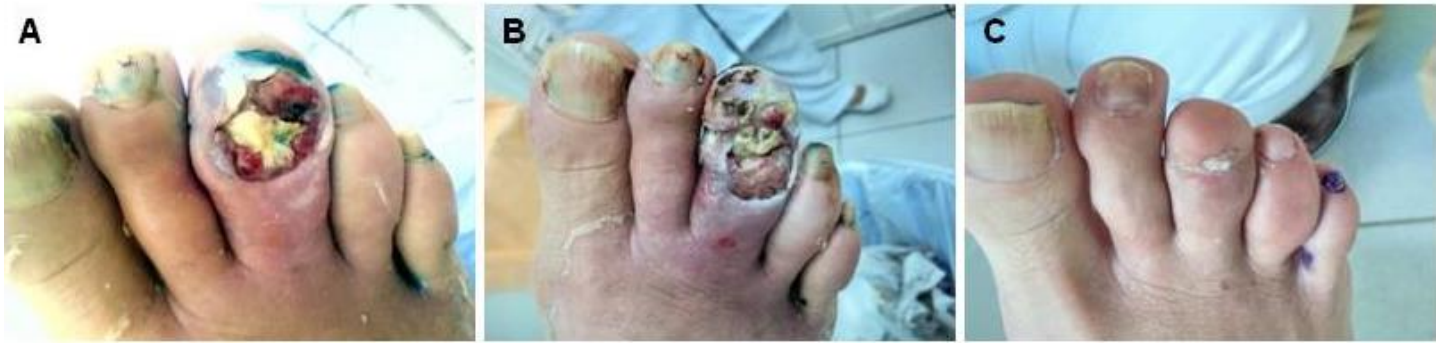
A reliable method to prevent amputation in the feet of diabetic patients based on a low-cost Photodynamic Therapy protocol

Tardivo et al, A clinical trial testing the efficacy of PDT in preventing amputation in diabetic patients, *PDPDT* 2014, 11 (3), 342–350.

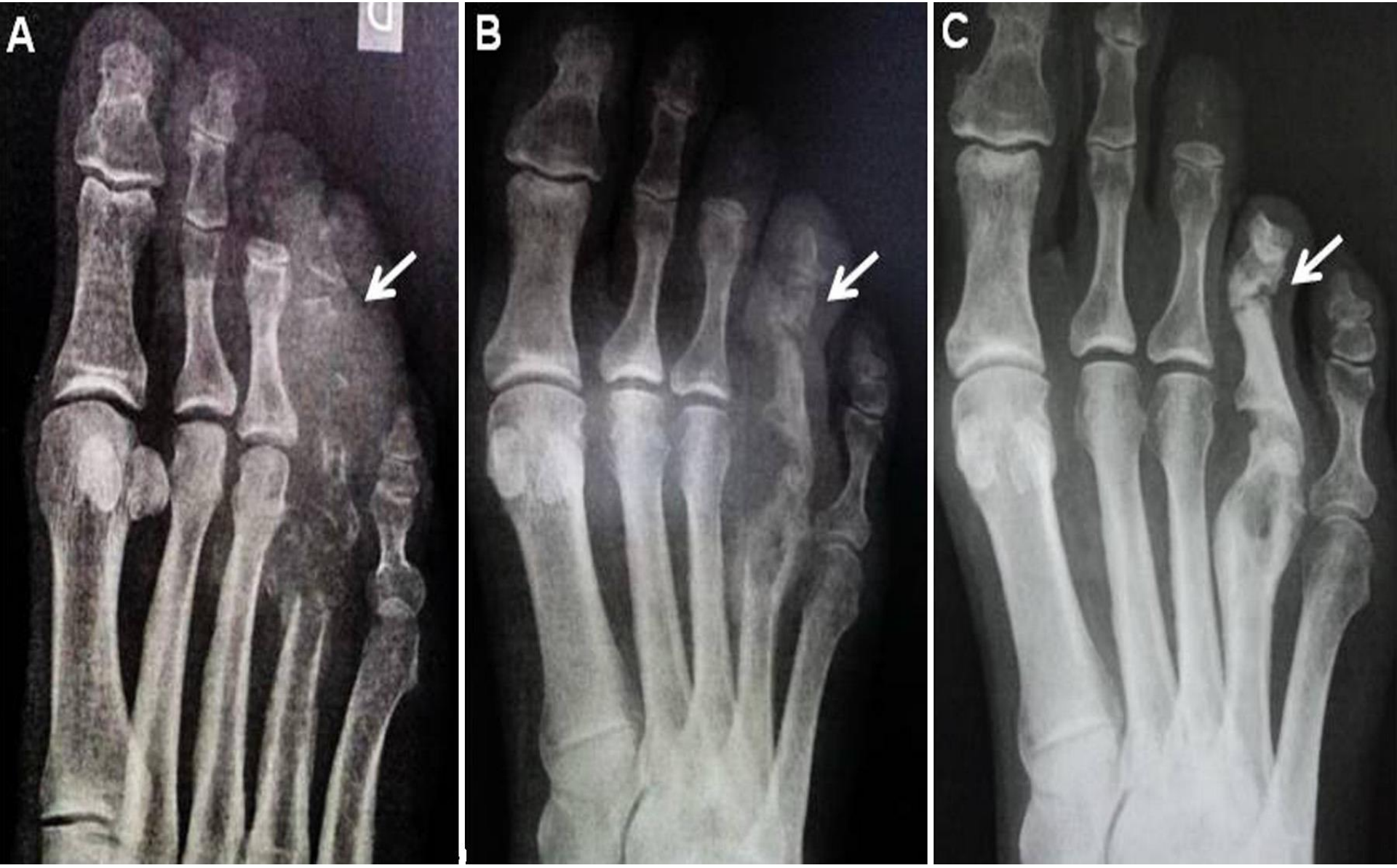
MB 1mM
DMMB 50μM

PDT
Photodynamic
Therapy





Bone regeneration???

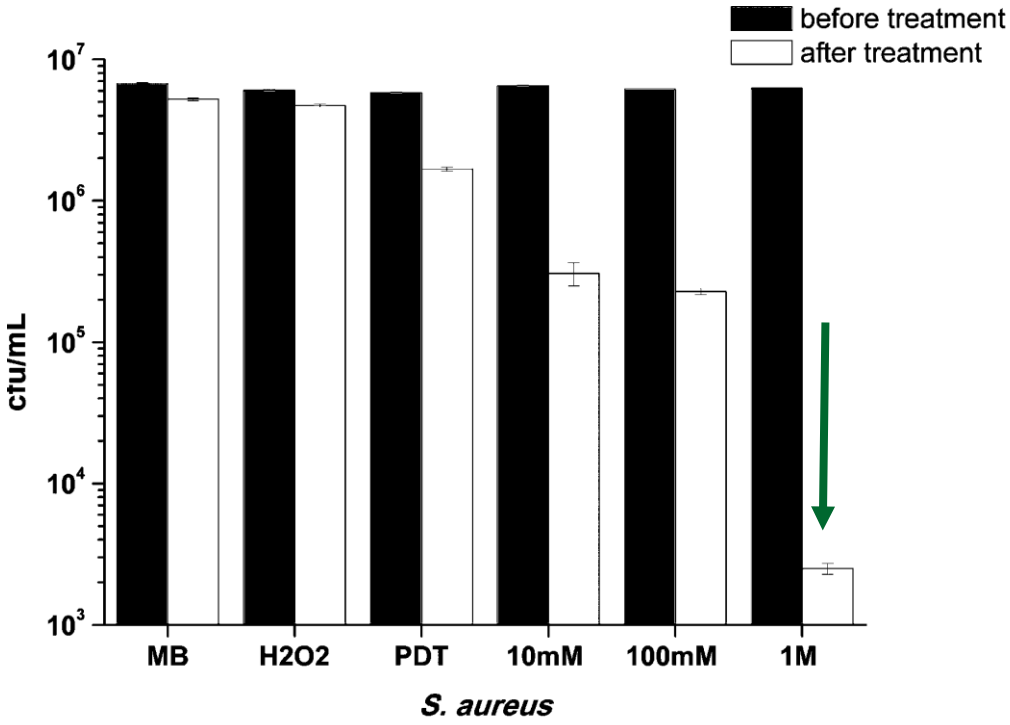


Can PDT become more efficient?

More potent photosensitizers…

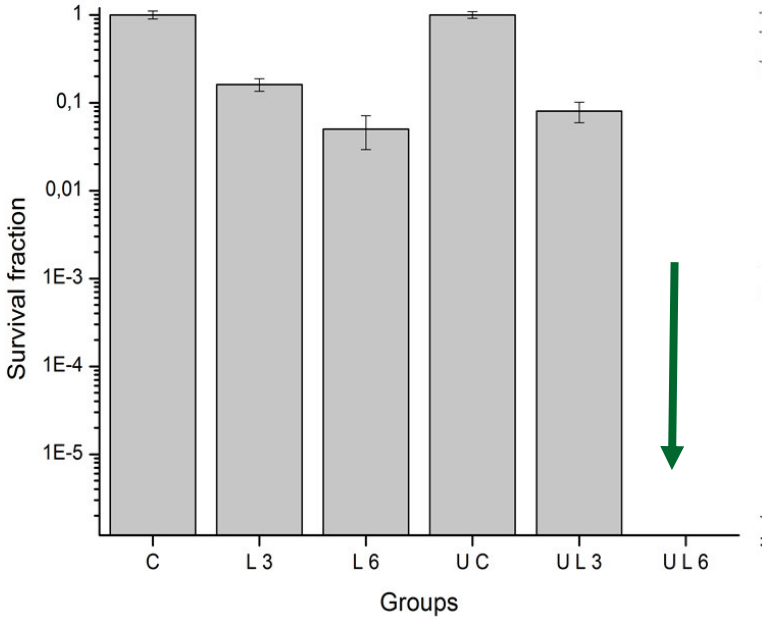
Synergistic action!

MB + H_2O_2



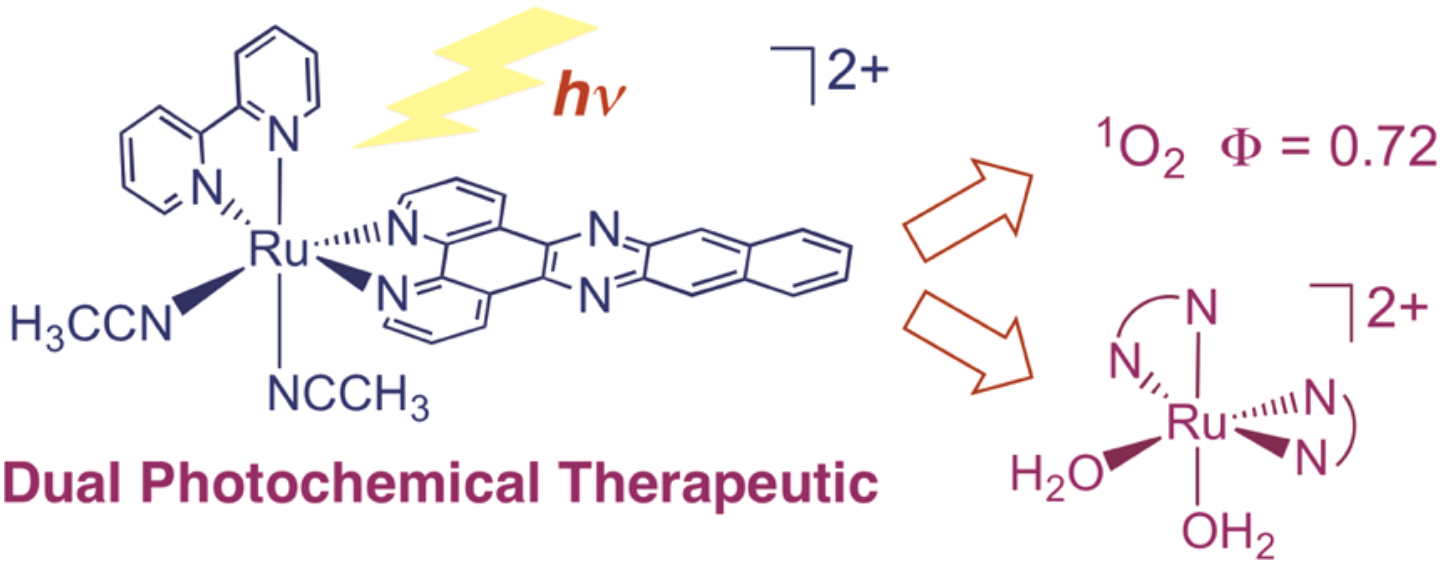
AS Garcez, et al *Photochemical & Photobiological Sciences 2011,* 10 (4), 483-490

MB + Urea



SC Nuñez et al *J. Photochem. Photobiol.* B 2015, in press

Discover of new action mechanisms and improving the efficiency of PDT



Journal of the American Chemical Society

Article

Table 1. Toxicity Data in the Dark and upon Irradiation for 1−3

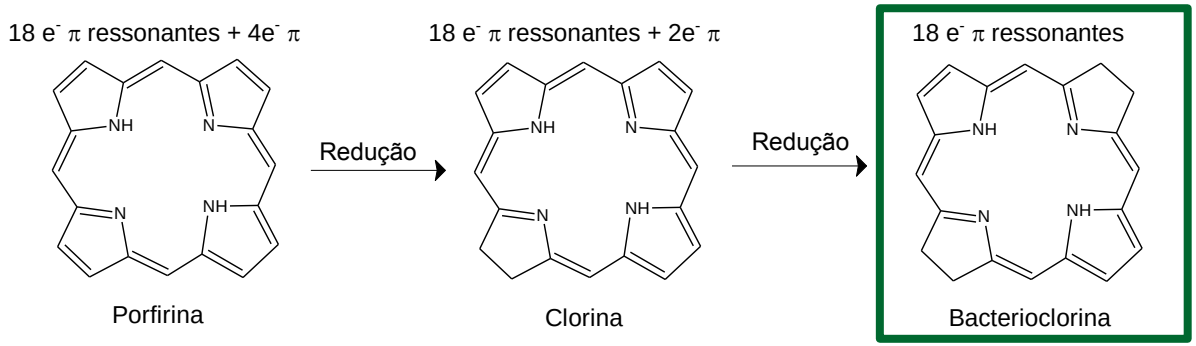
complex	RA[a]	$IC_{50}^{dark}/\mu M$[b]	$IC_{50}^{irr}/\mu M$[b]	PI[c]	PI_{cor}[d]
1	1	110 ± 28	0.39 ± 0.06	282 ± 69	282 ± 69
2	0.17	244 ± 23	223 ± 94	1.1 ± 0.4	6.4 ± 2.3
3	0.64	334 ± 74	0.47 ± 0.02	711 ± 132	1110 ± 206

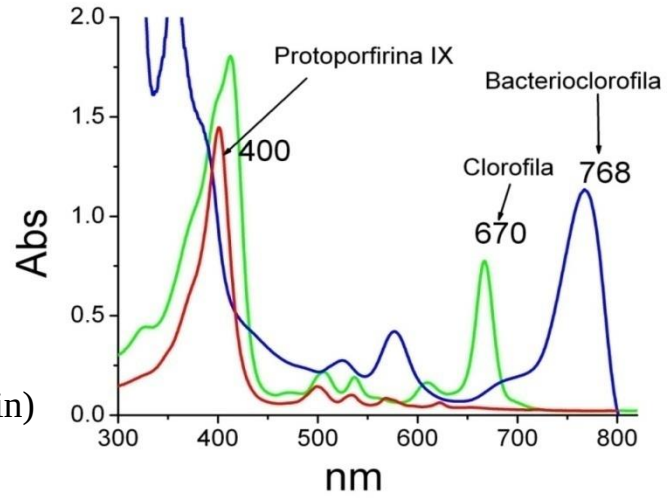
[a]Molar absorptivity relative to that of 1 at the irradiation wavelength for phototoxicity studies (466 nm). [b]IC_{50} represents the concentration required to attain 50% cell death; IC_{50}^{irr} value determined by irradiating the cell culture with a 466 ± 20 nm LED for 20 min and then incubating for 48 h; errors determined from two or three experimental trials. [c]Phototoxicity index: $PI = IC_{50}^{dark}/IC_{50}^{irr}$. [d]Corrected PI value: $PI_{cor} = PI/RA$.

B.A. Albany et al Marked Improvement in Photoinduced Cell Death by a New Tris-Heteroleptic Complex with Dual Action: Singlet Oxygen Sensitization and Ligand Dissociation. *The Journal of the American Chemical Society* **2014**, 136, 17095.

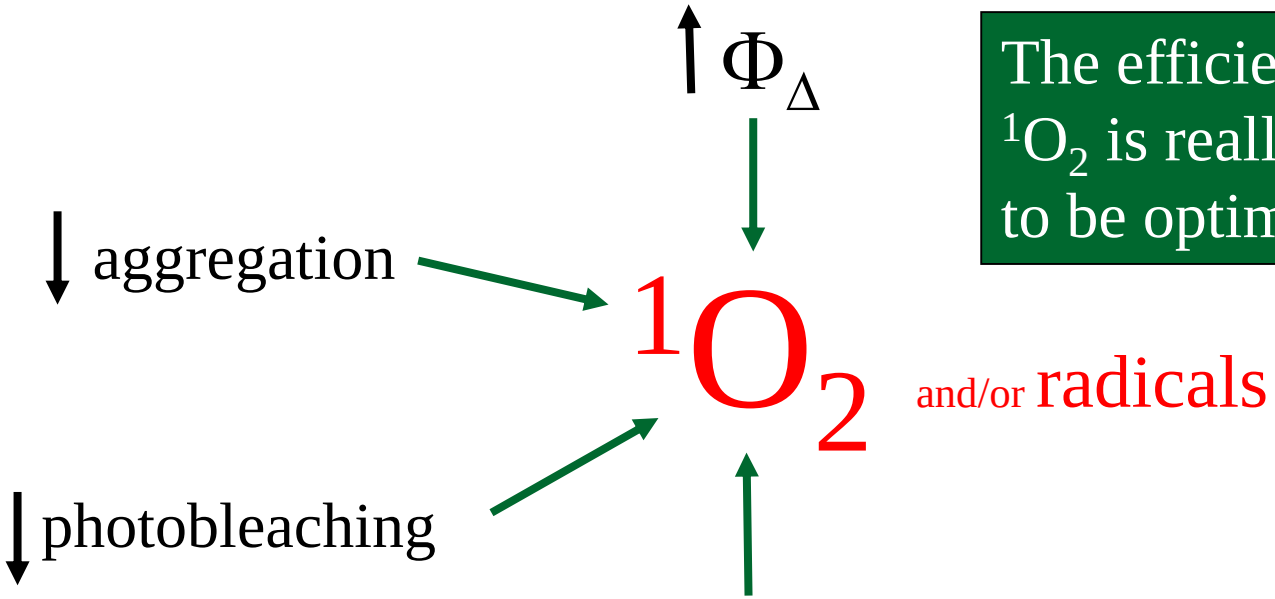
Can PDT become more efficient by improving selectivity?

DEVELOPMENT OF NEW DRUGS ACTIVATED BY LIGHT

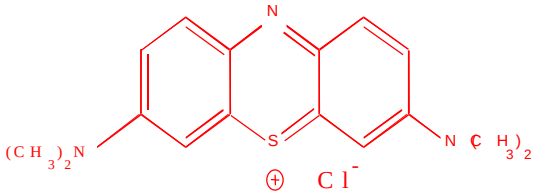




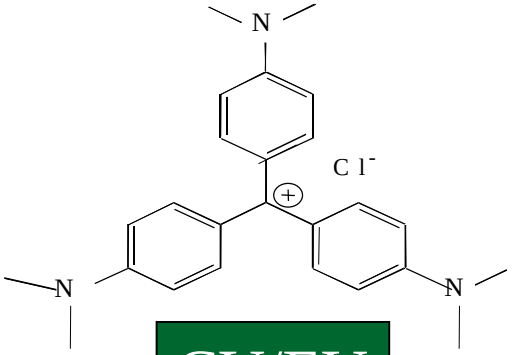
Macrocyclic aromatic porphyrin and its derivatives (chlorine and bacteriochlorin)



The efficiency of generation of $^1O_2$ is really the best parameter to be optimized?



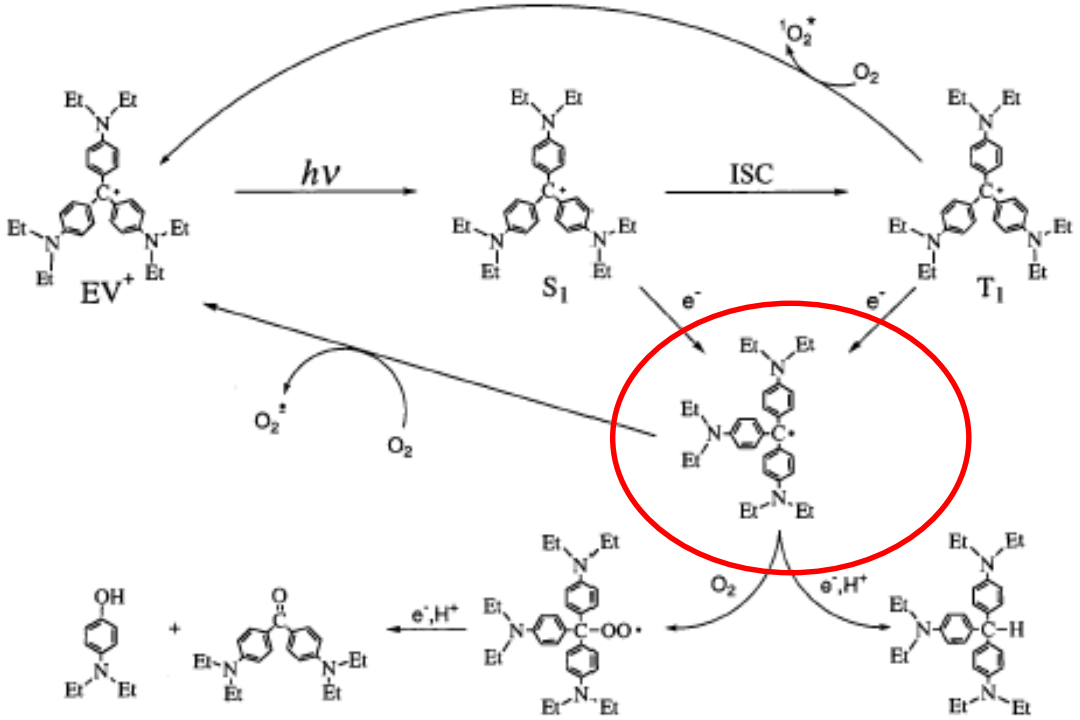
AM$^+$



CV/EV

Type II e/ou type I(?): ϕ_T ~0.5

Junqueira et al *Phys Chem Chem Phys* **2002**, 4, 2320.



Type I and Type II: ϕ_T << 0.01

Baptista & Indig *J Phys Chem* **1998,** *102*, 4678.

Calculations

1. Power density/Energy per photons: photons per time

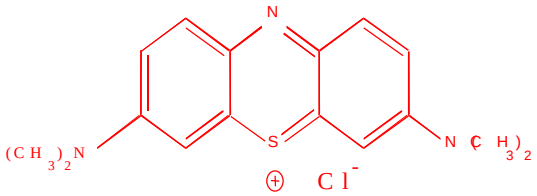
2. Absorption cross section $\sigma = (\mu/\rho) m_a / N_A$

μ/ρ is the mass absorption coefficient

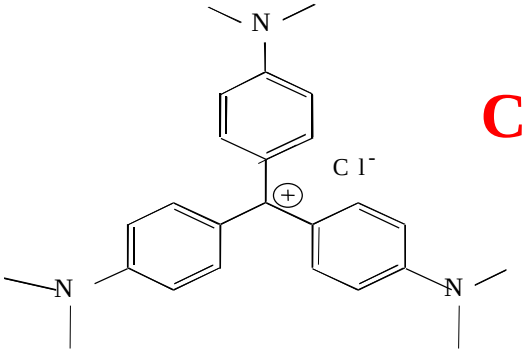
m_a is the atomic molar mass in g/mol

N_A is Avogadro´s number

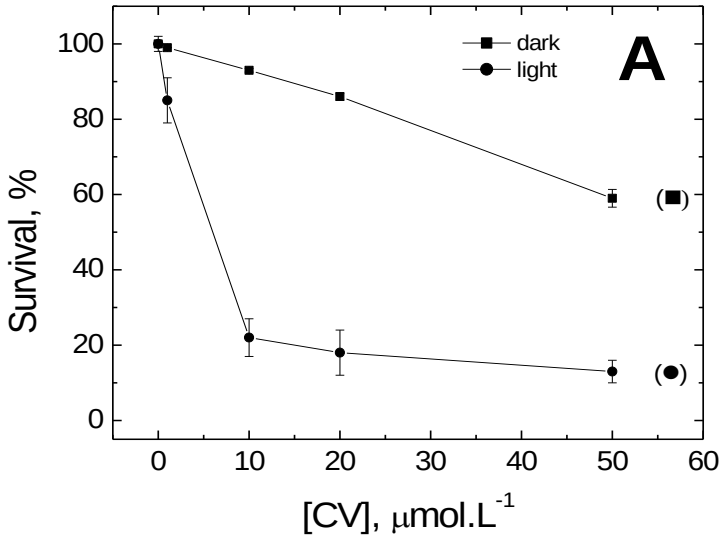
3. Quantum yield: number of processes of interested divided by the number of photons absorbed

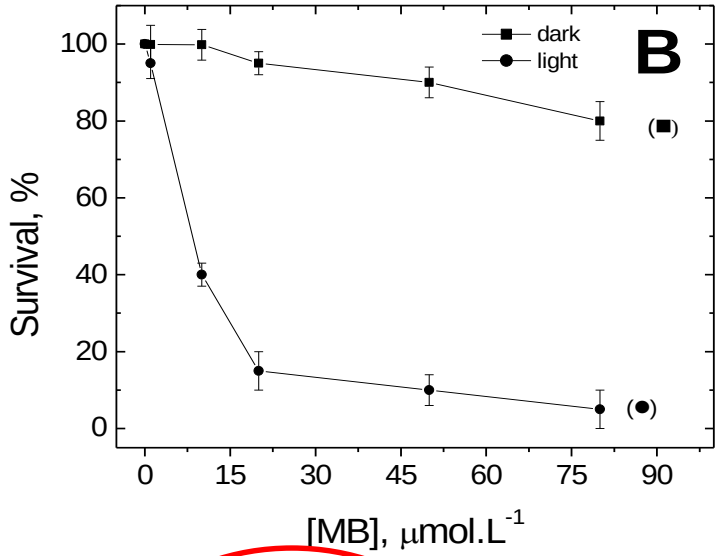


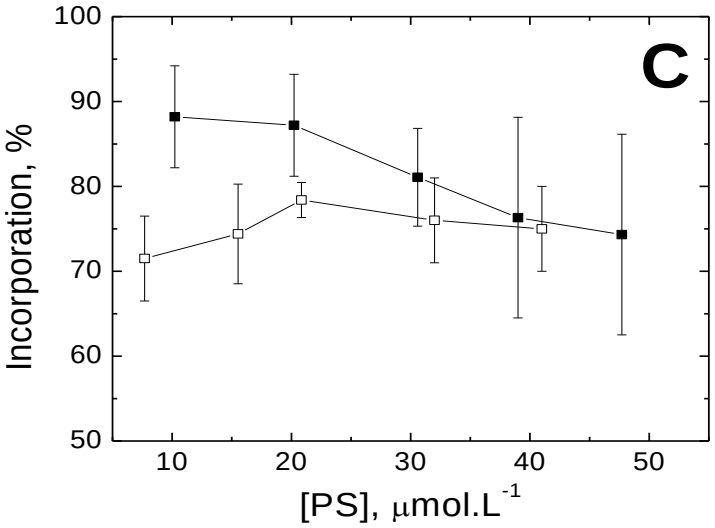
- Photon energy (600µm)=3.31 10^(-19) J Joules (ou seja mais ou menos dois eV).
- Power density of 1 KW/m^2 or dose of 1 KJ/s/m^2 sends 10^3/(3.31 10^(-19)) = 3.02 10^(21) photons/ m^2/s
- Cross section of MB is 2.17 10^(-20) m^2. MB exposed to this photon flux absorbs 65.6 per second.
- Given that quantum yield is 0.52, each MB produces 34.1 singlet oxygen per second.
- 1 µM MB produces 34 µM Singlet oxygen per second por segundo numa luz de 1KW/m^2.
- 1 µM MB is equivalente 6.02 10^20 m^3, or 602 moléculas por µm^3 (fL).
- In 1 fL, 1 µM MB at 1KW/m^2 produces 34X602=20468~**20K singlet oxygen per second.**

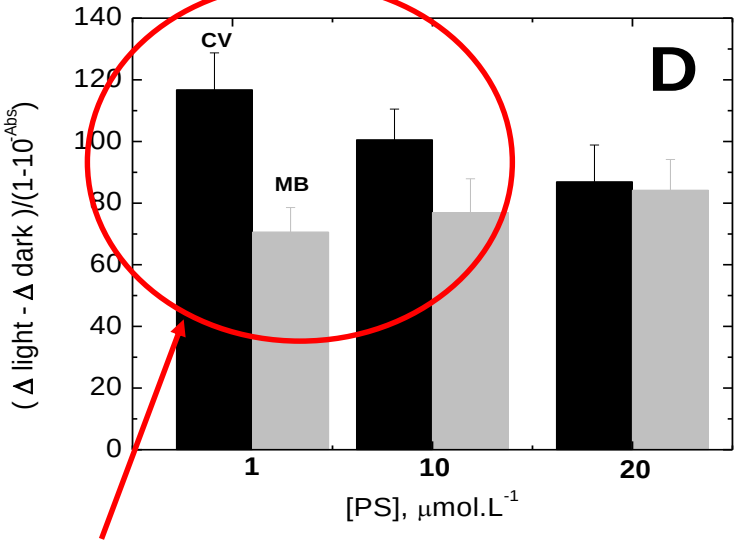


CV produces about 20 singlet oxygen per second

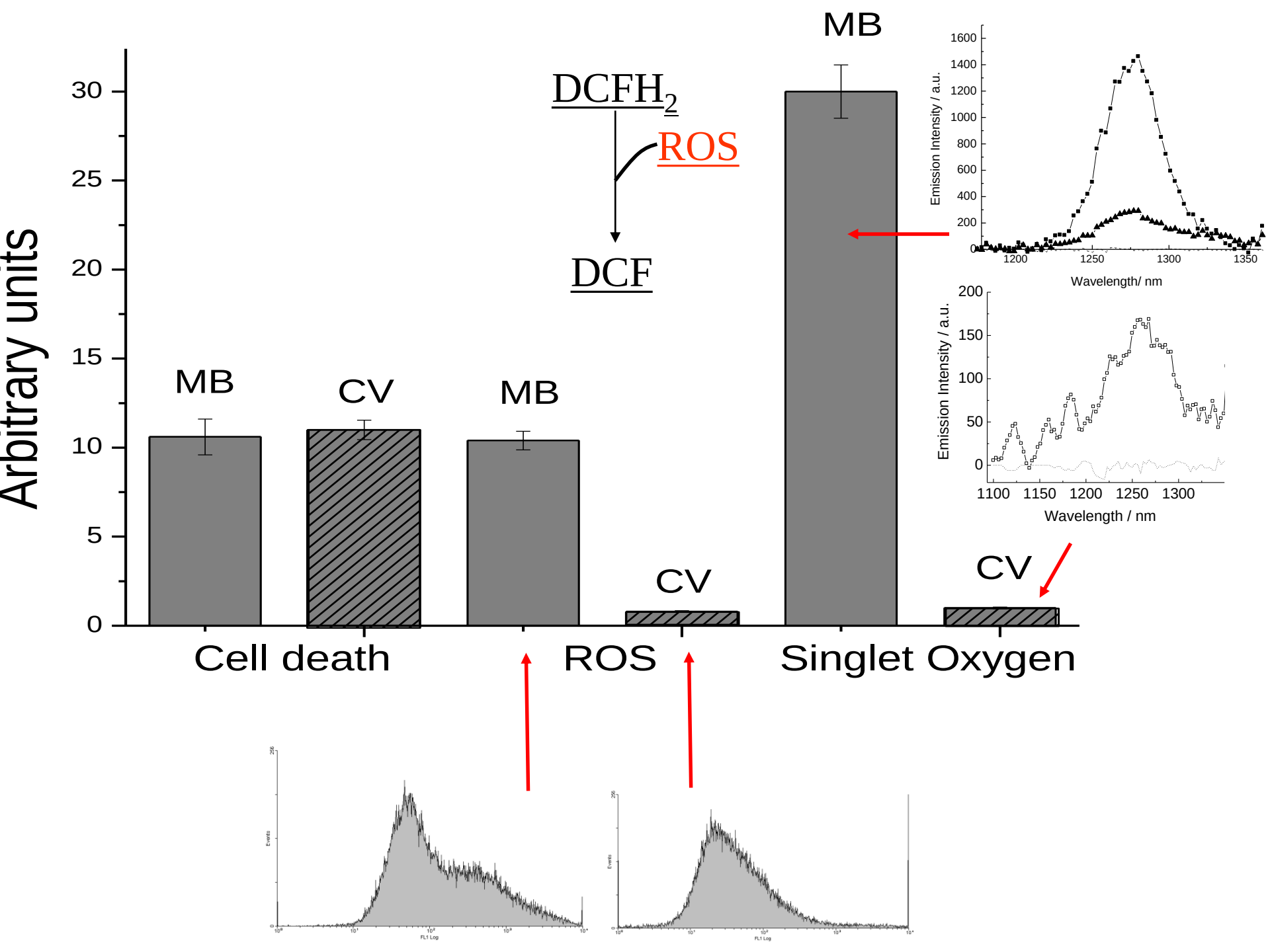


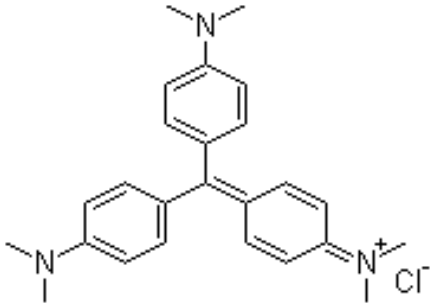


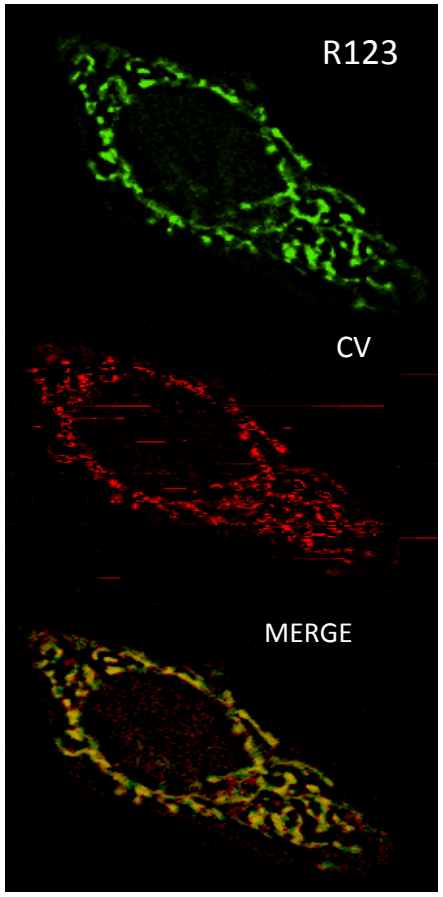




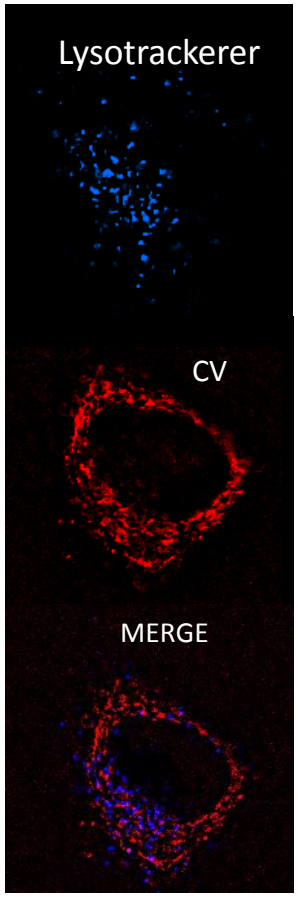
Reduction of MB


Arbitrary units
30
25
20
15
10
5
0
MB
CV
MB
CV
MB
CV
Cell death
ROS
Singlet Oxygen
DCFH2
ROS
DCF
Emission Intensity / a.u.
1600
1400
1200
1000
800
600
400
200
0
1200
1250
1300
1350
Wavelength/ nm
Emission Intensity / a.u.
200
150
100
50
0
1100
1150
1200
1250
1300
Wavelength / nm
Events
FL1 Log
Events
FL1 Log

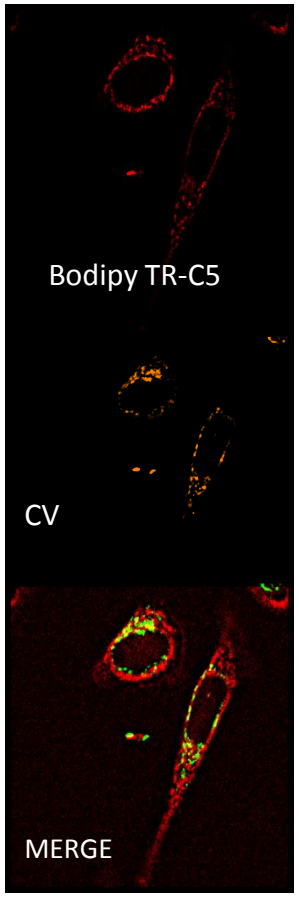

N
N
N+
Cl-


R123
CV
MERGE

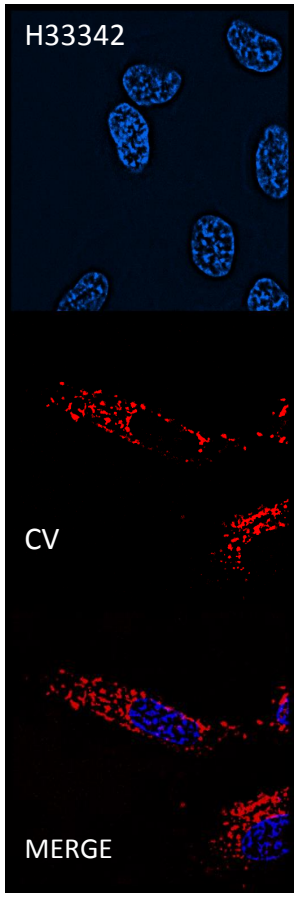
A


Lysotrackerer
CV
MERGE

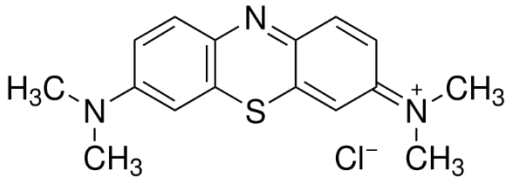
B


Bodipy TR-C5
CV
MERGE

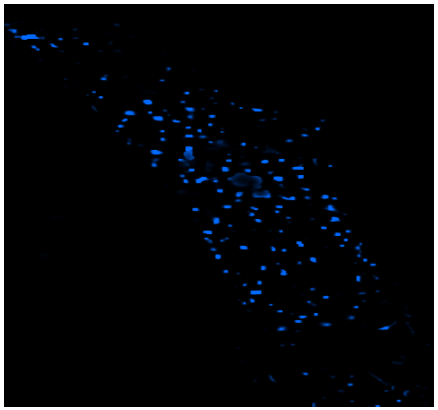
C

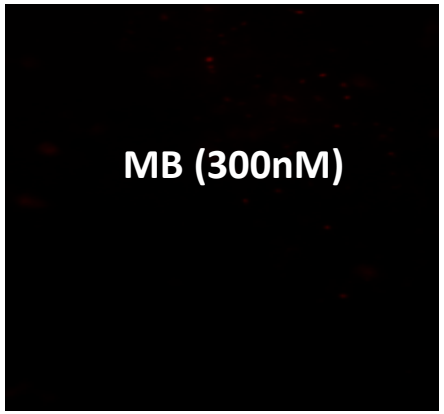

H33342
CV
MERGE

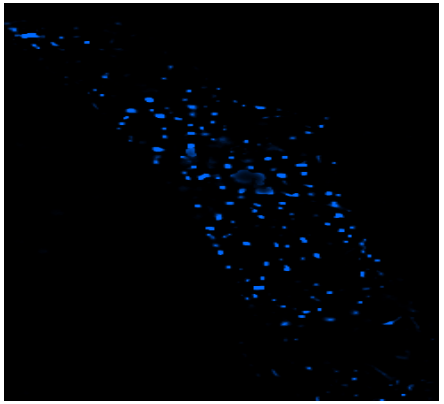
D



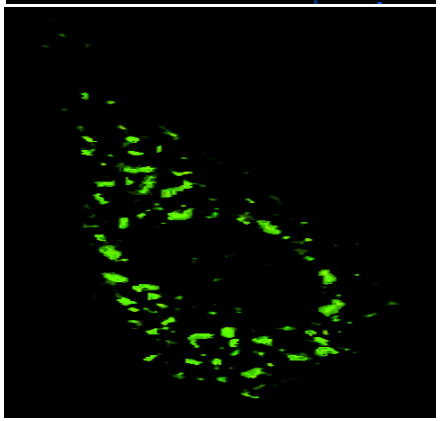
$$AM^{+} \xrightarrow{2e+H^{+}} AMH$$

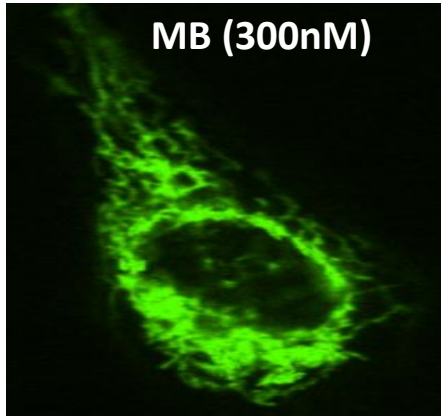


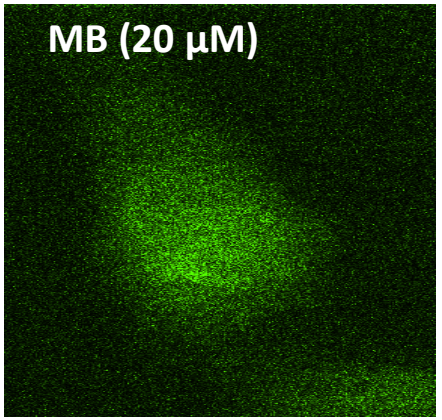




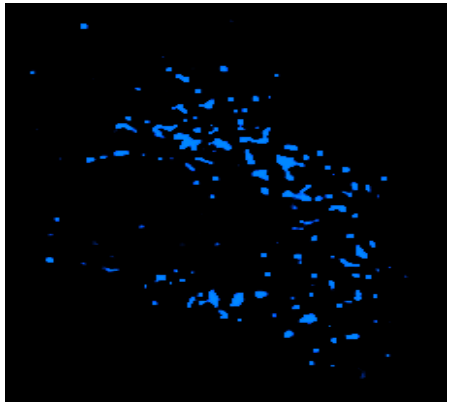
Lisosome

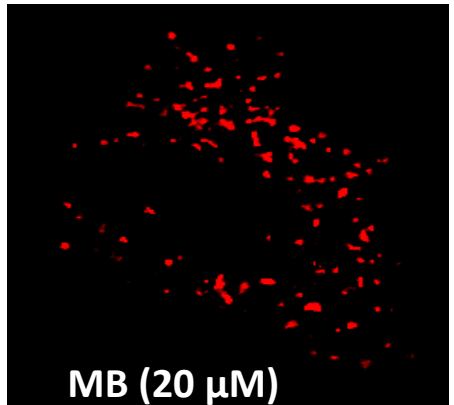


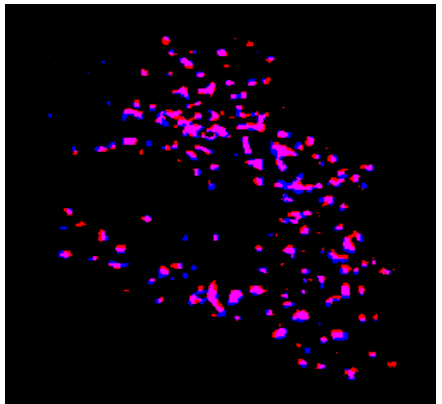




mitochondria





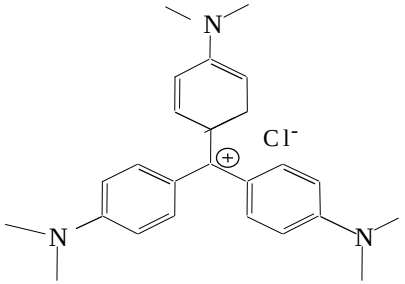


lisosomo

CV

↓ PHOTOCHEMISTRY YELD

LOCALIZATION MITOCHONDRIAL



$\phi_T << 0.01$

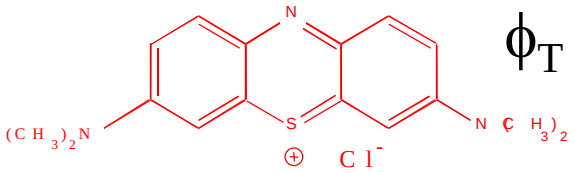
CRISTAL VIOLETA - CV

MB

↑ PHOTOCHEMISTRY YELD

MITOCHONDRIAL (Reduction)

+ DIFUSED DISTRIBUTION



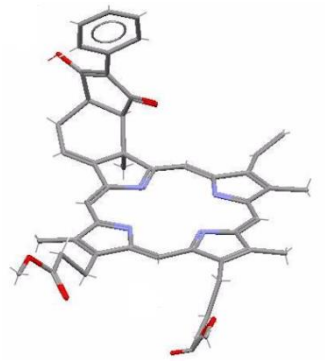
$\phi_T \sim 0.5$

Against the paradigm ↑ ROs ⟶ ↑ cell death

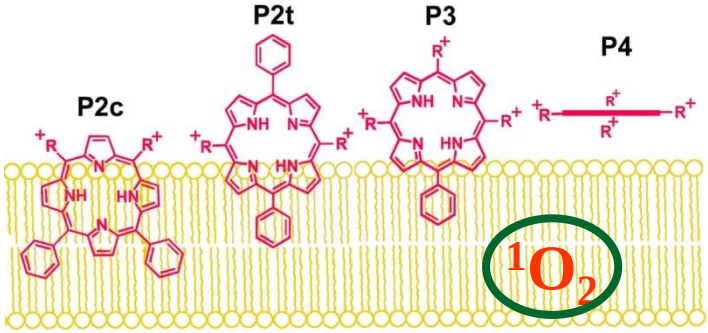
Oliveira CS et al *Free Radic Biol Med* **2011**, 51, 824.

G. Cilento Award **Carla Santos de Oliveira IAPS**

Structure x activity



Amphiphililic



Engelmann, F. M. et al *J Bioenerg Biomembr* **2007** 39(2), 175.

Engelmann, F.M. et al *Int J Pharm* **2007**, 329, 12.

Cordeiro et al *J. Phys. Chem B* **2012** 116(50):14618.

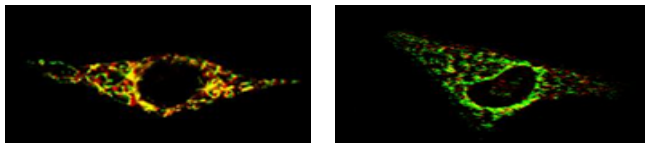
Inhibited aggregation

Oliveira K et al *J. Org Chem* 2009, 74, 7926.

Uchoa, AF et al *J. Org. Chem.* **2011** *76*, 8824.

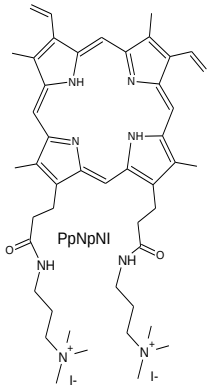
Patent INPI# 018110004875, 2011.

Chelated zinc



Pavani et al *Photochem. Photobiol. Sci.* **2009**, 8, 233.

Pavani et al, *Photochem Photobiol* **2012**, 88: 774.

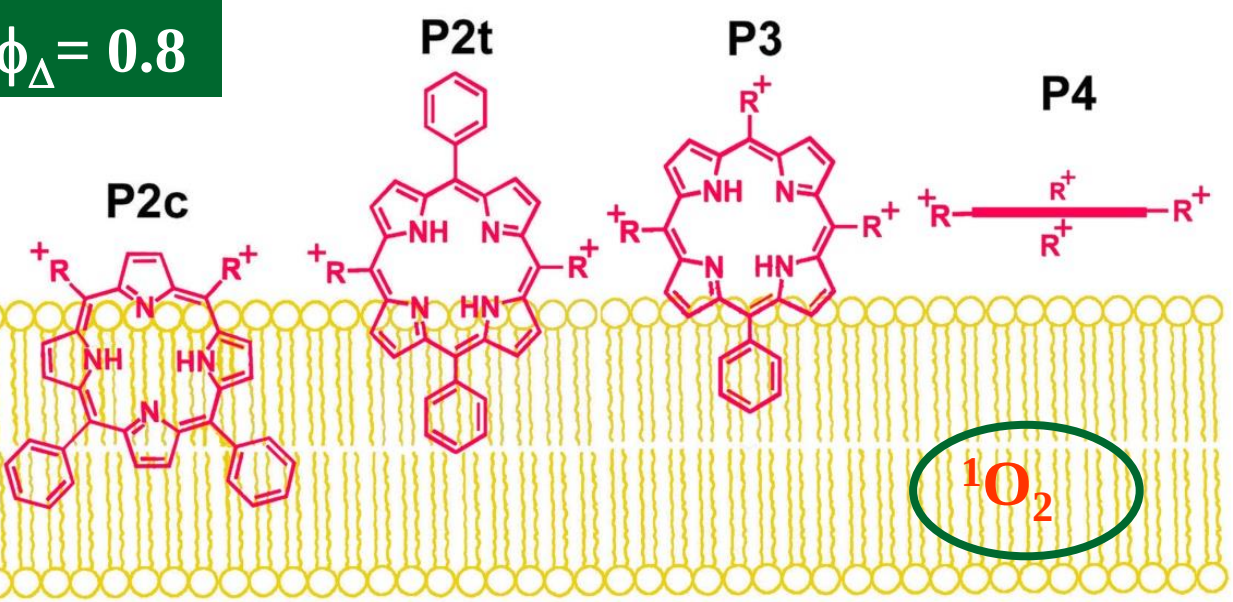


Cell localization

Patente de invenção. Uchoa, A.F.; Baptista, M.S. INPI # 018080052150, 2008.

Uchoa, AF et al *JPP* **2010**, 14, 832.

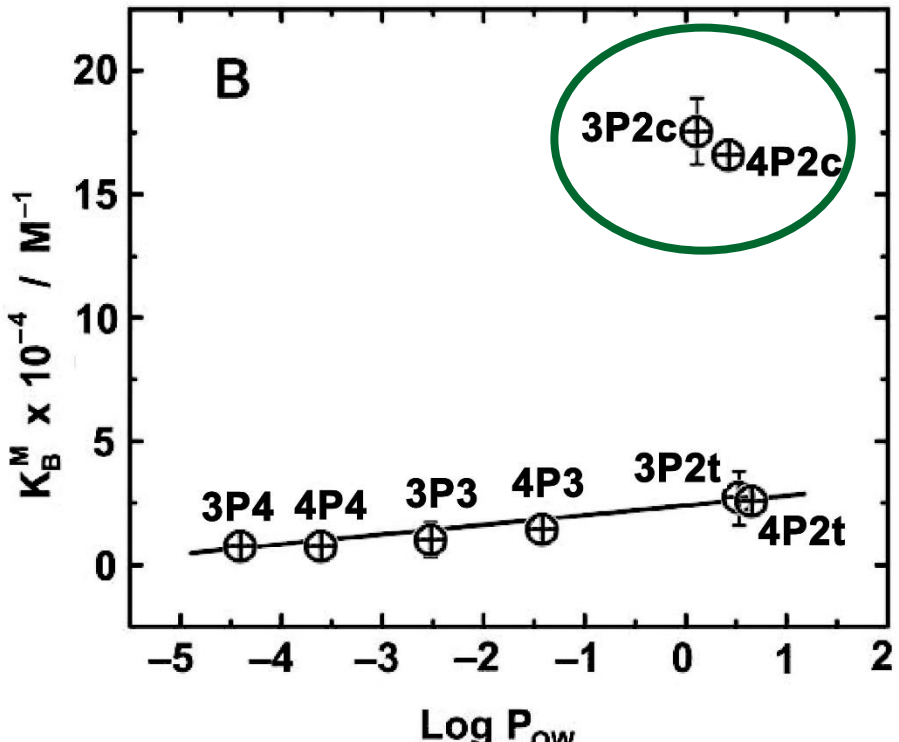
$\phi_\Delta = 0.8$

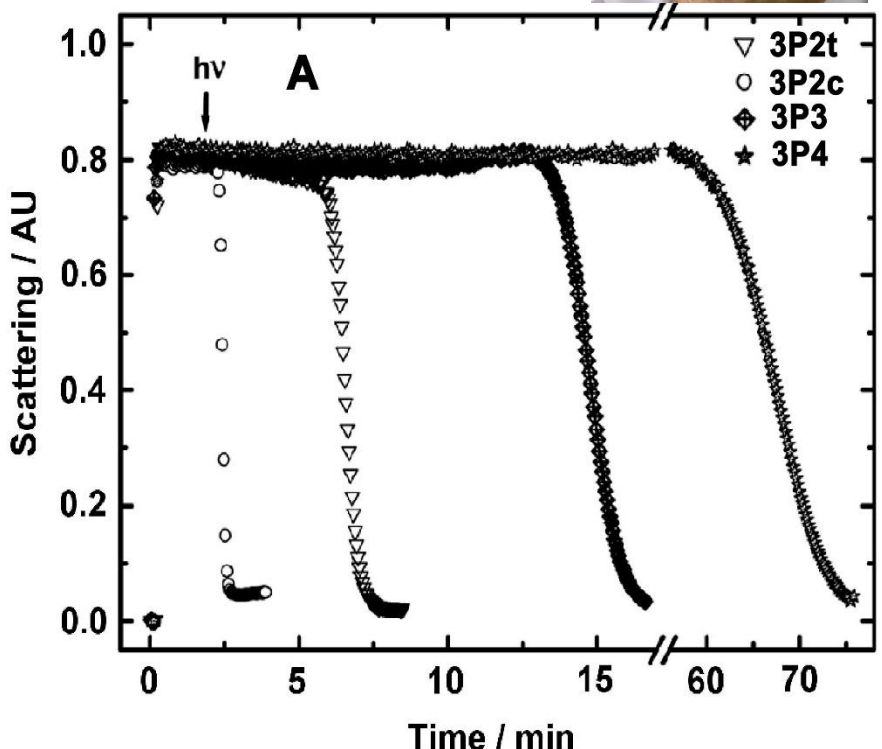


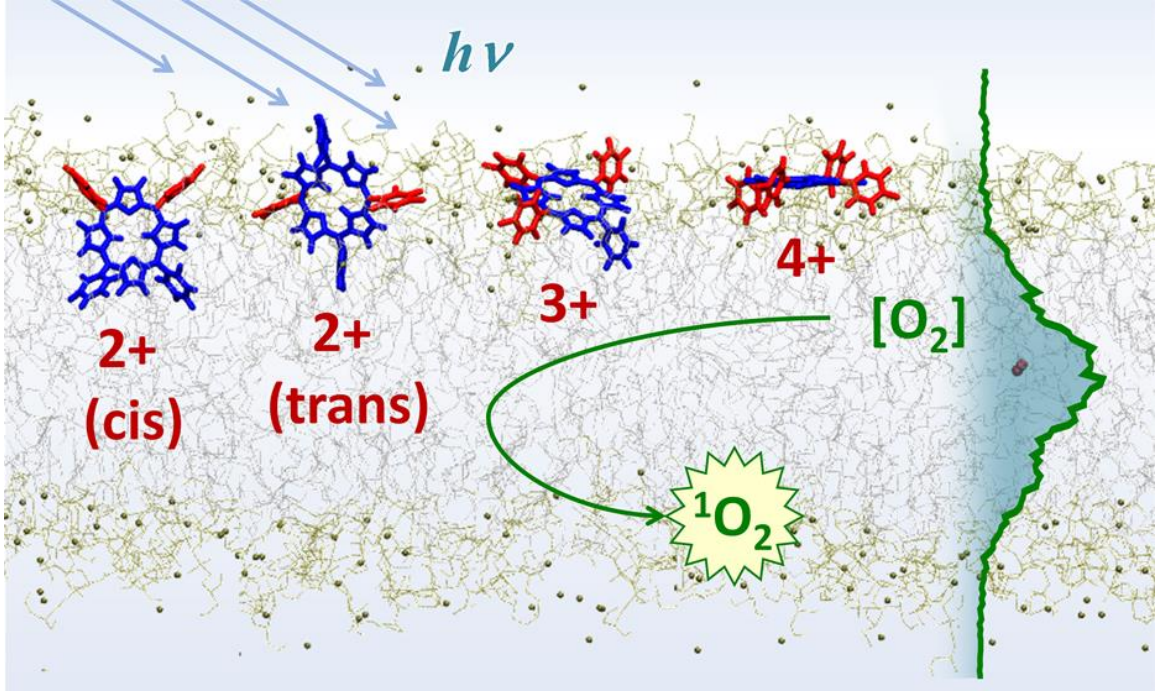
Amphiphilic molecules have higher affinity for membranes and generate $^1O_2$ close to biological targets

Engelmann, F. M. et al *J Bioenerg Biomembr* **2007** 39(2), 175.

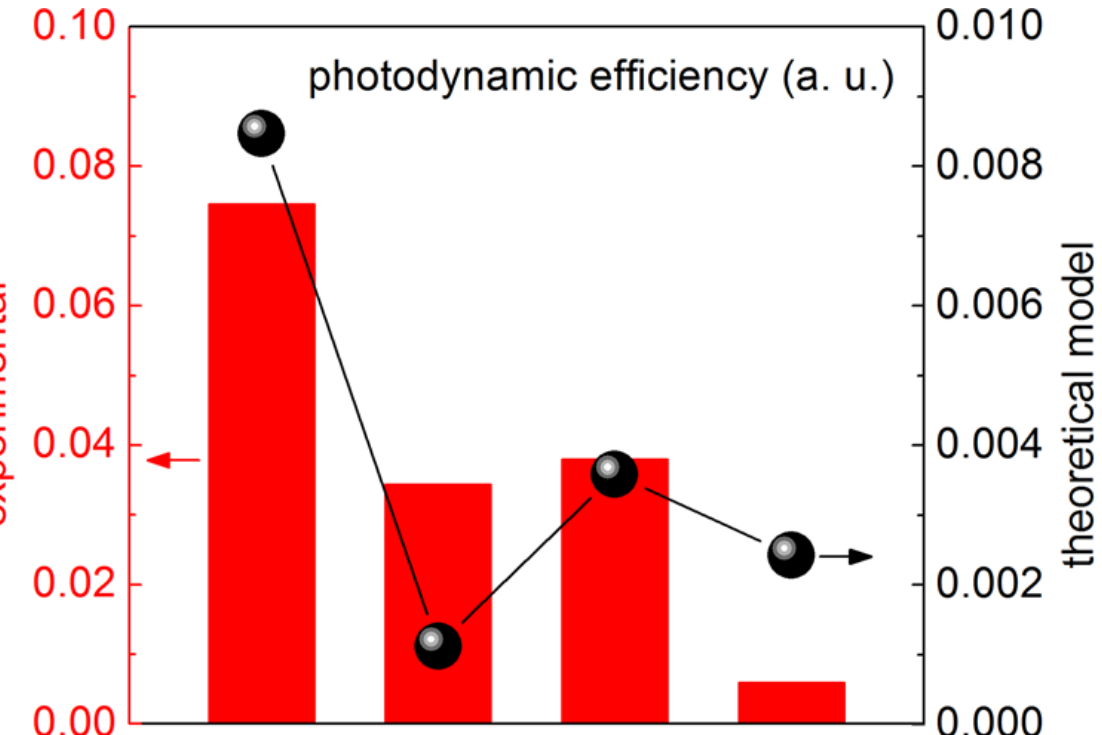
Engelmann, F.M. et al *Int J Pharm* **2007**, 329, 12.







Dynamical simulations agree and explain these results



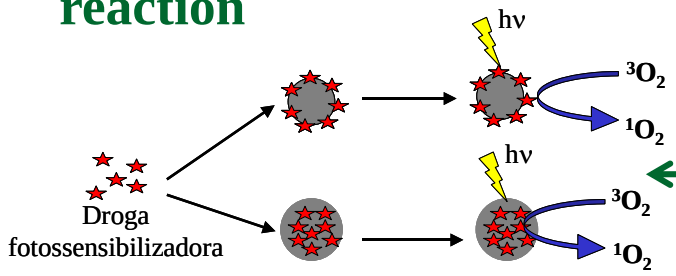
Cordeiro, Miotto and Baptista Photodynamic Efficiency of Cationic meso-Porphyrins at Lipid Bilayers: Insights from Molecular Dynamics Simulations *J. Phys. Chem. B* **2012**,116, 14618.

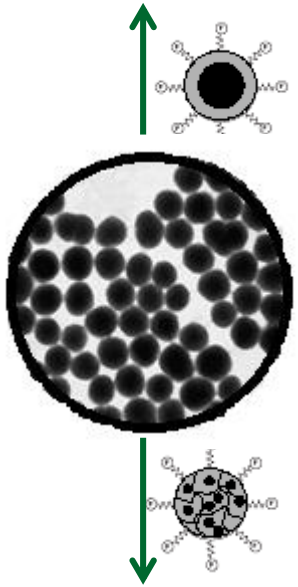
Using the nanoparticles to obtain more efficient photosensitizers

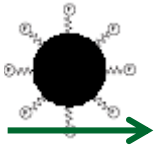
Magnetic properties

Haddad et al *Progr Coll Interf Sci* **2004,** 128, 232.
Duarte et al *Nanotechnology* **2006,** 17**,** 5549.
Tada et al *Langmuir* **2007,** *23*, 8194.

Control of excited state reaction



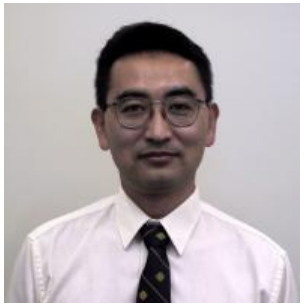




Decrease agreggation

Rossi et al *Langmuir* **2008,** 24, 12534.
Silva et al *Phys Chem Chem Phys*, **2011**, 13, 14946.

Rodrigues et al *Chemistry Letters* **2002**, 6, 604.
Rodrigues et al *J Non-Crystal Sol* **2002,** 304**,** 116.
Rodrigues et al *Adsorption* **2005**, 11 (5-6): 595.
Rodrigues et al *J Photochem Photobiol A* **2006**, 218.
Tada DB et al *JNN* 2010, 10, 3100.



Controlled release and cell death mechanism

Patente de invenção: Deda DK, Toma HE, Baptista, MS, Araki K, INPI # 018100015608, 2010.
Deda DK et al *Int J Pharm* **2009**, 376, 76.
Deda DK et al *J. Porphyr. Phtalocyan.***2012,** 16, 55..
DK Deda, et al *Journal of Biomedical Nanotechnology* 2013, 9 (8), 1307.
Tada et al *Journal of Biomedical Nanotechnology* 2014, 10, 519

Journal of Materials Chemistry B

COMMUNICATION

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Silica nanoreactors from silylated riboflavin for efficient singlet oxygen delivery†

Cite this: *J. Mater. Chem. B*, 2014, **2**, 4221

Received 31st January 2014
Accepted 23rd April 2014

DOI: 10.1039/c4tb00170b

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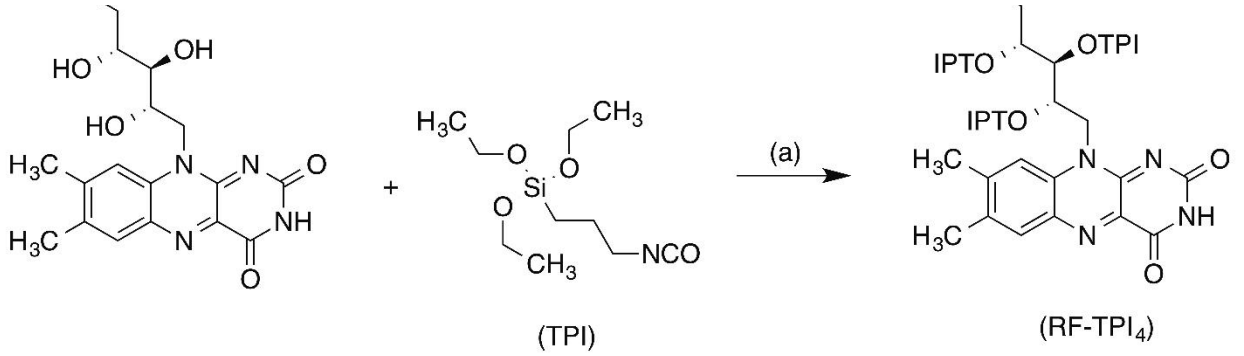
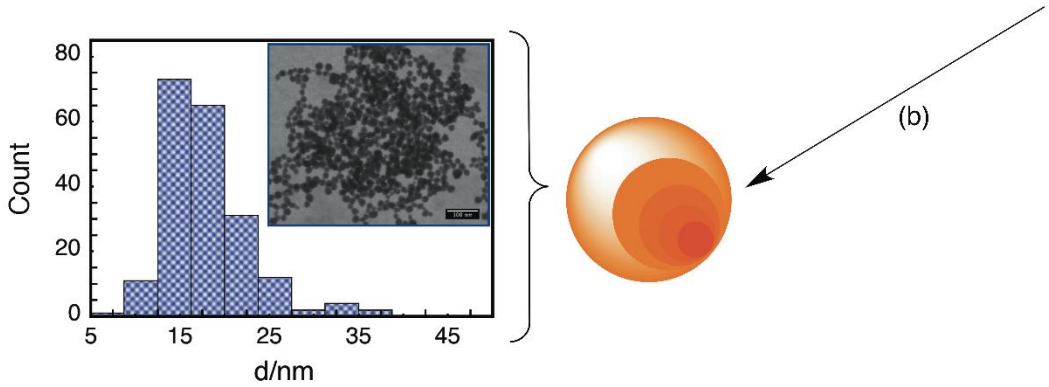
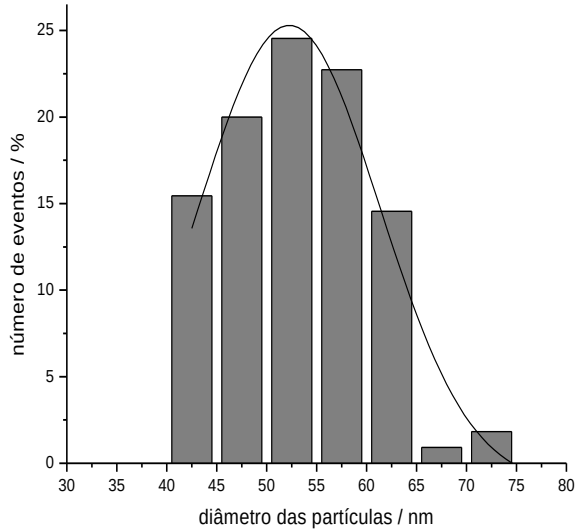
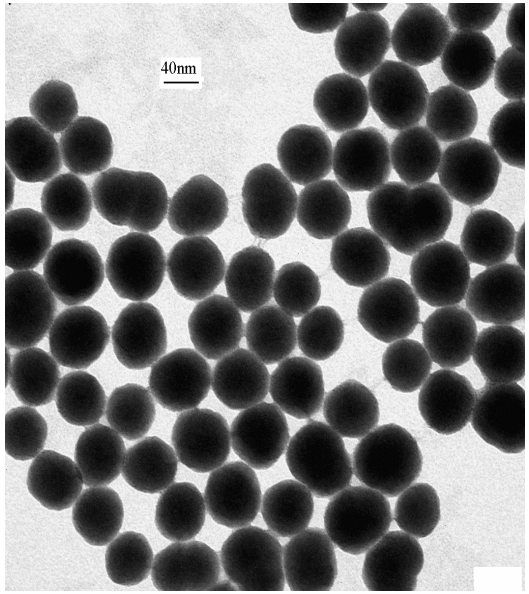
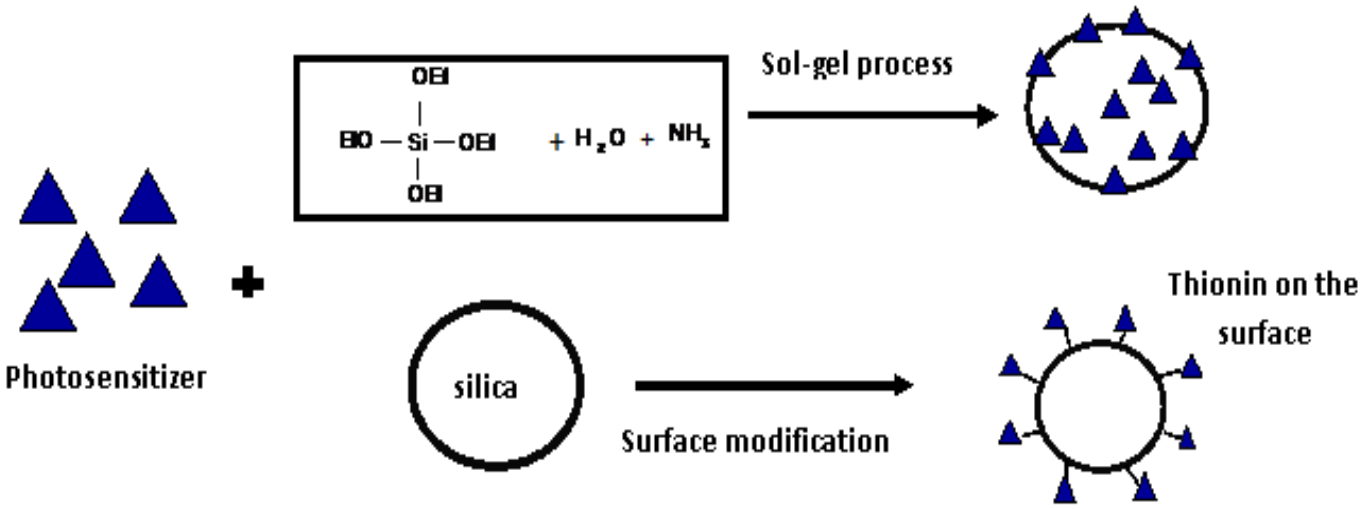


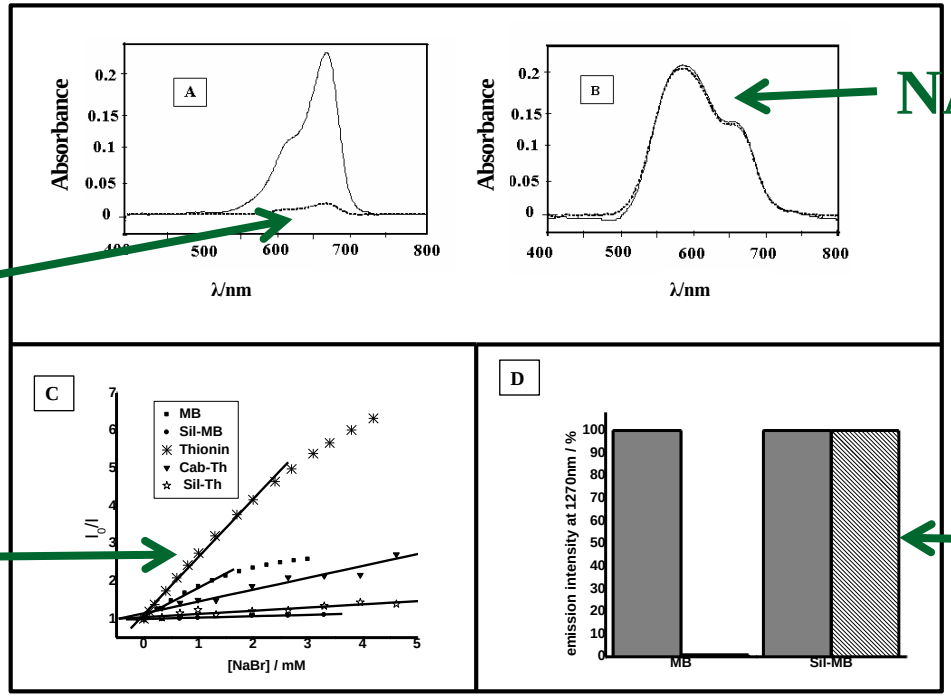


It is possible to control PHOTOCHEMISTRY by nanoparticle architecture









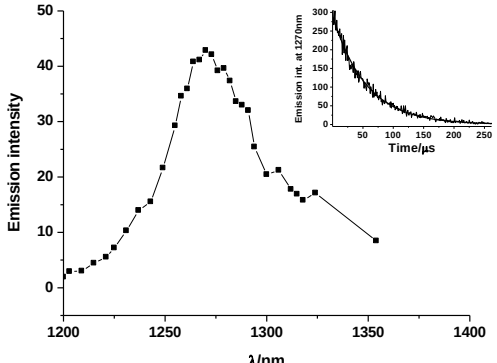
NADH

NADH

Br⁻

Asc⁻

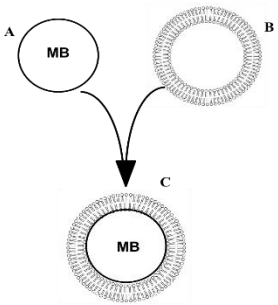
Partícula	D/M	ϕ
Nano 1	1,6	0,02 ± 0,01
Nano 2	0,76	0,05 ± 0,01
Nano 3	0,0	0,4 ± 0,1



Tada DB et al *JNN* **2010**, 10, 3100.

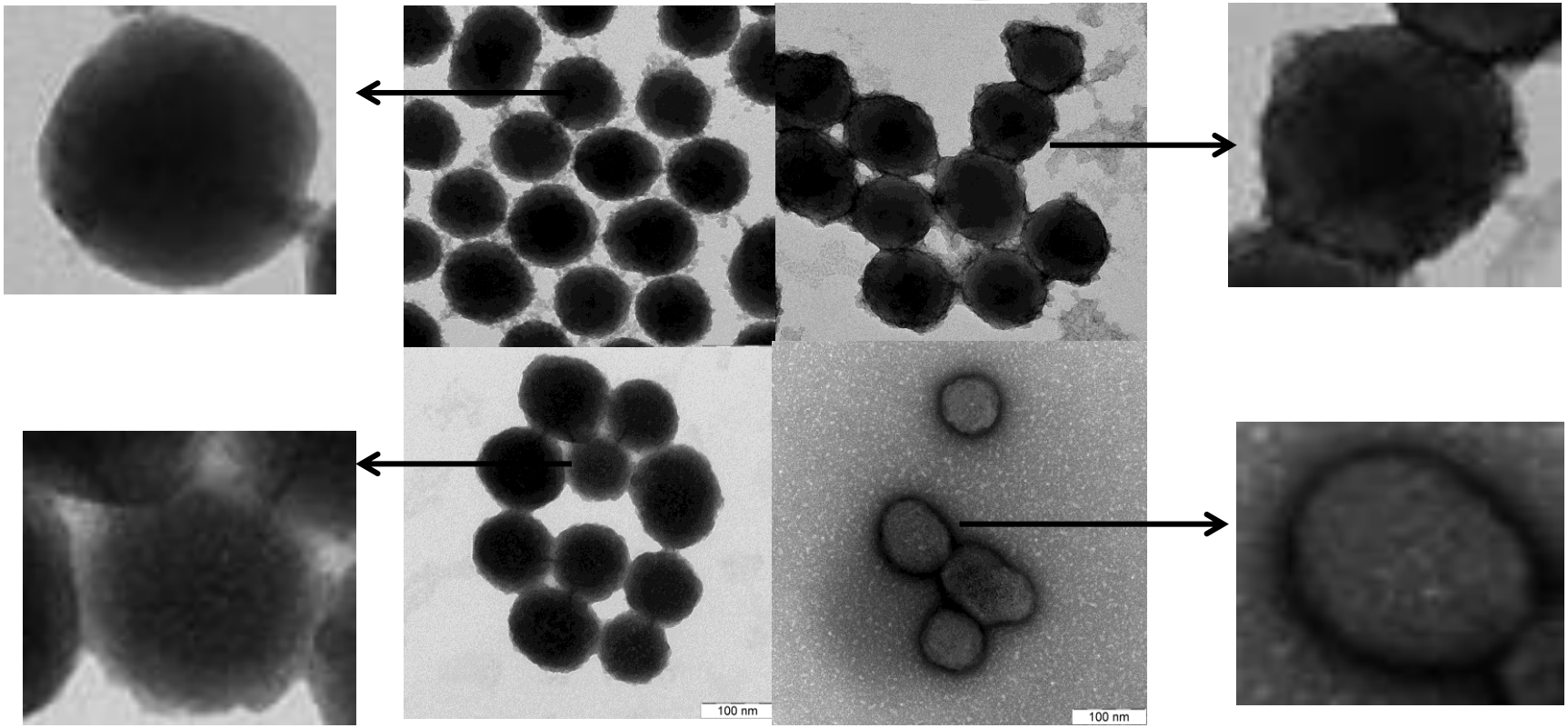
Tada DB, Tese de Doutorado, IQUSP, 2008.

It is possible to control cell LOCALIZATION by nanoparticle architecture



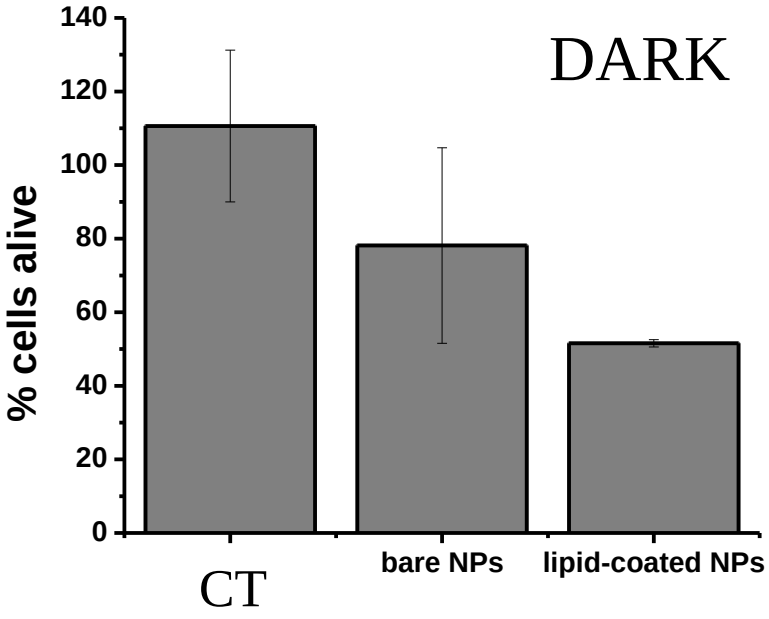
A MB

MB B



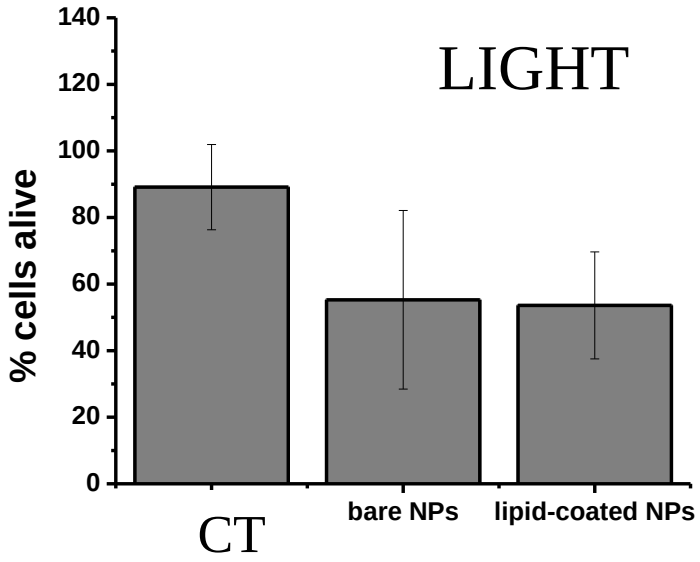
C MB

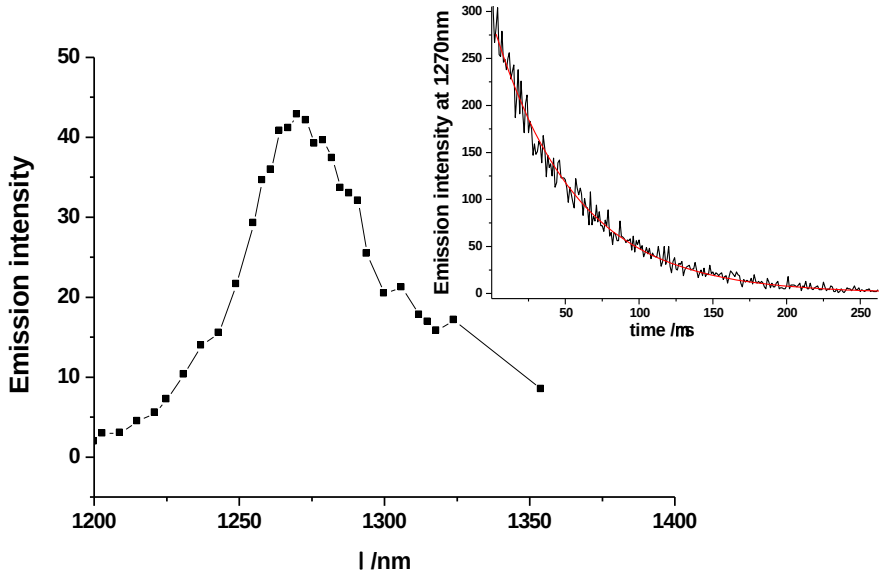
MB D

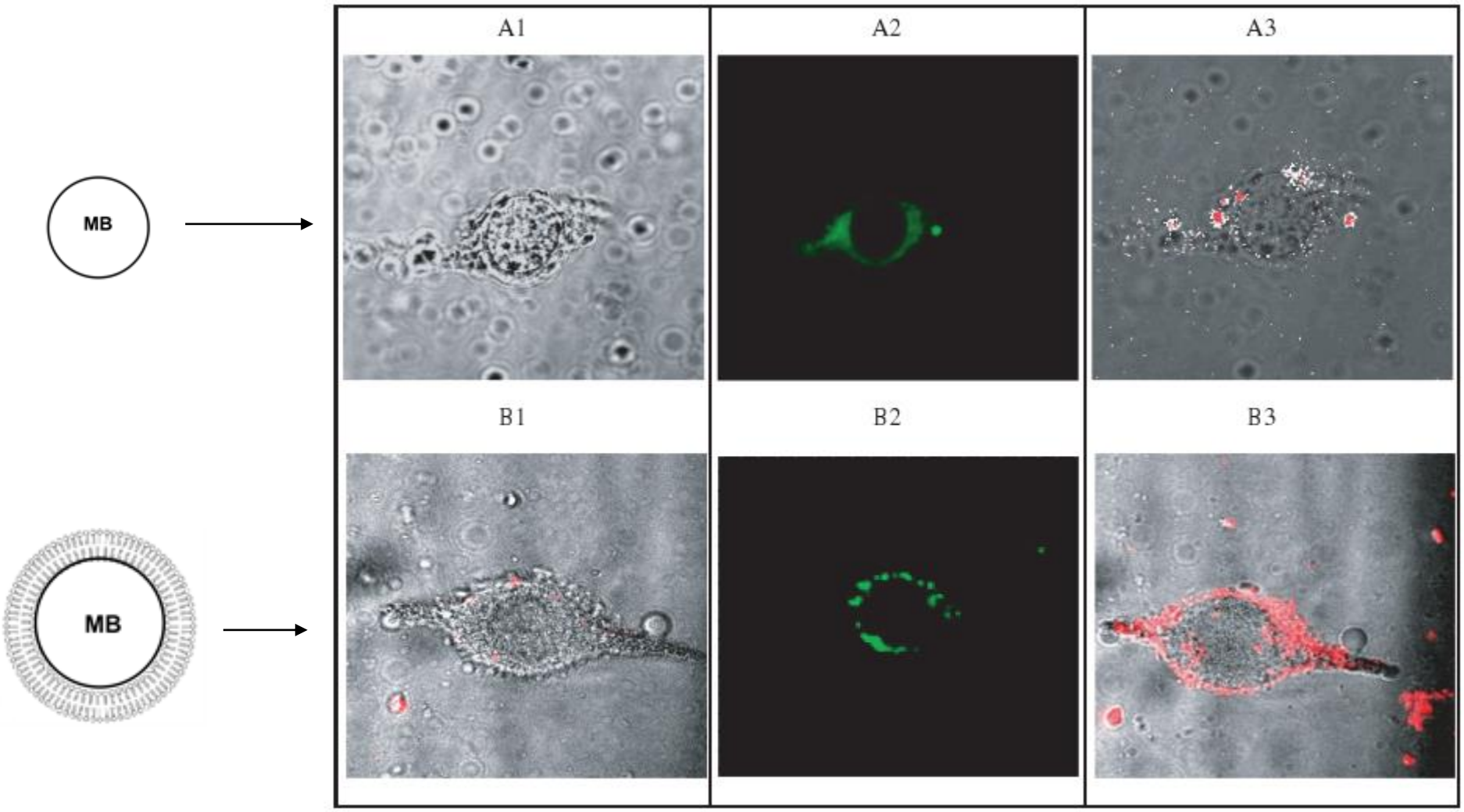


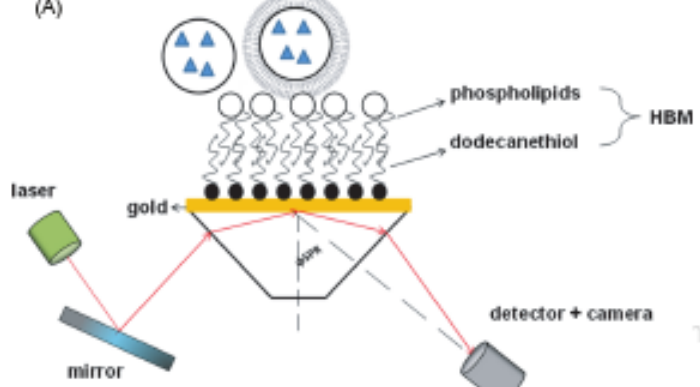
Bare nanoparticles are less toxic than lipid-coated in the dark

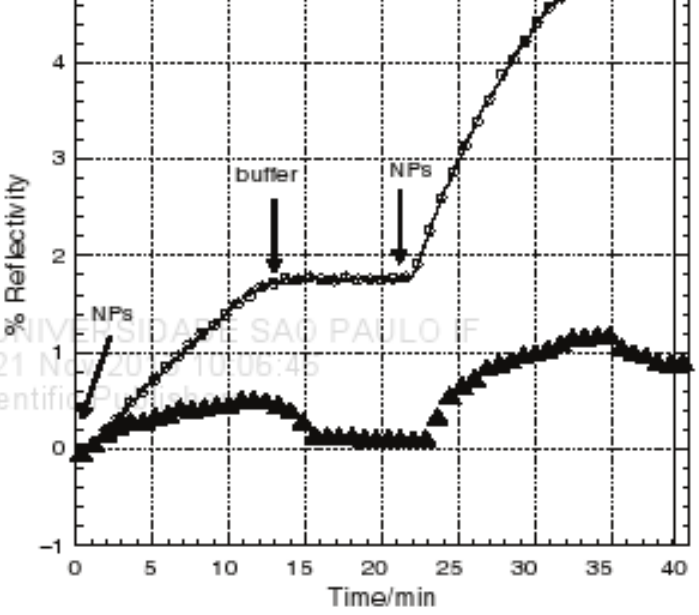
Under irradiation both generate single oxygen and kill cells!

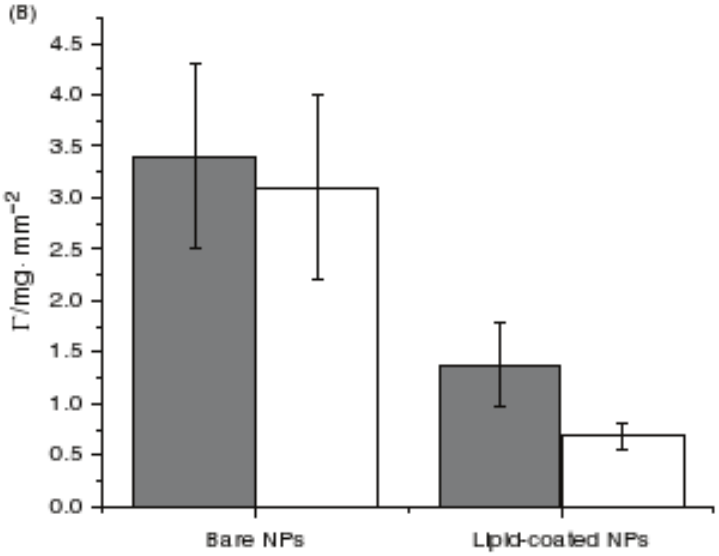


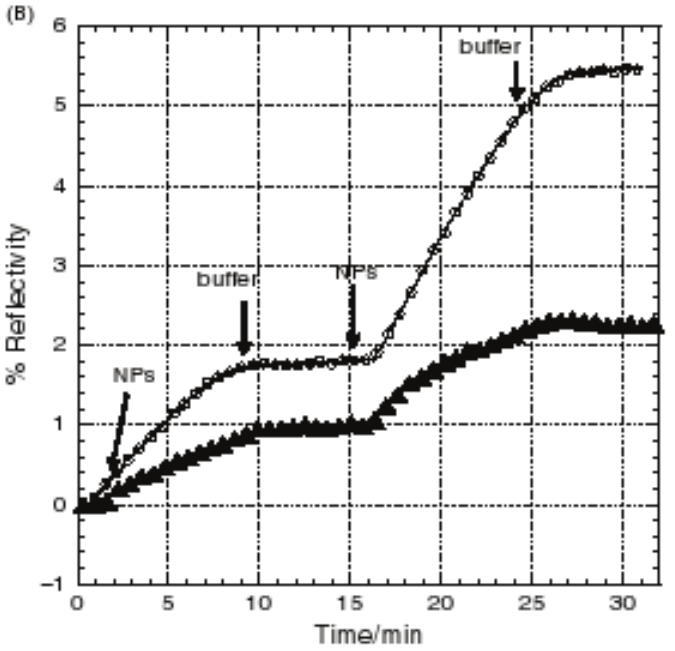


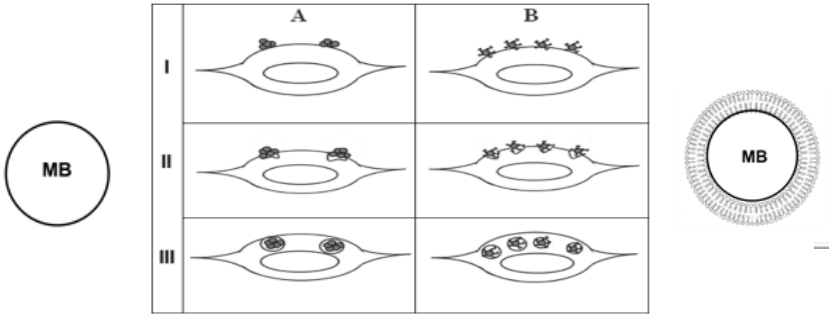

MB
MB
A1
A2
A3
B1
B2
B3

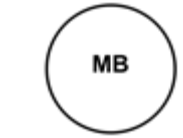


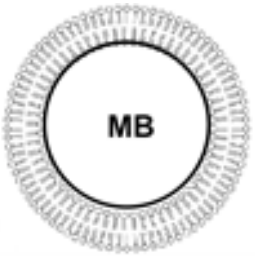


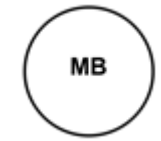


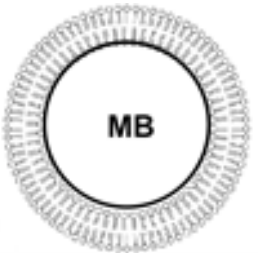








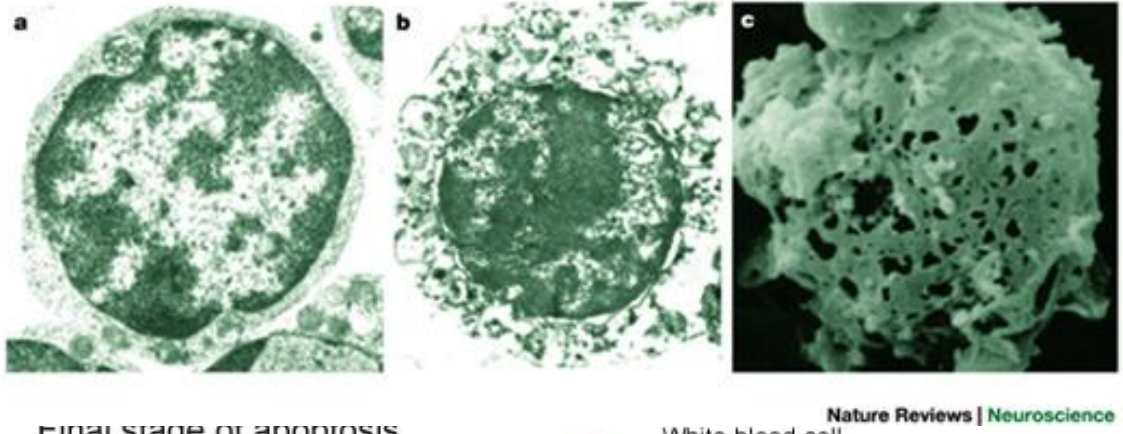




Tada et al *Journal of Biomedical Nanotechnology* 2014, 10, 519

Can PDT become more efficient by improving selectivity?

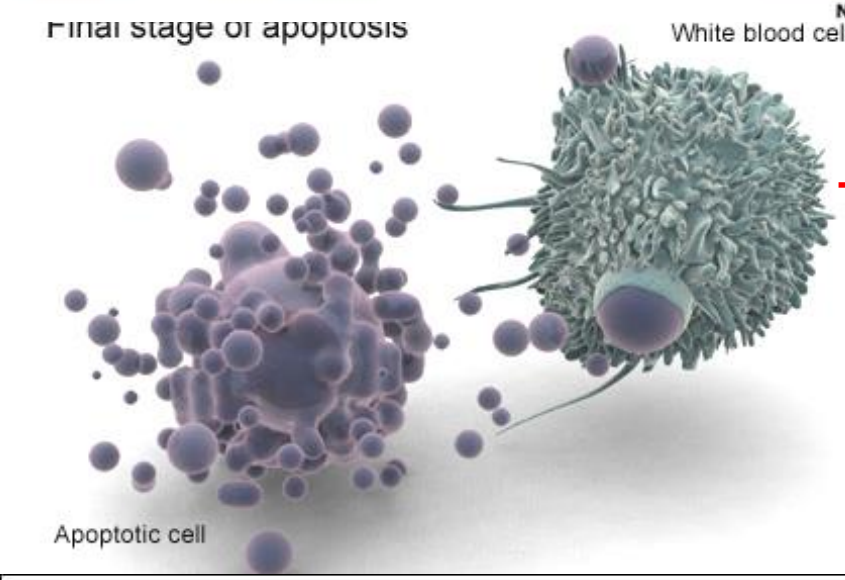
Yes, we should search for ways to activate or inhibit specific biological routes!



NECROSIS

MEMBRANE INTEGRITY LOST

UNCONTROLLED DNA DEGRADATION

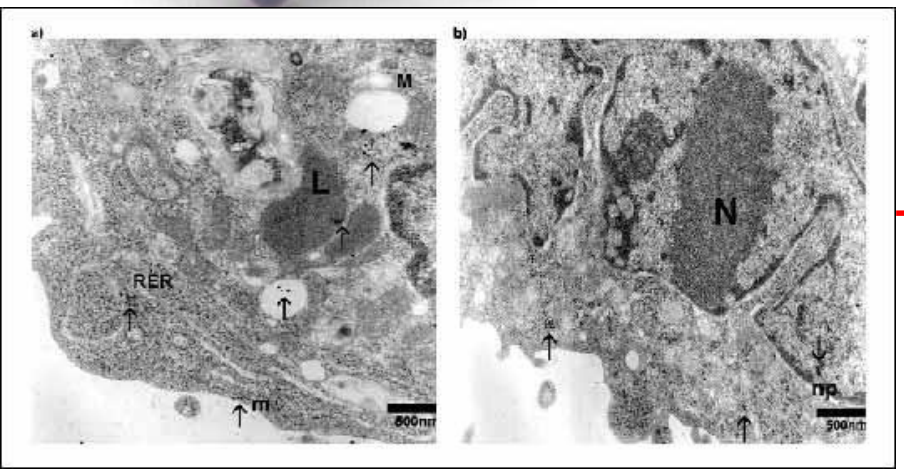


APOPTOSIS

MEMBRANE PRESERVED

CONTROLED DNA

CYTOSKELETON CRUMBLED

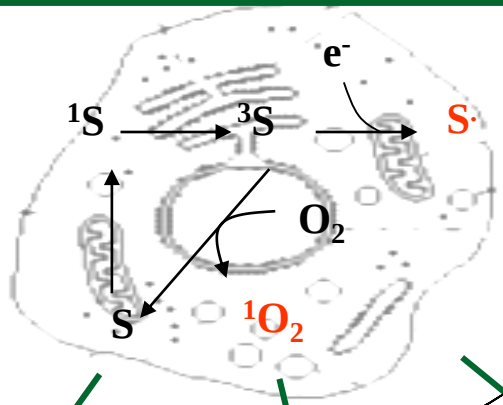


AUTOFAGY

ACIDIC VACUOLOS ACCUMULATE

CYTOSKELETON IS KEPT

Effect of the amount and location of the ROS in cell homeostasis



Magnitude of damage

survival / proliferation

Viability: CV, MTT, LA, BE, IP growth of colonies

Necrosis:

Integridade da membrana citoplasmática: AT, IP, LD

Senescense

Ki67

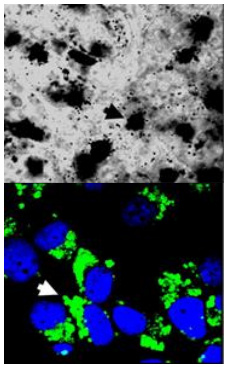
SA-β-Gal

Autophagy

IP/BE x LA

NR

LC3B-II



Glycosilases

Proteases

Lipases

Apoptosis

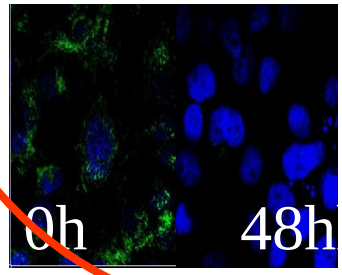
Anexin x PI

Cytochrome c

Mitochondrial potential

DNA sub G1

Anti-caspase 3



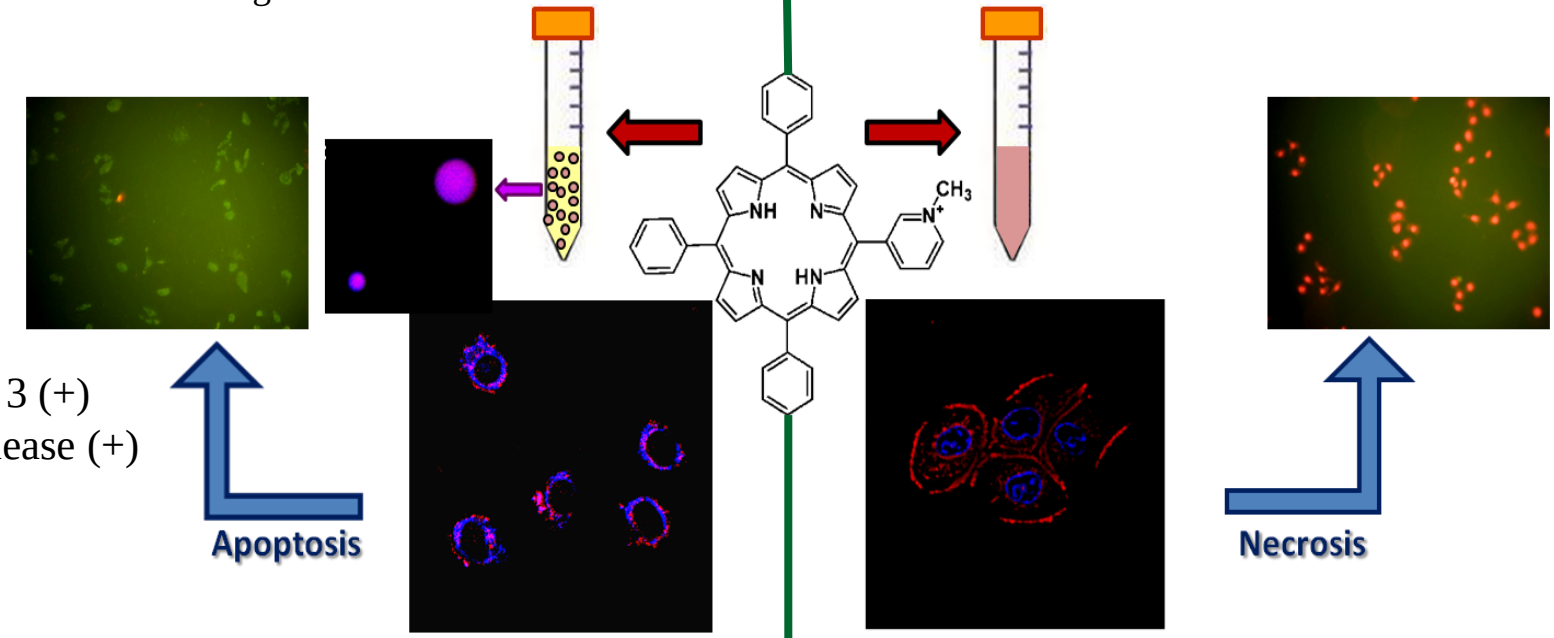
Controlling the mechanism of cell death

Porphyrin in Coacervated nanocapsules.

Menger and Sykes *Langmuir* **1998**, 14, 4131.

Isopropyl myristate, almond oil, Tween 20, xanthan gum, and sodium sulfate atelocolagen

Porphyrin in DMSO



Caspase 3 (+)
Cyt c release (+)

Apoptosis

Necrosis

650 nm

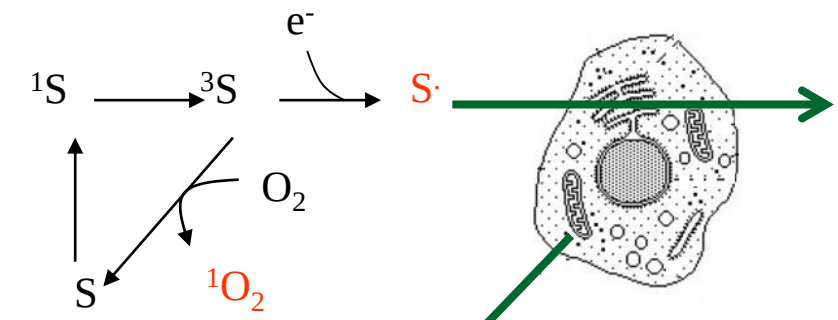
DK Deda, et al *Journal of Biomedical Nanotechnology* **2013**, 9 (8), 1307-1317.

Patent of invention: Deda DK, Toma HE, Baptista, MS, Araki
INPI # 018100015608, 2010.

Daiana Kotra Deda, PhD, IQUSP, 2011

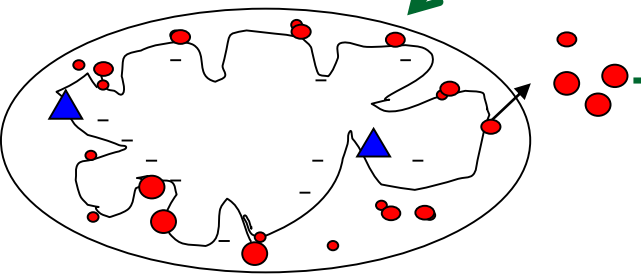
Release of cit*c* from mitochondria is fundamental for apoptosis

Li et al . *Cell* **1997** 91, 479–489.
Wallace DC *Science* **1999**, *283*, 1482-1488.
Green & Reed *Science* **1998**, *281*, 1309-1312.



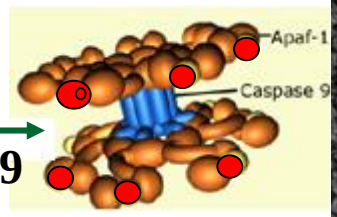
Photodamage—Release of cit*c*—apoptosis

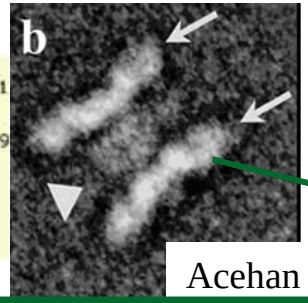
Agostinis et al *Photochem. Photobiol. Sci.* **2004**, 3, 721.
Krammer et al *Current Parmaceutical Design* **2005**, 11, 1151.



Apaf1

Procaspase 9





Activate effector caspase
Caspase 3

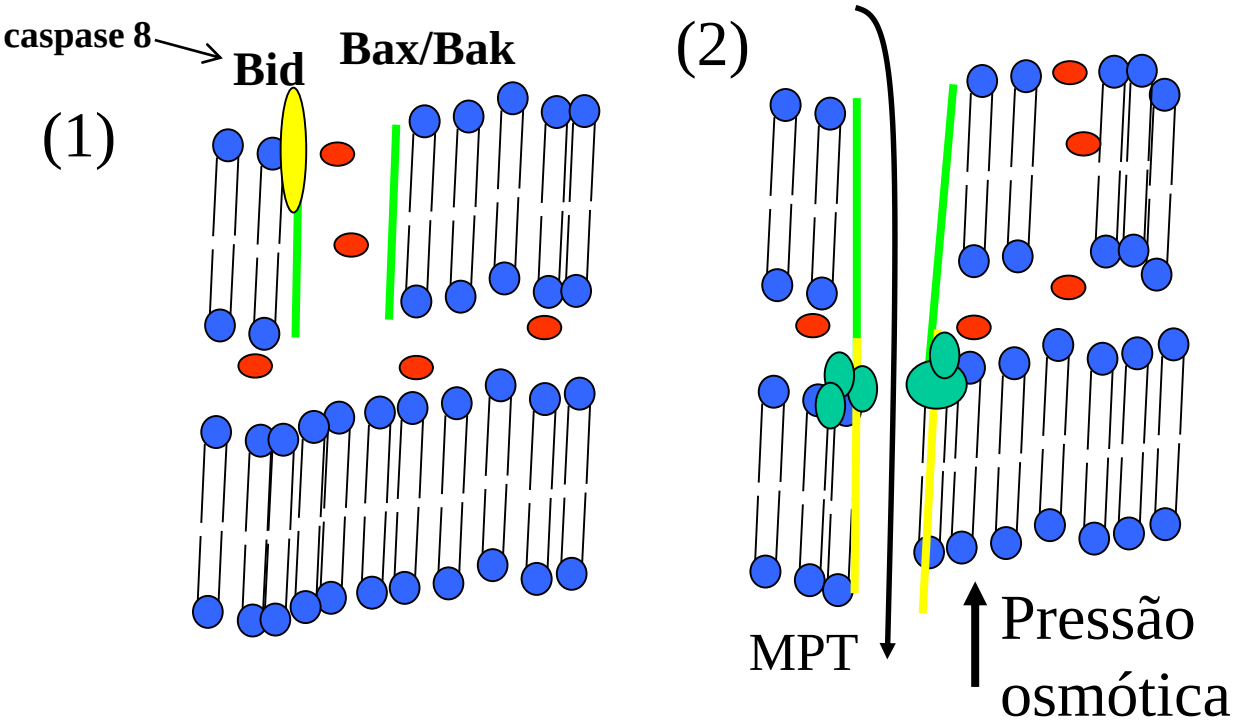
Acehan et al, *Mol Cel* **2002**, 9, 423

Apoptosomo: cit-c+APAF1+ATP/dATP+procaspase9

Why is cit c released from mitochondria? Is it a damage in protein or lipid?

AIF is carbonilated before being clived by calpain!
Norberg et al *FRBM* **2010**, 48 791.

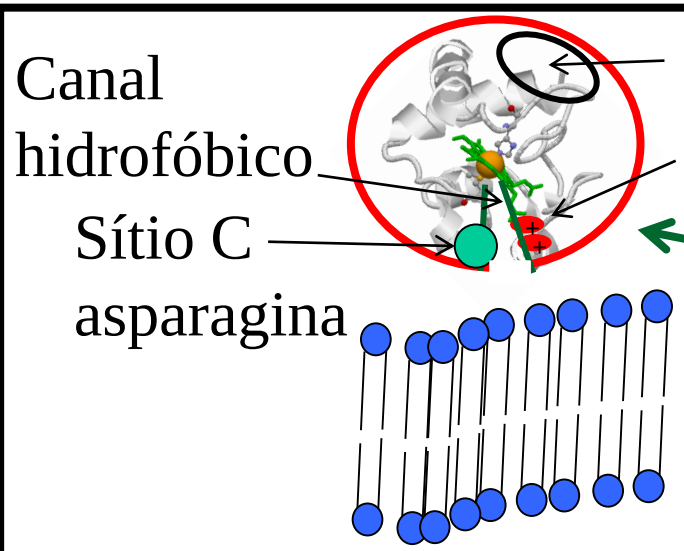
Liberação de citc da mitocôndria durante apoptose



Garrido *Cell Death and Differentiation* **2006** 13, 1423.

Kowaltowski *FEBS Letters* **2001**, 495, 12

Como a afinidade é alterada em função da foto-oxidação do citc e dos lipídeos da membrana?



Forças eletrostáticas,
Ligação de hidrogênio,
Efeito hidrofóbico
Interações lipídeo/proteína

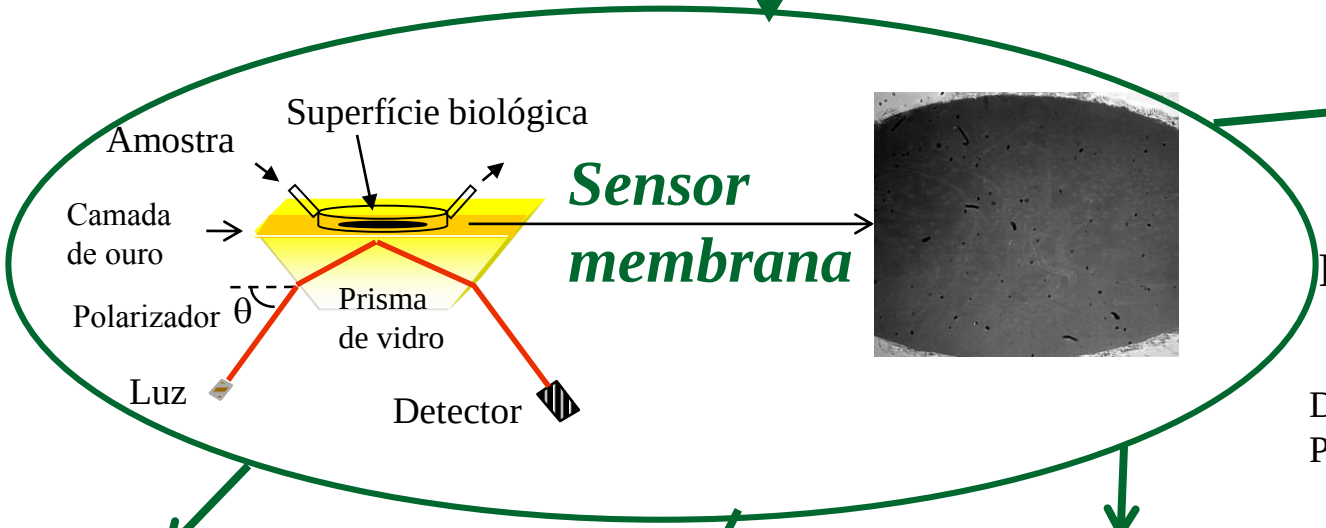
Tuominen et al *J Biol Chem* **2002**, 277, 8822

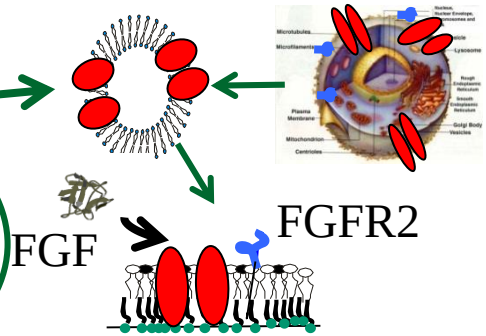
How to meaure mass of adsorbed protein (ng/mm^2)?

Homola J *Anal Bioanal Chem* **2003**, 377, 528.

Surface Plasmon Resonance (SPR): ultra sensitive balance

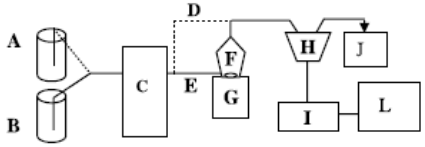
Castellana & Cremer *Surface Science Reports* **2006** ,61, 429.





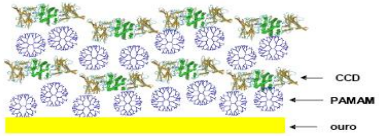
Dr. Gilson Masahiro Murata
Prof. Hugo Aguirre Armelin

Concentration grandients



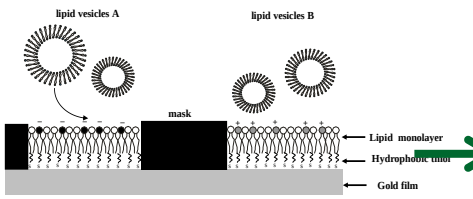
Tumolo T et al *Anal Biochem* 333 **2004**, 237.

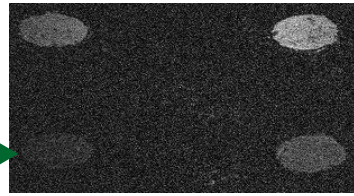
Selfassembled



-Zucolotto V *et al Appl Surf Sci* **2005**, *246 (4) 397.*
-Zucolotto V *et al Biosens Bioelectr* **2006**, *21, 1320.*

Membrane mimics

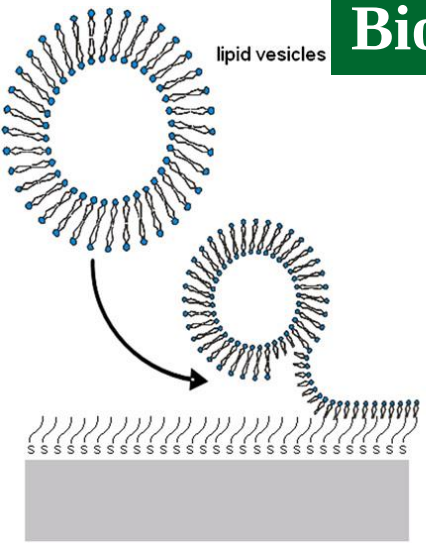


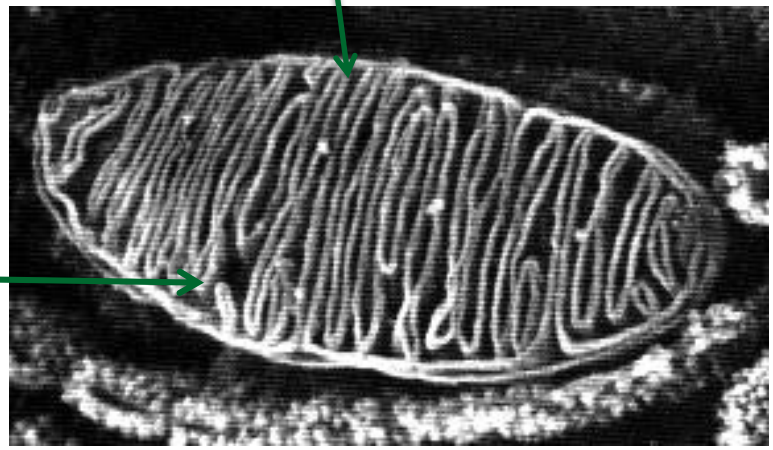


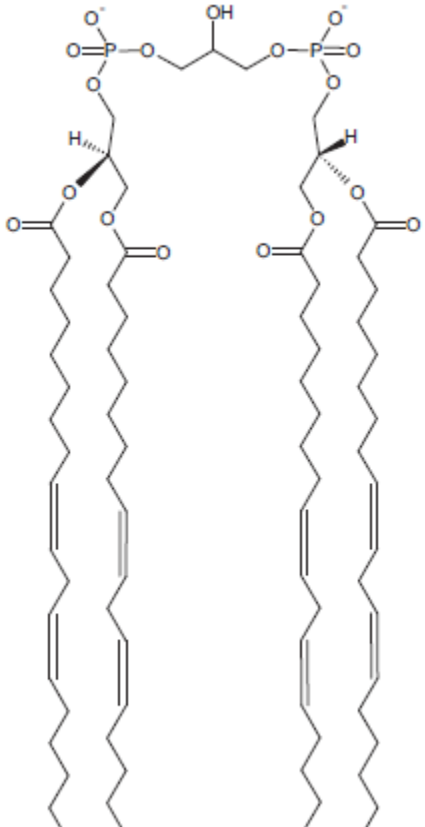
Moraes ML *Mat Sci Eng C* **2008**, 28, 467.
Suraniti et al *Langmuir* **2007,** *23*, 6835.
Tumolo T et al *Coll Surf B: Bio* **2012, in press**

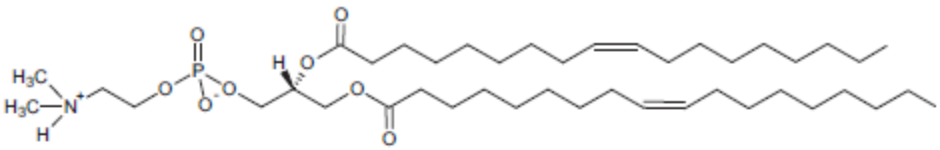
Tumolo T, Tese de Doutoramento, IQUSP, 2008.

Biosensor de membrana que imita a MMI (PE, PC, CL)

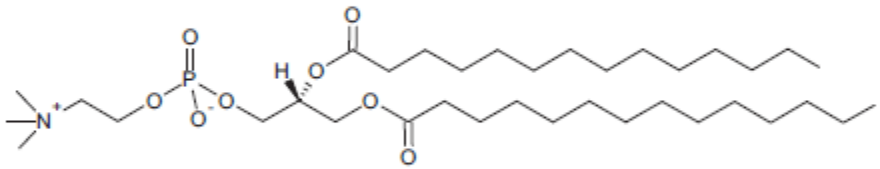








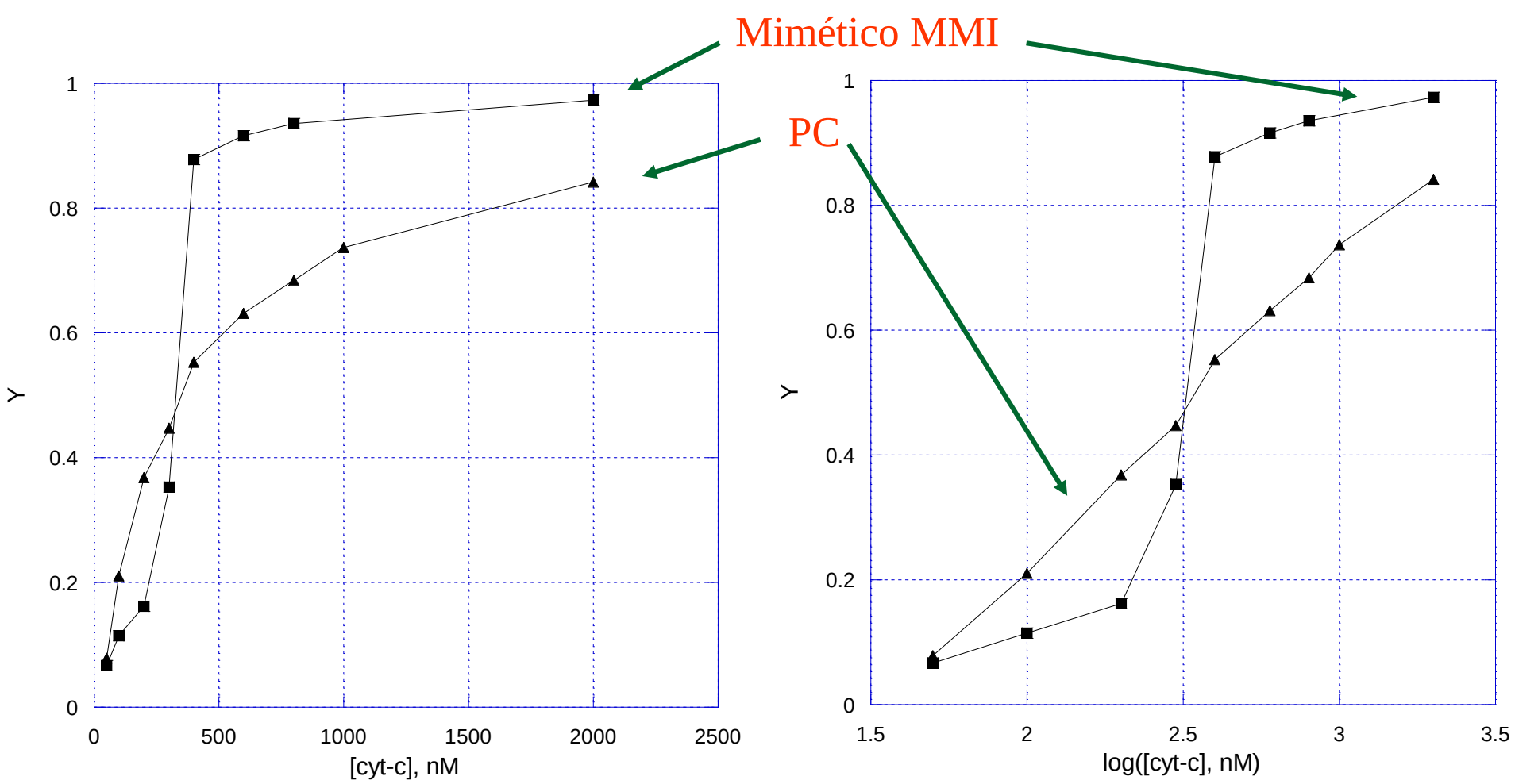
1,2-dioleoyl-sn-glycero-3-phosphatidylethanolamine-N,N-dimethyl (Dimethyl-PE)



1,2-dimyristoyl-sn-glycero-3-phosphatidylcholine (DMPC)

Tumolo T, Tese de Doutoramento, IQUSP, 2008

Tumolo T et al *Coll Surf B: Bio* **2012, in press**



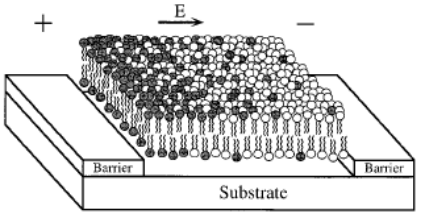
Y is the fraction of occupied sites

Cit c binding on negative mimetic membranes presents positive cooperativity

Electric fields can cause “lipid demixing”

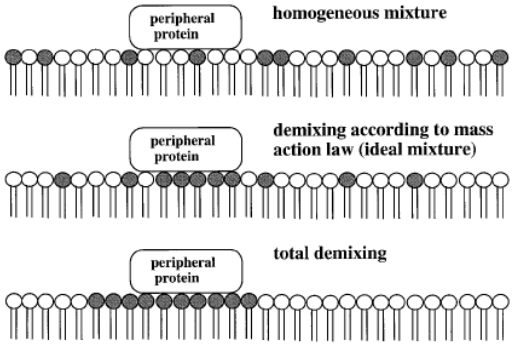
Groves, Boxer, McConnel et al *PNAS* 1998, 95, 935.

Electric field causes *lipid demixing*



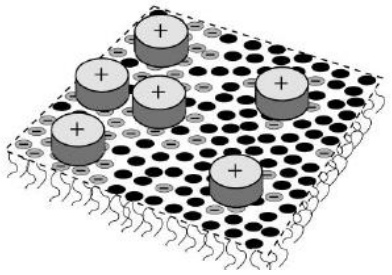
D. Marsh et al *Biophys.J.* 1999, 76, 2575.

Surface protein cause *lipid demixing* estimative of 8-13 lipids/domain in the case of cytc



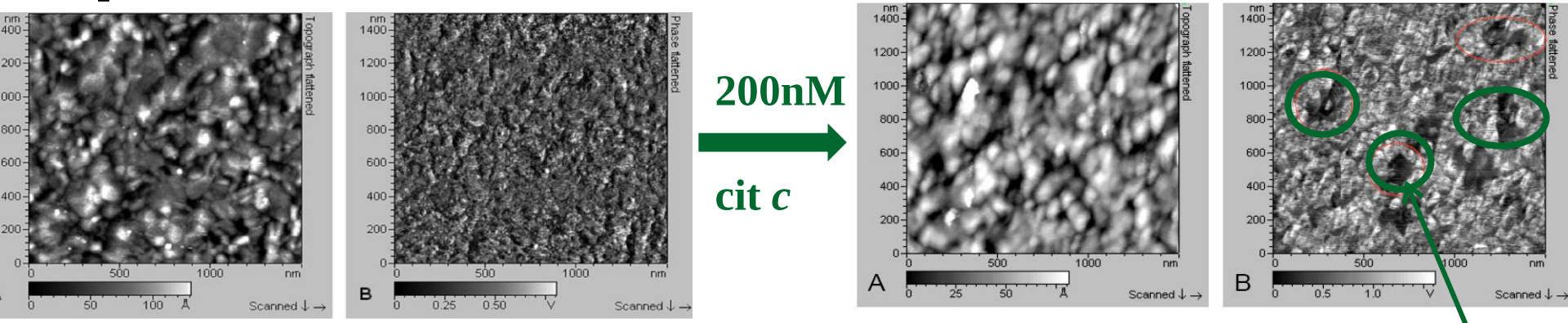
S. May et al *Biophys. J.* 2005, 88, 1702.

Improved calculation and predicted that domains are larger than few molecules (tenths)



AFM de HBM mimética da MMI

Amplitude **Contraste de fase** **Amplitude** **Contraste de fase**



200nM

cit *c*

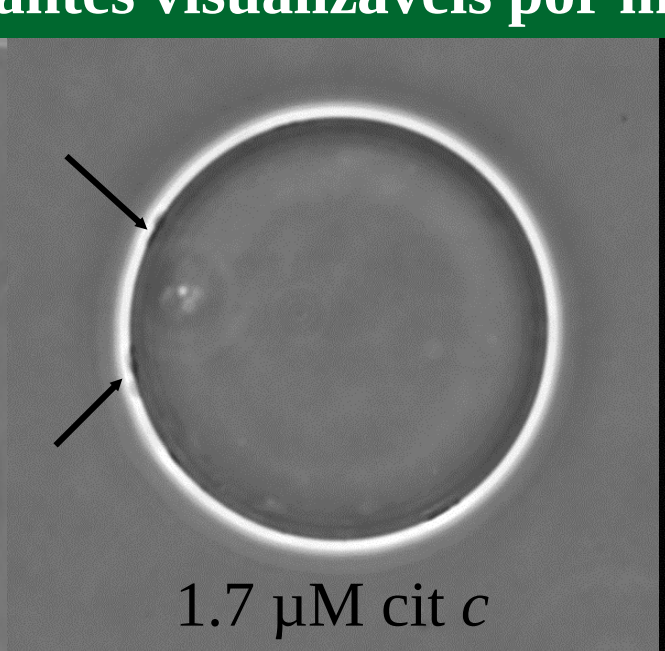
Tumolo T, Tese de Doutoramento, IQUSP, 2008

Regiões mais rígidas

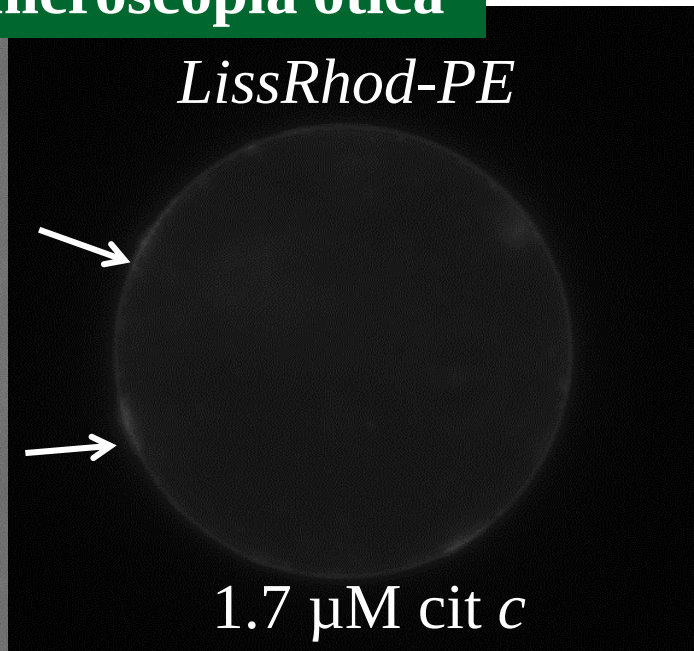
Costa, Rippel and Galenbeck *Polímeros: Ciência e Tecnologia* **2002**, 12,188.

Vesículas gigantes visualizáveis por microscopia ótica

mimética da MMI

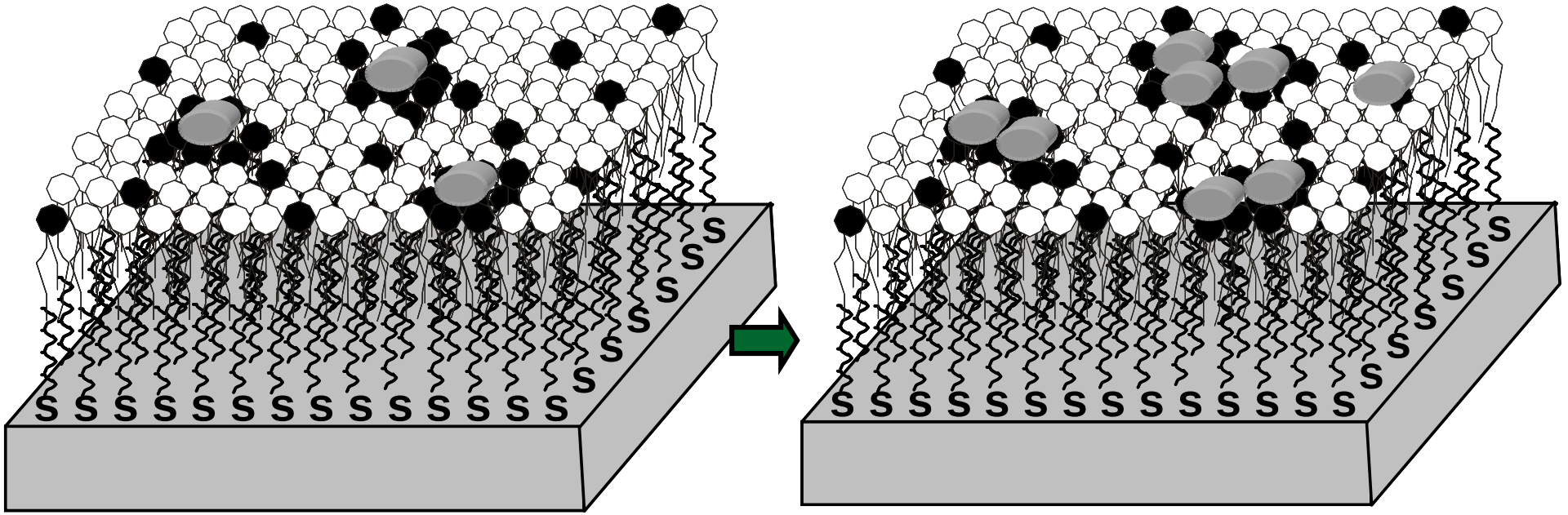


1.7 µM cit *c*



LissRhod-PE

1.7 µM cit *c*



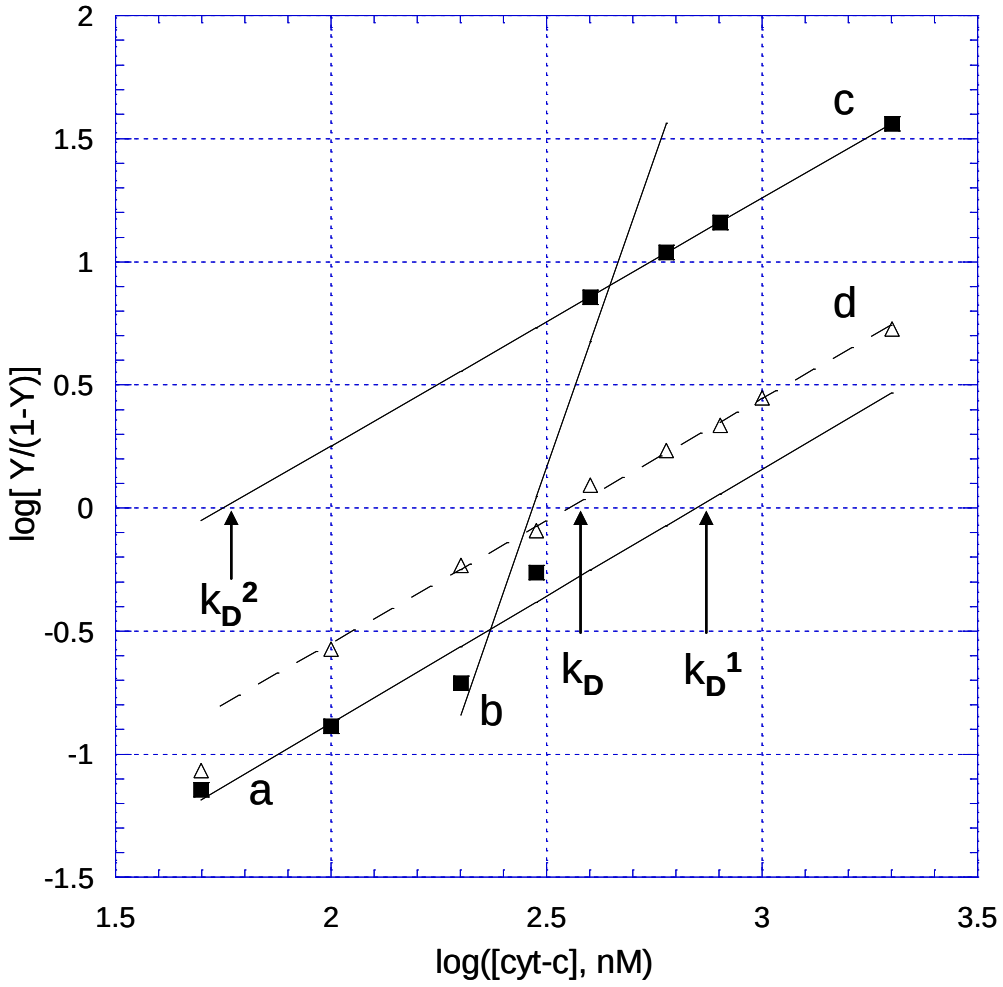
Cooperativity Hill model addapted to explain the interaction of charged proteins with heterogenous lipid mixture.
LIPID DIMIXING

Suraniti et al *Langmuir* **2007,** *23*, 6835.

$$\mathrm{L} + n\mathrm{C} \leftrightarrows \mathrm{LC}_n \qquad K_\mathrm{D} = \frac{[\mathrm{L}][\mathrm{C}]^n}{[\mathrm{LC}_n]} \tag{4}$$

$$Y = \frac{n[\mathrm{LC}_n]}{n([\mathrm{L}] + [\mathrm{LC}_n])} \tag{5}$$

$$\log\left(\frac{Y}{1-Y}\right) = n \log[\mathrm{C}] - \log K_\mathrm{D} \tag{6}$$



Interação cooperativa entre proteinas/peptideos e fosfolipideos pode modular uma variedade de processos em biologia celular

Suraniti et al *Langmuir* **2007,** *23*, 6835.

Afinidade aumenta devido a reorganização dos lipídeos

PC K_D=0,4 μM

Mimético MMI K_D^1 = 0,7 μM, **K_D^2=0,06 μM**, n=5,0

↑pH → Desprotona fosfato → K_D^1 ↓ Aumenta afinidade

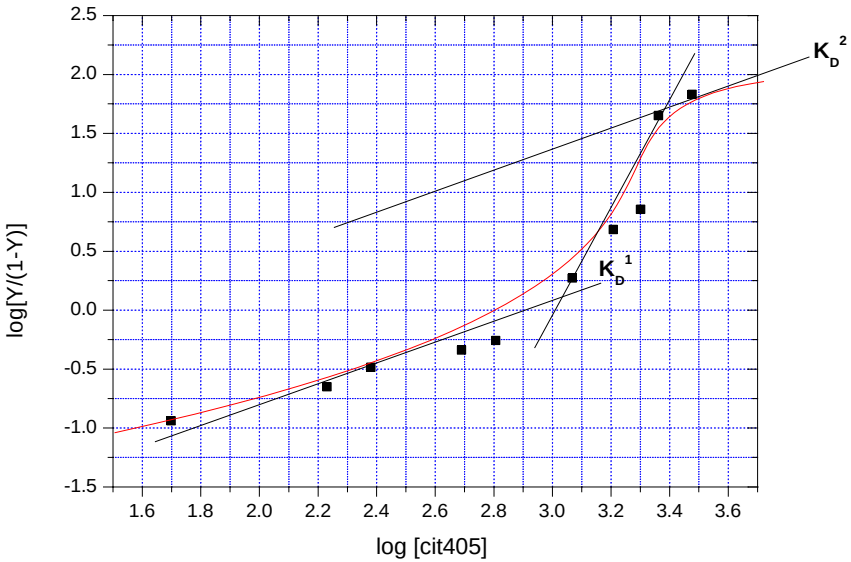
n ↓ Diminui cooperatividade

Tumolo T, Tese de Doutoramento, IQUSP, 2008

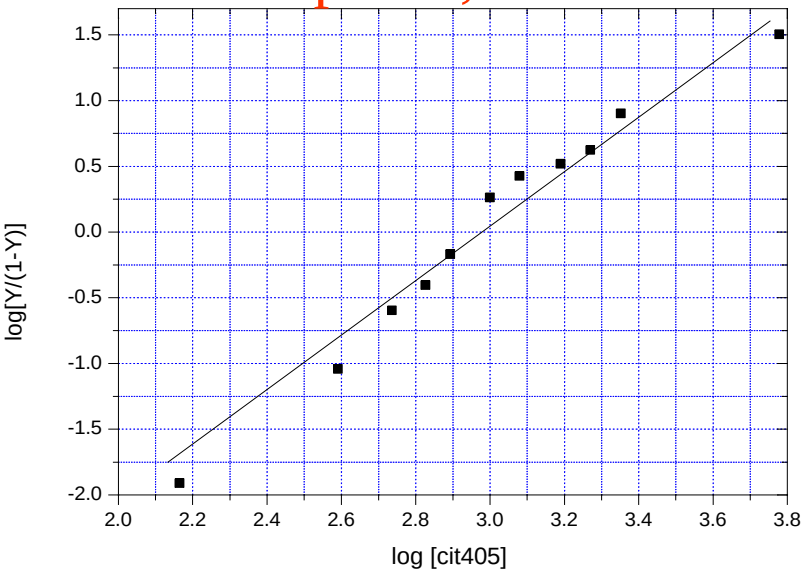
Citc + $^1O_2$ = *citc405.* Estevam et al *J Biol Chem* **2004**, 279, 39214.

Como citc405 interage com a membrana mimética da MMI?

pH=6,8



pH=8,0



Cytc: K_D^1=1.0, K_D^2=0.04, n~9

Cytc405: K_D^1=0.5, K_D^2=0.03, n~5

Cytc: K_D^1=0.6, K_D^2=0.03, n~5

Cytc405: K_D=0.7 n~0

Cooperatividade diminui e afinidade aumenta!

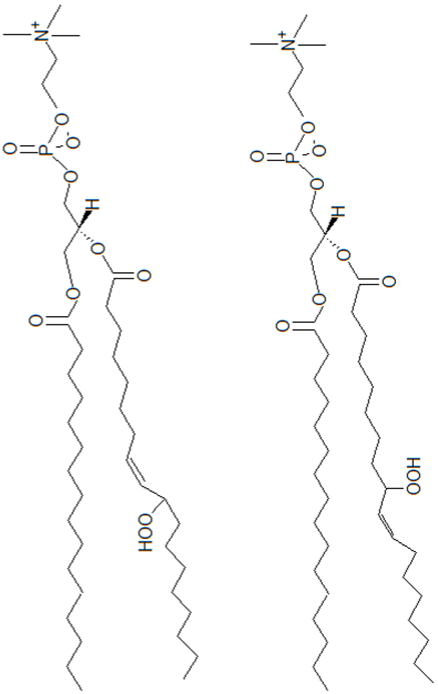
Fotooxidação do cit*c* não é responsável pela sua liberação da mitocôndria

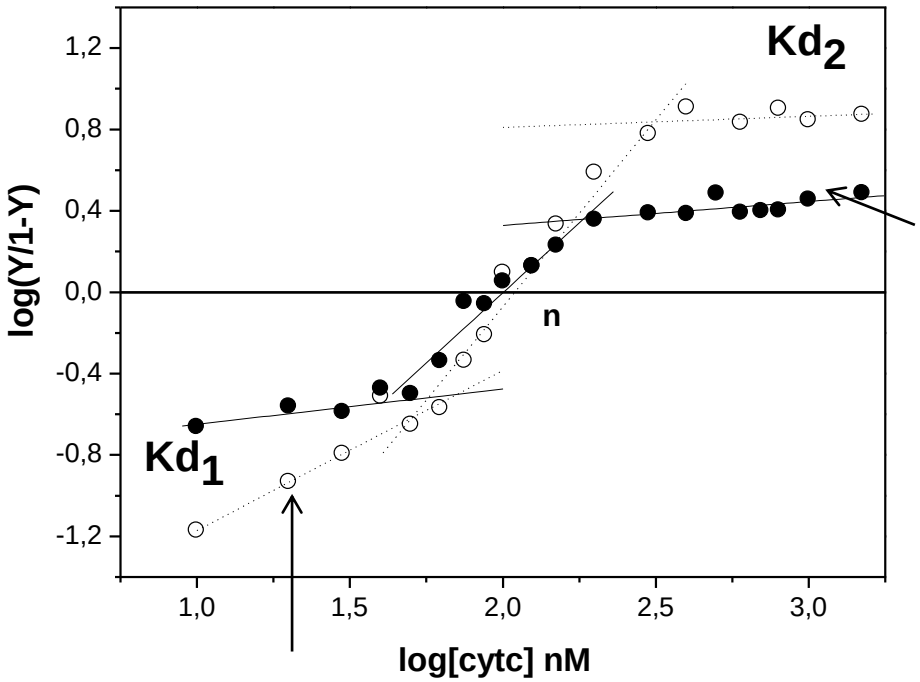
Tumolo T, Tese de Doutoramento, IQUSP, 2008

CL oxidation decreases cytc-membrane affinity

Ott et al *PNAS* **2002**, 99, 1259
Petrosillo *FASEB J* **2003**,17,2202

What happens on the presence of POPC-OOH ?





control

pH=7.4

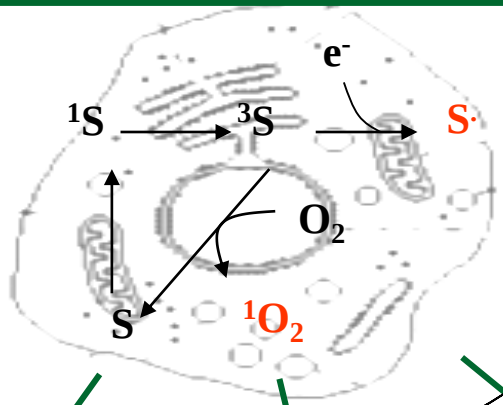
~25% de hidroperóxidos de POPC

controle: K_D^1=1, K_D^2=0,01, n~2,0
Hidroperóxidos: K_D^1=0.5, K_D^2=2x10^2 n~1,6

Dra.Kawai
Pos-doc IQUSP

Decrease in affinity *due to the presence of lipid hydroperoxide*

Effect of the amount and location of the ROS in cell homeostasis



survival / proliferation

Viability: CV, MTT, LA, BE, IP growth of colonies

Magnitude of damage

Necrosis:

Integridade da membrana citoplasmática: AT, IP, LD

Senescense

Ki67

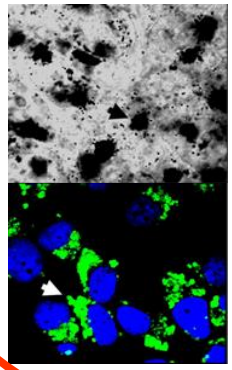
SA-β-Gal

Autophagy

IP/BE x LA

NR

LC3B-II



Apoptosis

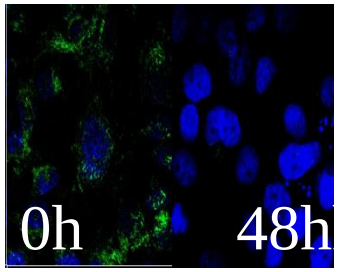
Anexin x PI

Cytochrome c

Mitochondrial potential

DNA sub G1

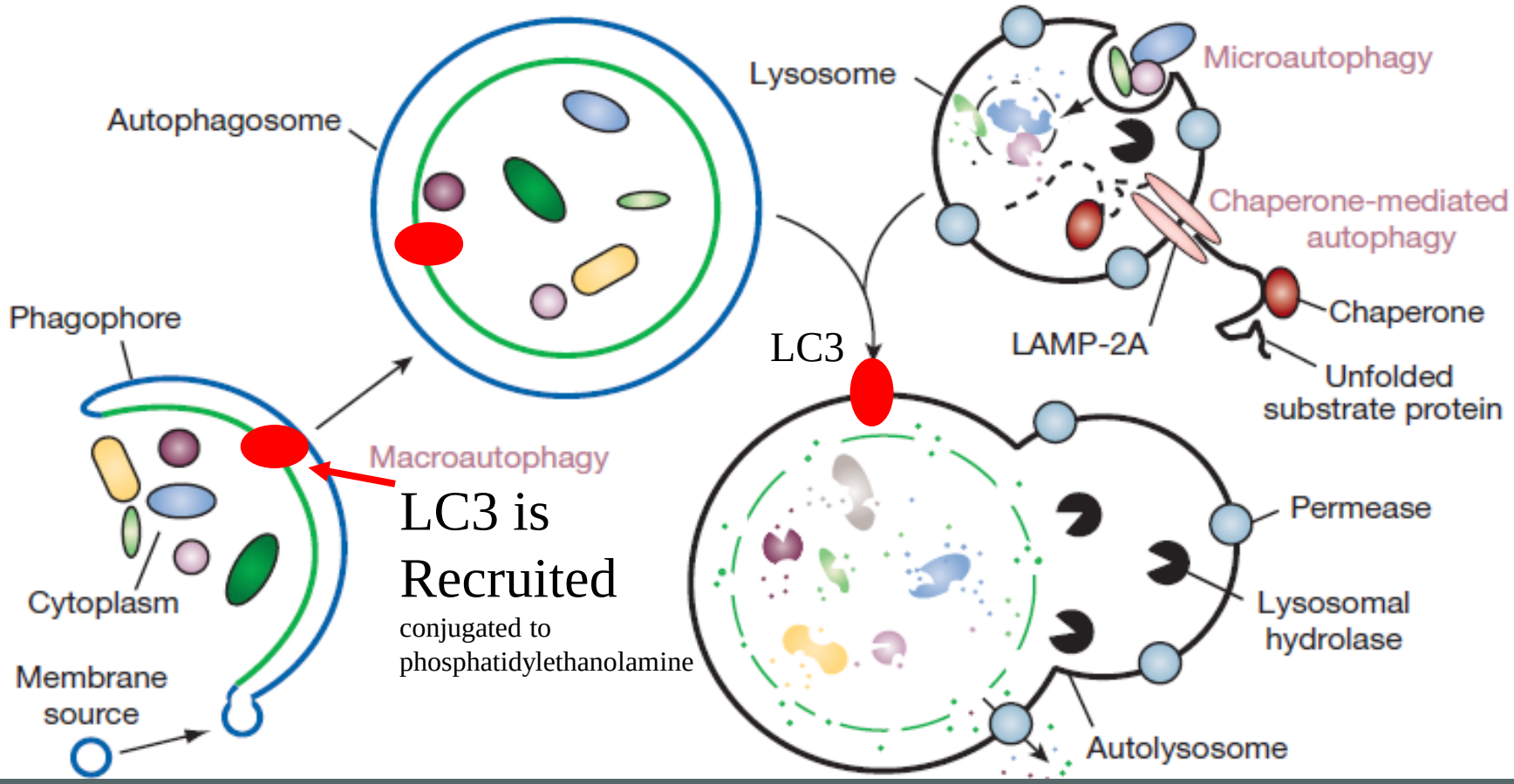
Anti-caspase 3



Pavani et al *Photochem Photobiol* **2012,** 88, 774.

Deda et al *J Biomed Nanotec* **2013**, 9, 1307.

NATURE|Vol 451|28 February 2008
Noboru Mizushima[1], Beth Levine[2], Ana Maria Cuervo[3] & Daniel J. Klionsky[4]



Excess

- Metabolic colapse
- Induction of cell death

Steady state condition

Homeostasis

- Removal of damaged organellas
- Retard cell aging

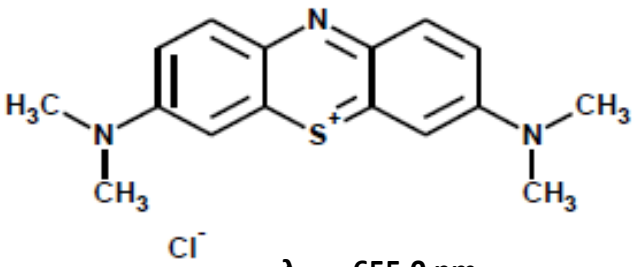
Loss of autophagy

- Acumulus of cell damage
- Acelerated aging

Photophysical properties are similar but DMMB binds more efficiently and causes more damages to the membranes

Methylene Blue (MB)

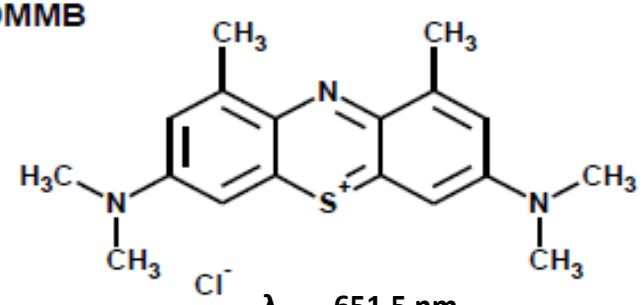
MB



$\lambda_{máx=}$ 655,0 nm

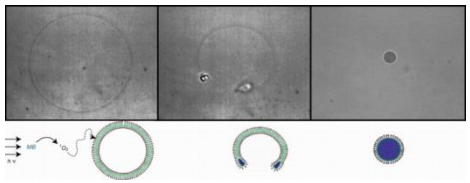
1,9- Dimethyl Methylene Blue (DMMB)

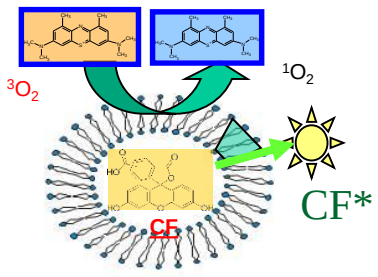
DMMB



$\lambda_{máx=}$ 651,5 nm

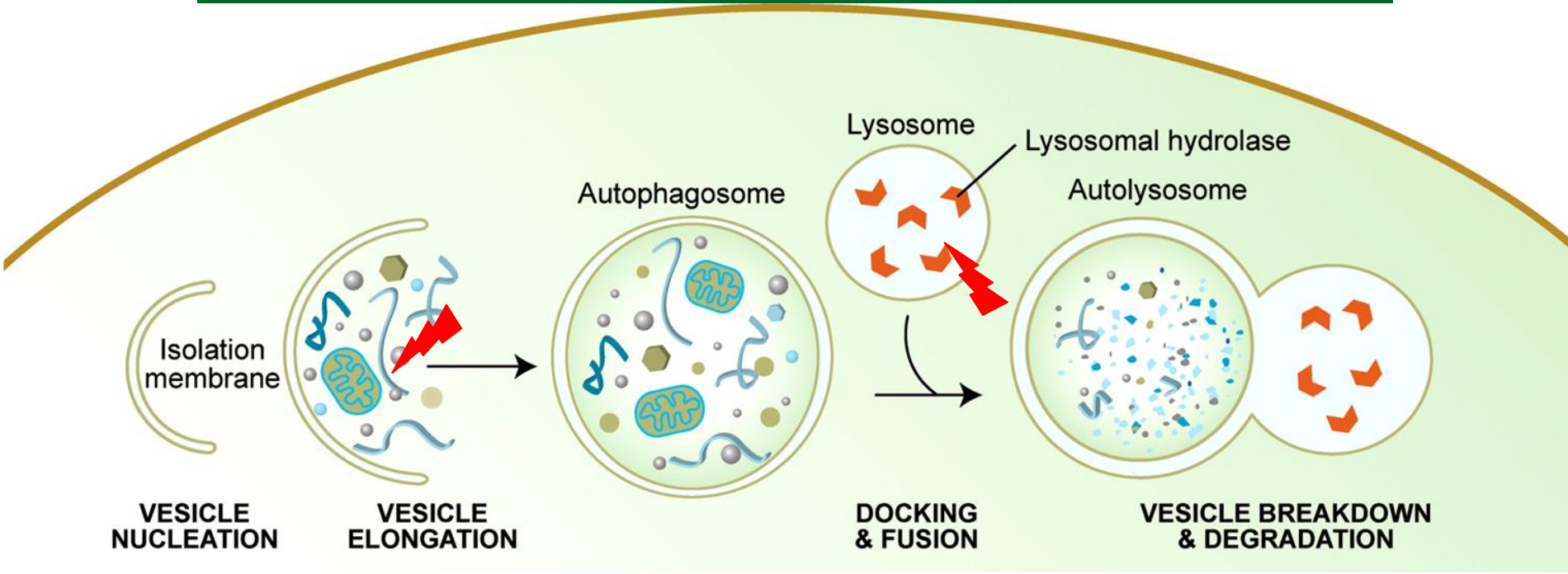
PS	Φ_Δ	Φ_f	τ / μs	Membrane Binding (%)	Membrane damage (%)
MB	0,52	0,04	9,3 ± 0,2	1,01 ± 0,004	2,9 ± 0,2
DMMB	0,59 ± 0,08	0,045 ± 0,005	10,4 ± 0,1	**50 ± 1**	**92 ± 4**





Bacellar et al *Photochem Photobiol* **2014**, 90, 801.

AUTOPHAGY by PDT: parallel damage in mitocondria and lisossomes



Damage in the mitochondrial and lisosome membranes

Inhibition of the autophagic flux: activation of cell death pathway

At lower concentrations MB does not induce autophagy because it is reduced in mitochondria. At higher concentrations, dye localizae inespefically!

Damages in membranes are really important for photoinduced cell killling!

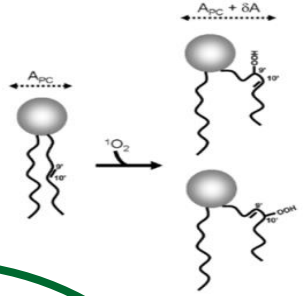
How can we study the photooxidation reactions on the membranes

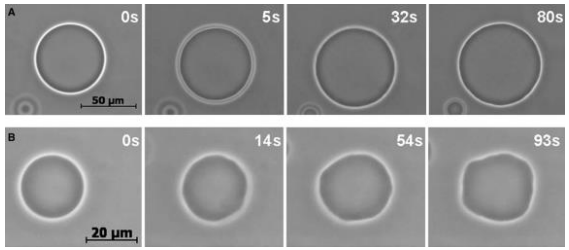
The membranes are important targets. Membrane damage and efficiency define the mechanism of cell death photoinduced!

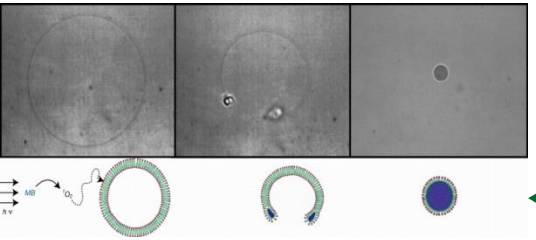
Look at the dynamics of membranes (giant unilamellar vesicles) with Optical microscopy fluorescence and phase contrast interference contrast / reflection

Expansion of area per lipids

Riske et al *Biophys J* **2009**,*97*, 1362.
Mertins et al *Biophys J* **2014**, 106,162.
Weber et al *Soft Matter* **2014**,10, 4241.

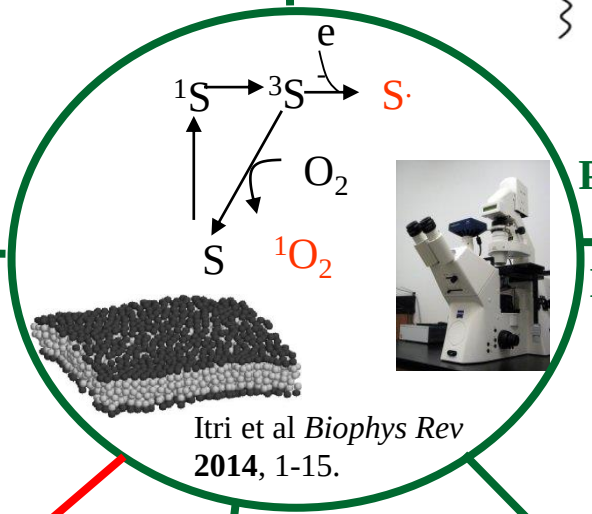






Kinetics of photodestruction

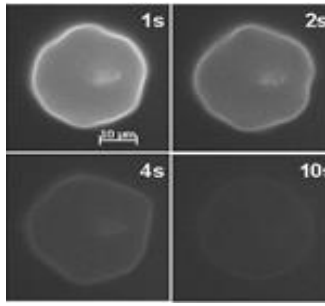
Caetano et al *Langmuir* **200**7, 23, 1307.



Itri et al *Biophys Rev* **2014**, 1-15.

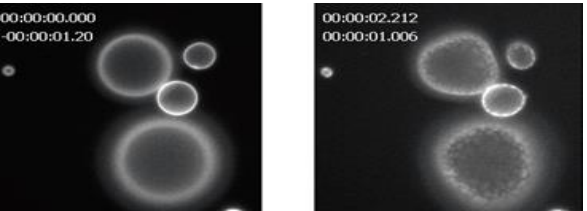
Photobleaching

Loosing contrast



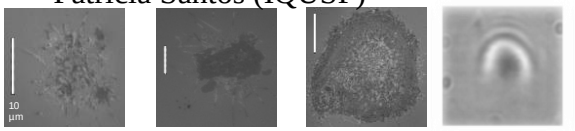
Organization and domains

Haluska et al *Biochim Biophys Acta. Biomemb* **2012**, *1818*, 666

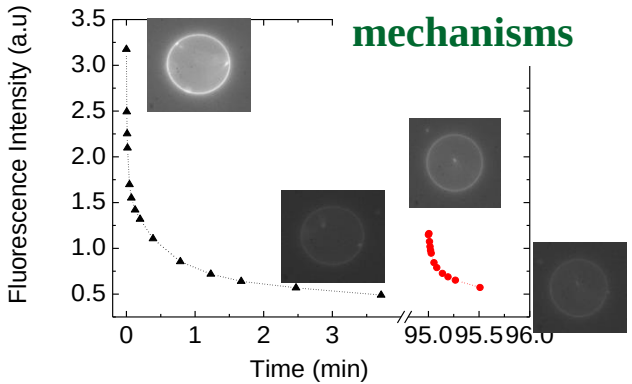


PDT and cell adhesion:

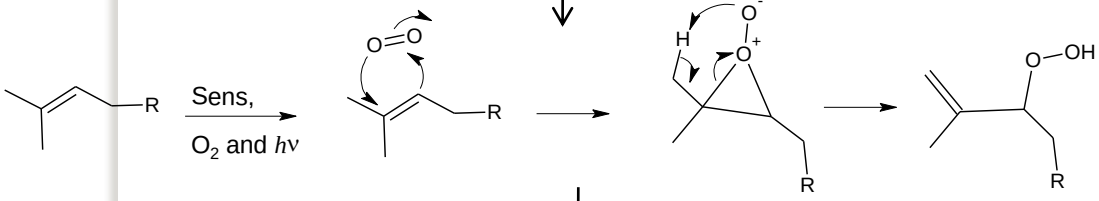
Patricia Santos (IQUSP)

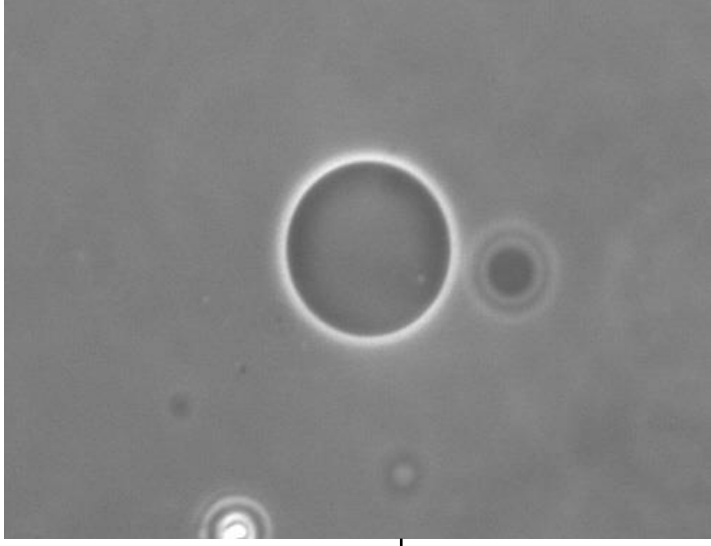


Photochemical mechanisms

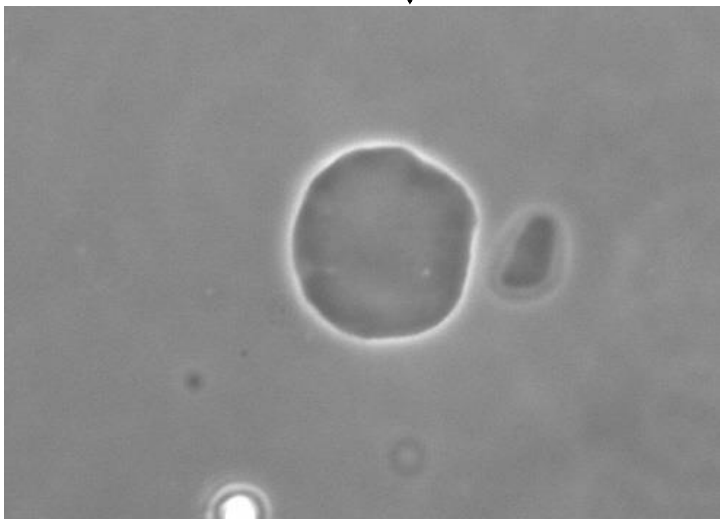


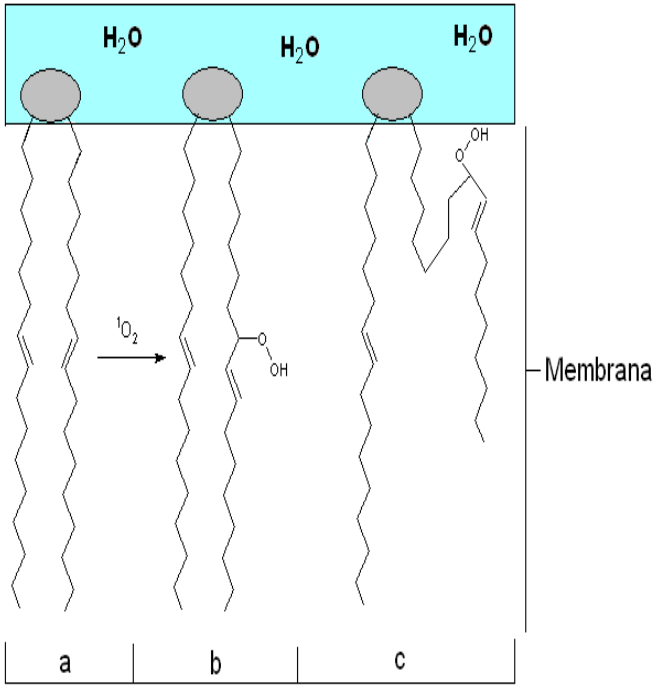
Hidroperóxidos

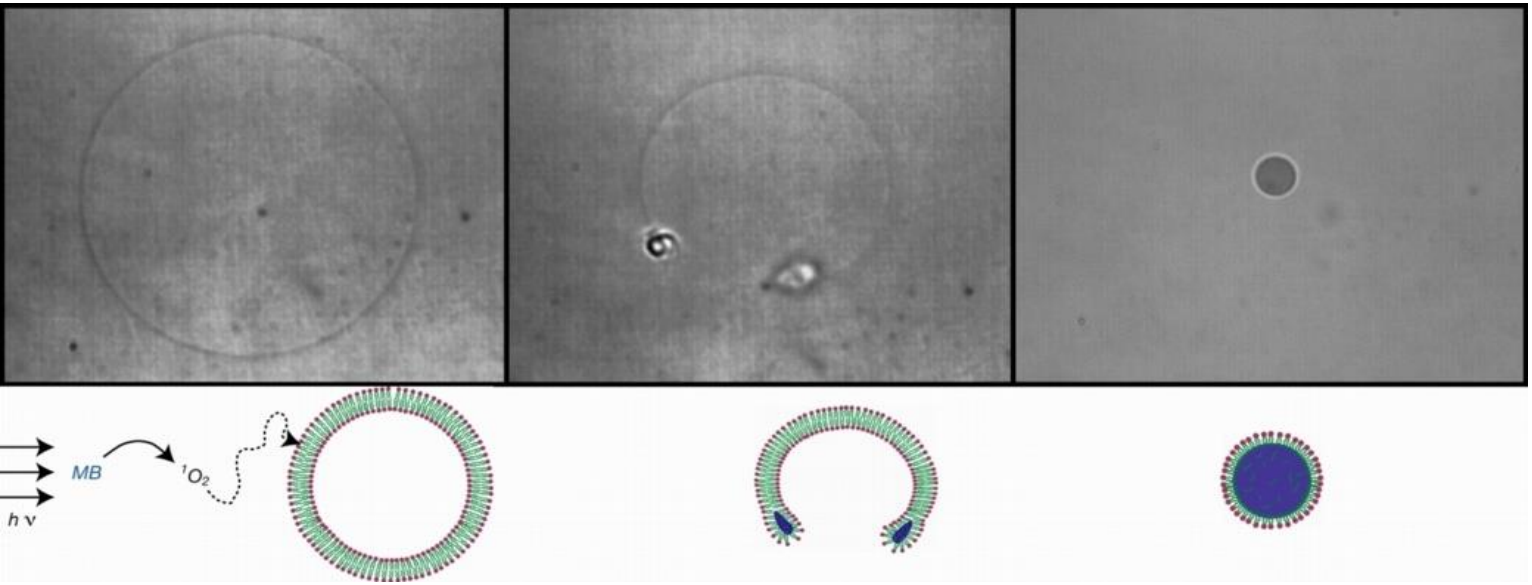


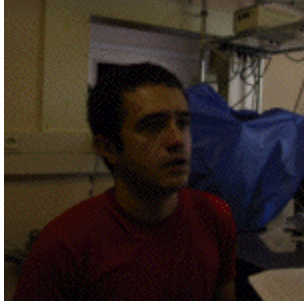


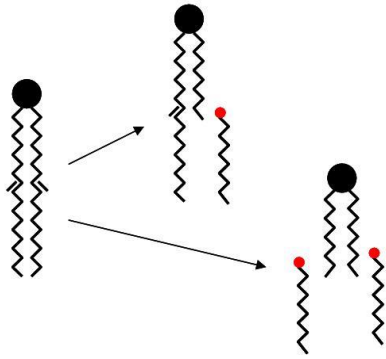
Aumento da área por lipídeo



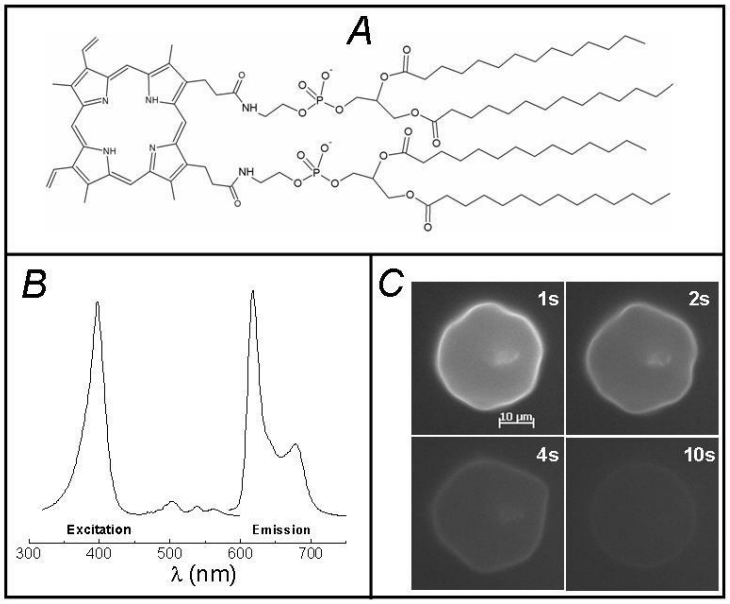


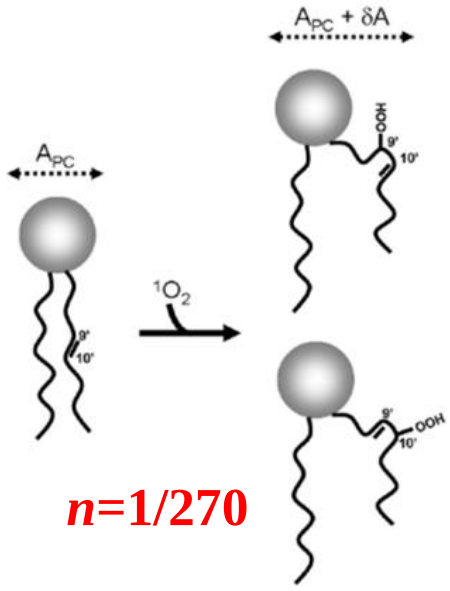






Caetano et al *Langmuir* **200**7, 23, 1307.



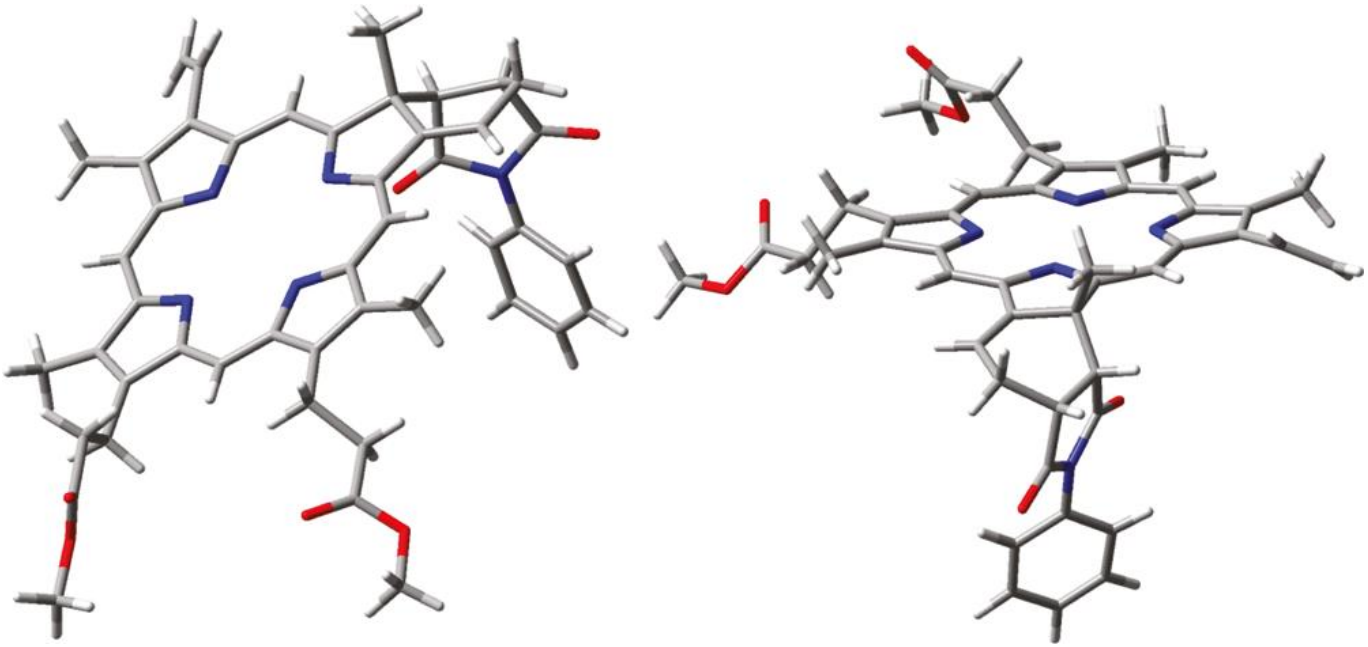


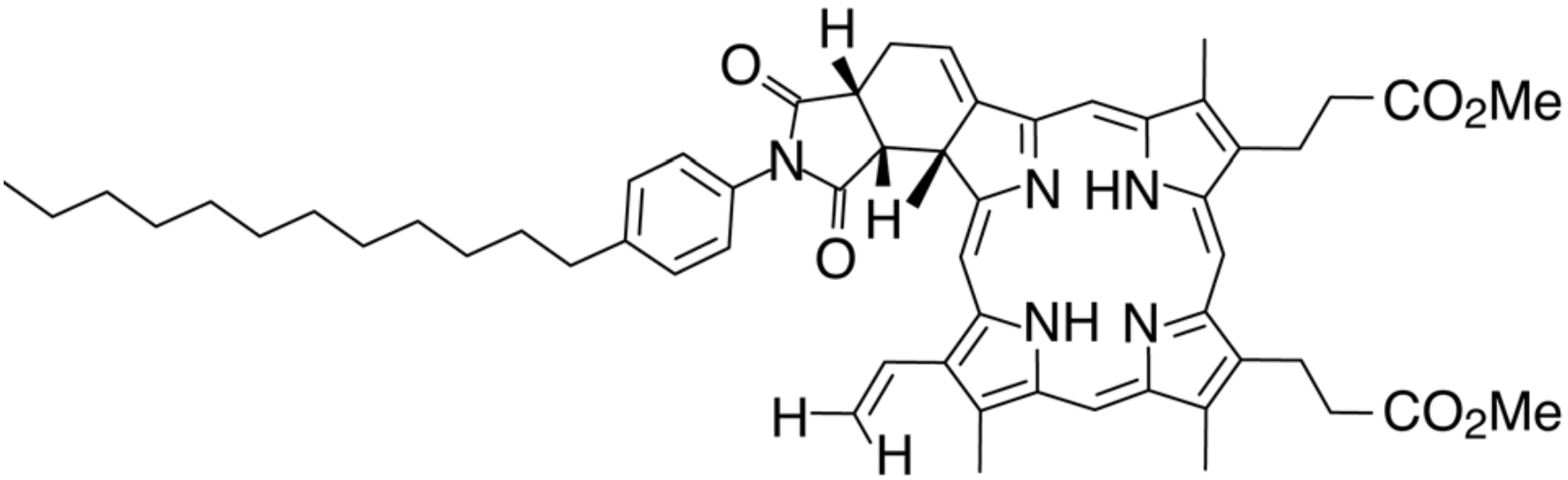
***n*=1/270**

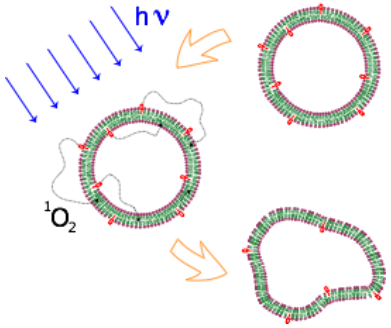
8% expansion
60% hydroperoxides

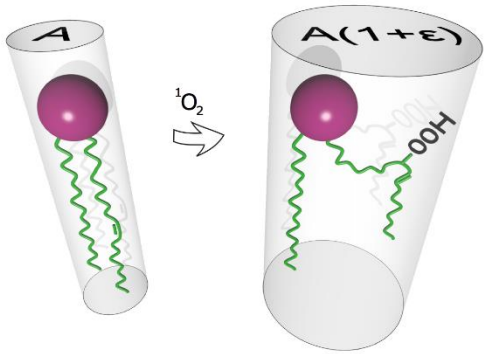
Riske et al *Biophys J* **2009**,*97*, 1362.

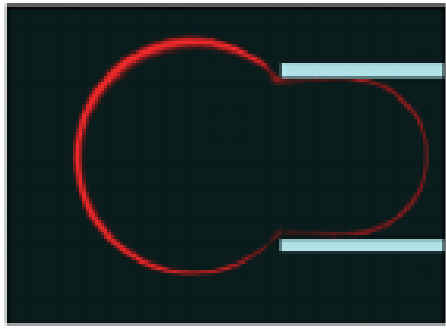
Adjaci Uchoa et al. Chlorin Photosensitizers Sterically Designed To Prevent Self-Aggregation
J. Org. Chem. **2011**, 76, 8824.





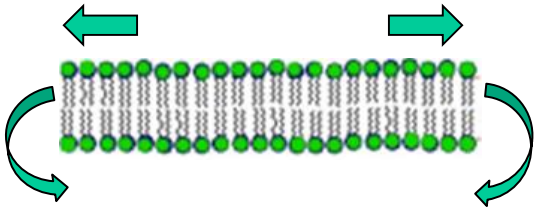




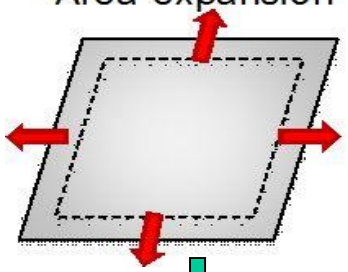


Reaction efficiency: 1:5
$k=3x10^6\ M.s^{-1}$

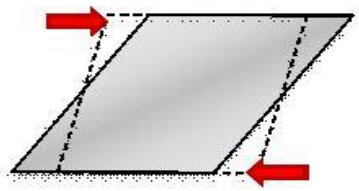
Weber et al *Soft Matter* **2014**,10, 4241.



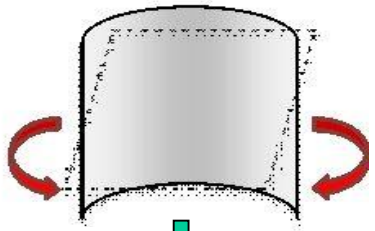
Area expansion



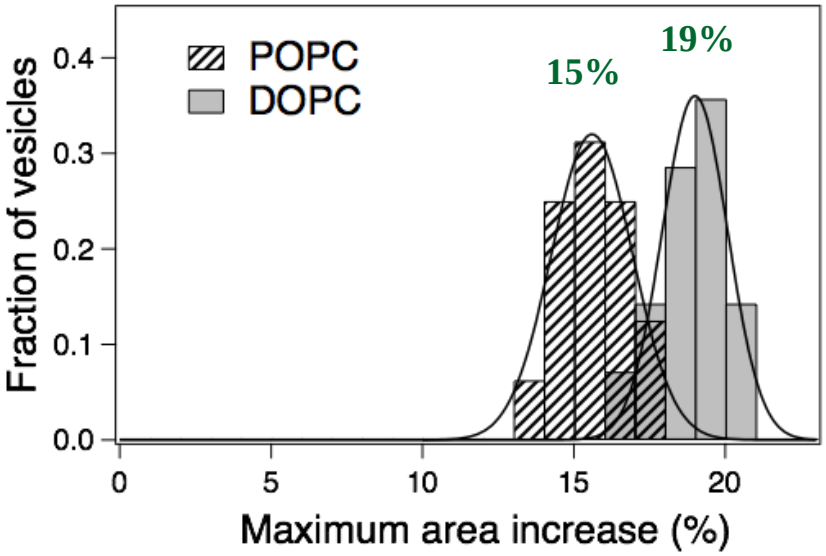
Stretching

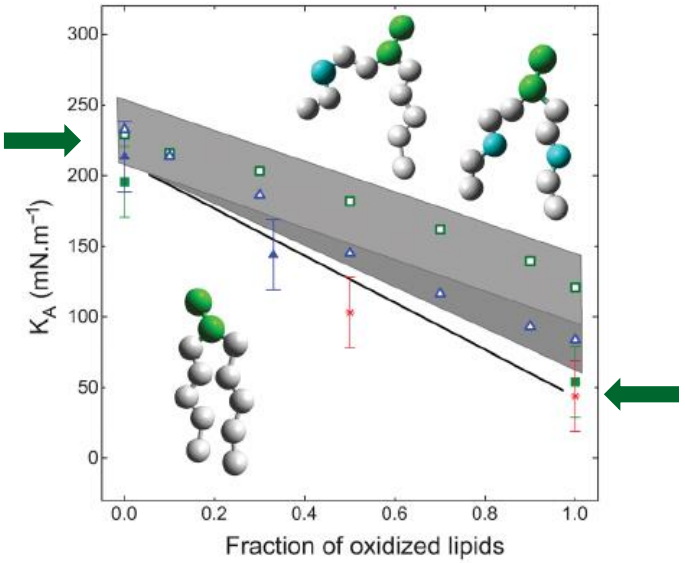


Bend

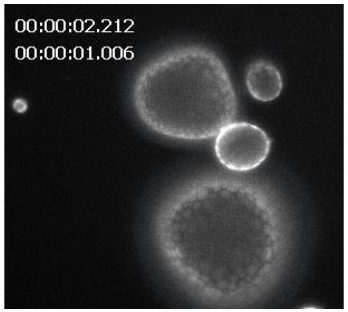


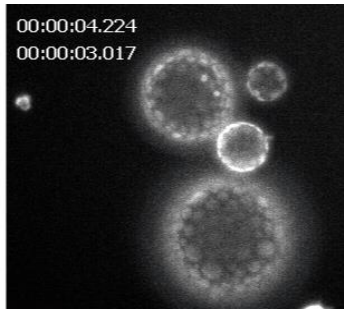
?





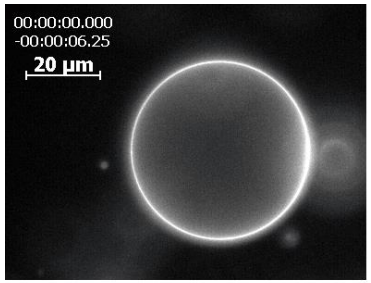
Ternary phase diagram and GUVs: POPC: DPPC: cholesterol (1%)PE-Porf

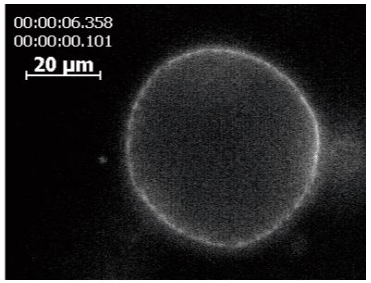


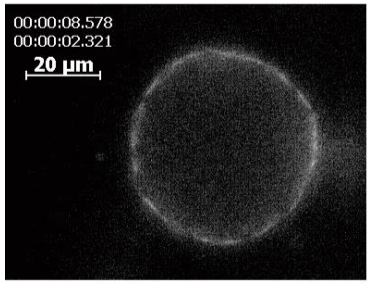


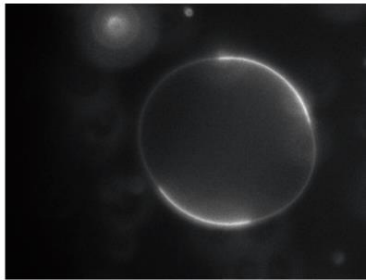
Photoinduced generation of domains Lo/Ld. POPC and cholesterol become slightly more fluid. Increases the areas of the line tension allowing phase separation (Cell signaling?)

Membranes that are prepared with lipid composition on the threshold between phases Ld + Lo and Lo have phase separation during photo-oxidation







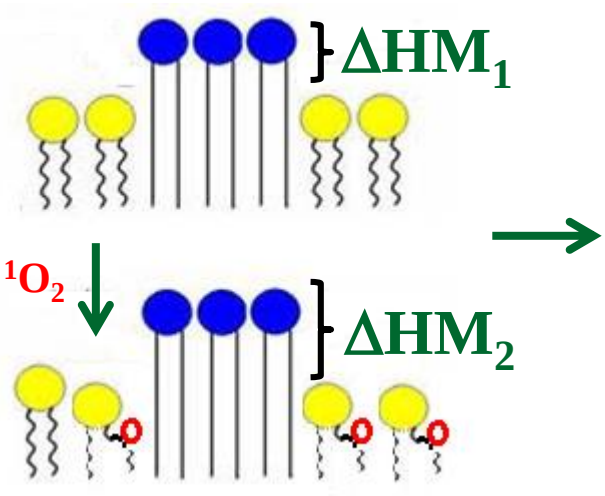


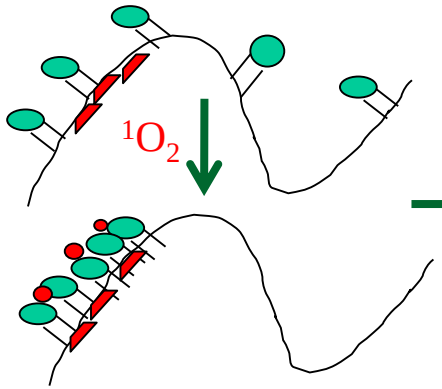
Haluska et al *Biochim Biophys Acta. Biomemb* **2012**, *1818*, 666.

Conclusion

1. Membranes respond to oxidative stress by increasing the area available for lipid and allowing the reorganization of these domains.

2. We intend to demonstrate the effect of this reorganization in signaling mechanisms related to oxidative stress





We can explain release of cyt c from mitochondria under oxidative stress...

Estevam et al *J Biol Chem* **2004**, 279, 39214
Suraniti et al *Langmuir* **2007,** *23*, 6835.
Tumolo T et al *Coll Surf B: Bio* **2012,** 91, 1-9.
Kawai C et al *J. Phys.Chem.B* **2014**, 118, 11863.

$\Delta HM_1 < \Delta HM_2$

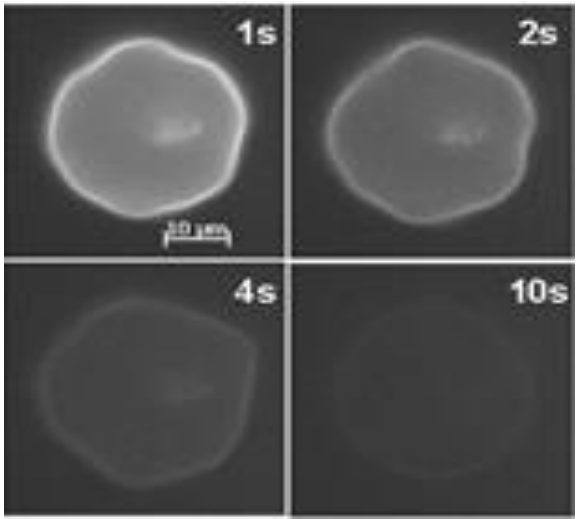
HM="hydrophobic mismatch"

Experimental systems and tools (Biophysical/Biochemical)

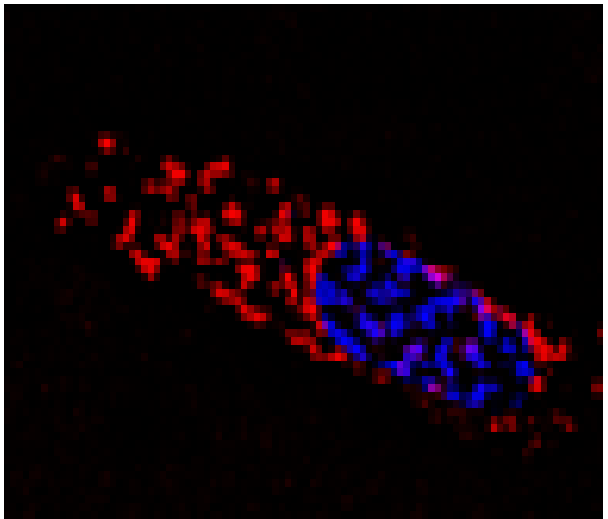
-Spectroscopy (UV, visible, IR), time-resolved
-Surface (SPR, Langmuir balance)
-Microscopy (micro and nano scales),
-Chemistry/Biochemistry (synthesis, purification, identification, blotting)

Solution

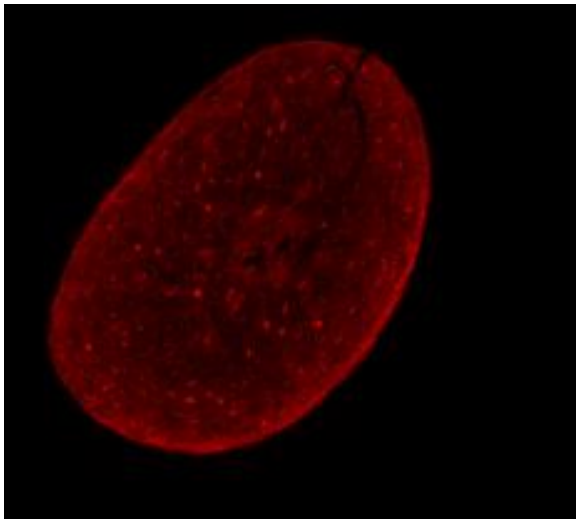
Membrane Mimics
GUVs, SUVs, HBM

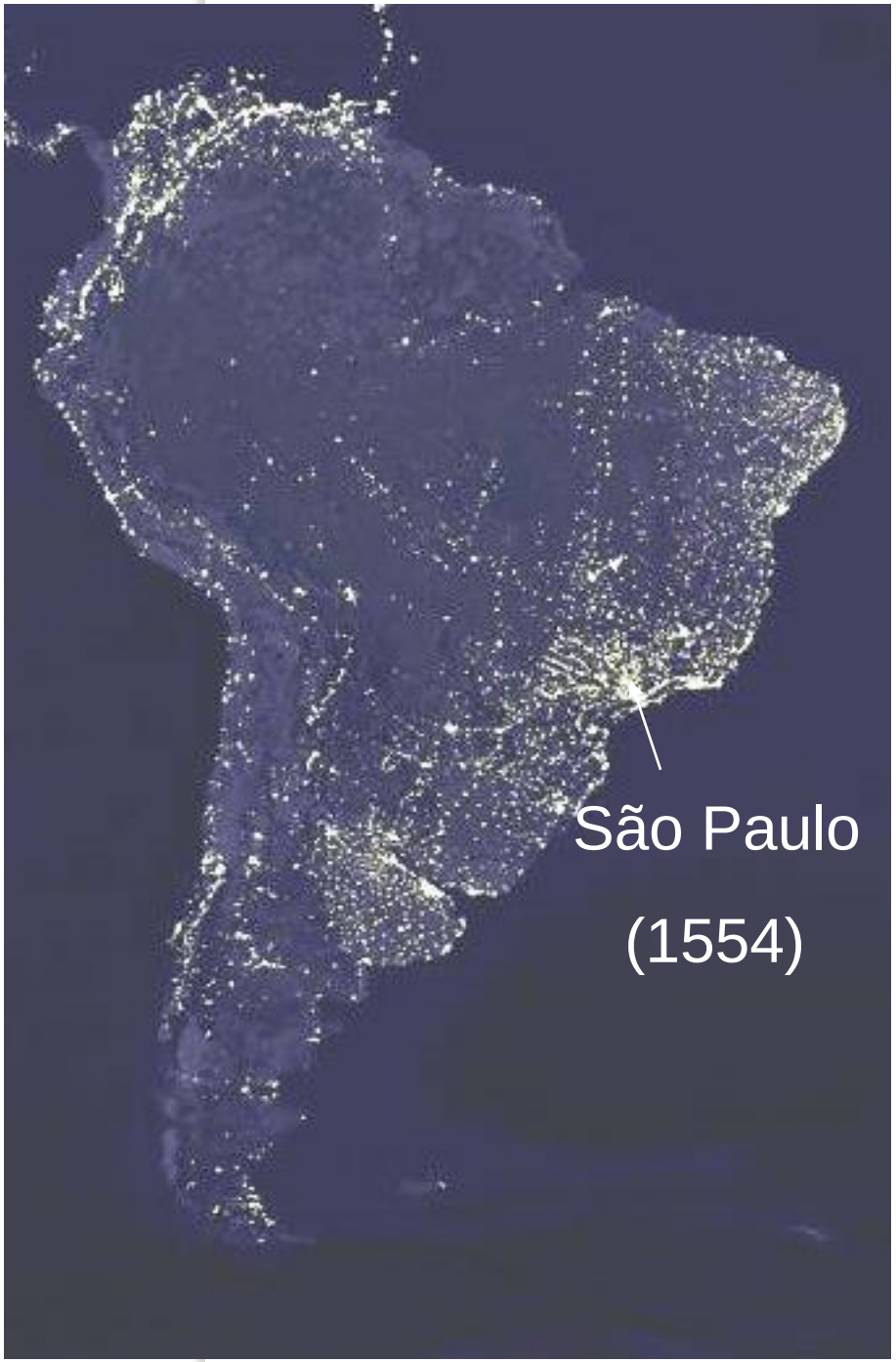


Cell culture: tumor,
Fibroblasts, keratinocytes



Tissues: hair & skin



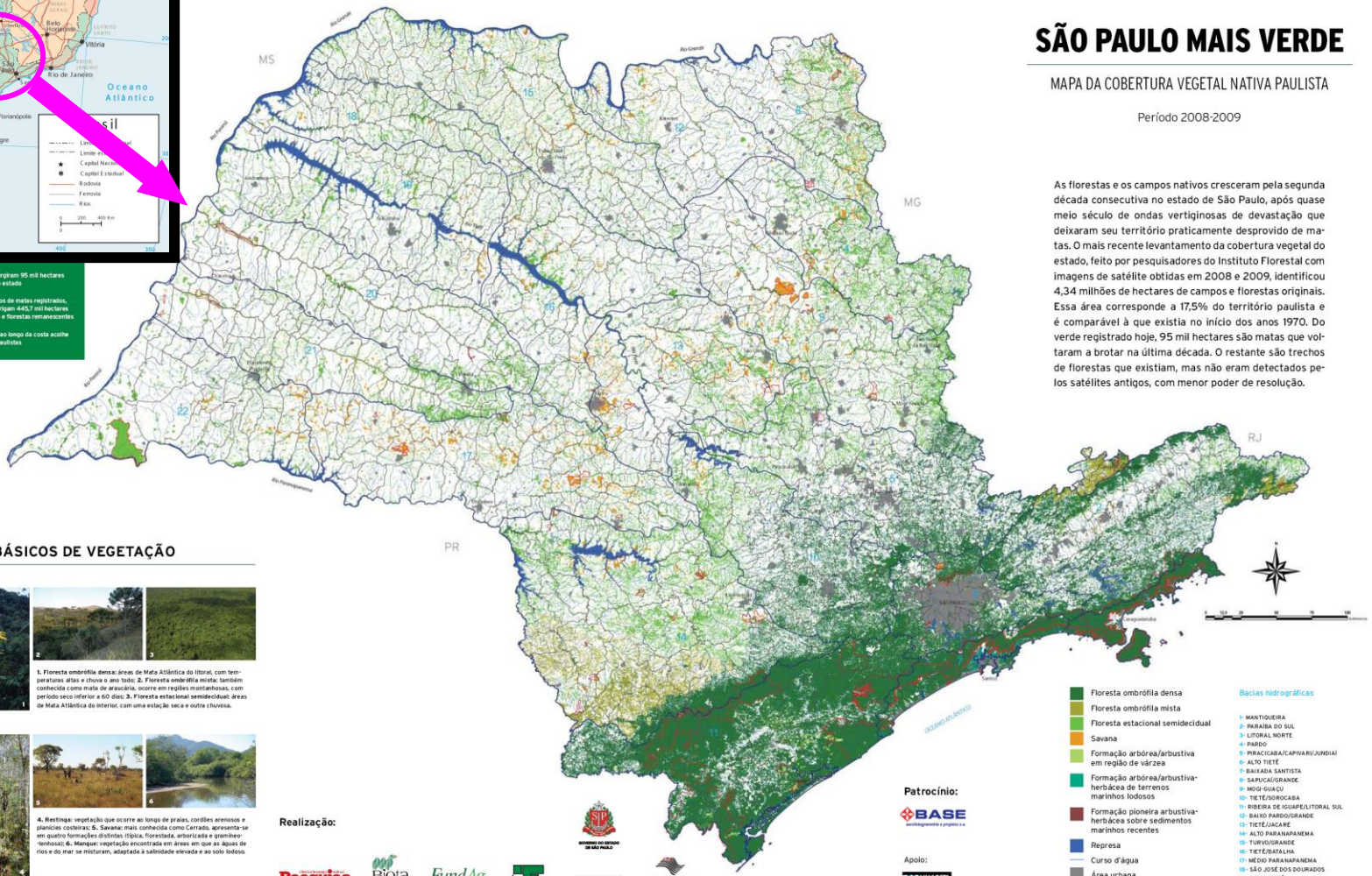

São Paulo
(1554)


South America
Brazil
(1500)
© edHelper.com

The State of São Paulo

32% of brazilian´s GNP

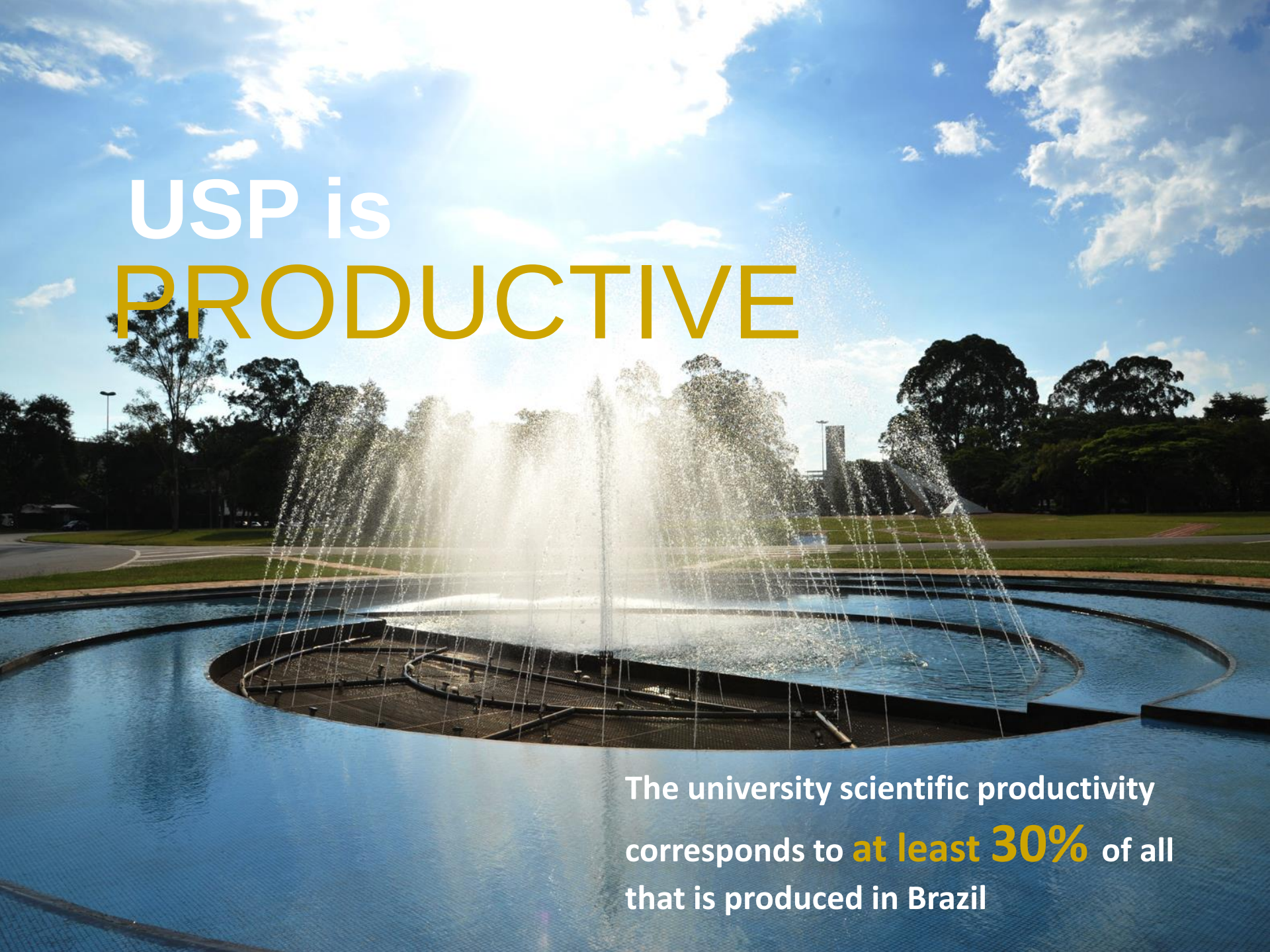
- Entre 2001 e 2009 surgiram 95 mil hectares de vegetação original no estado
- Dos 300 mil fragmentos de matas registrados, 184,5 mil são novos e abrigam 445,7 mil hectares (10% da área) de campos e florestas remanescentes
- Um estreito corredor ao longo da costa acolhe um terço das florestas paulistas

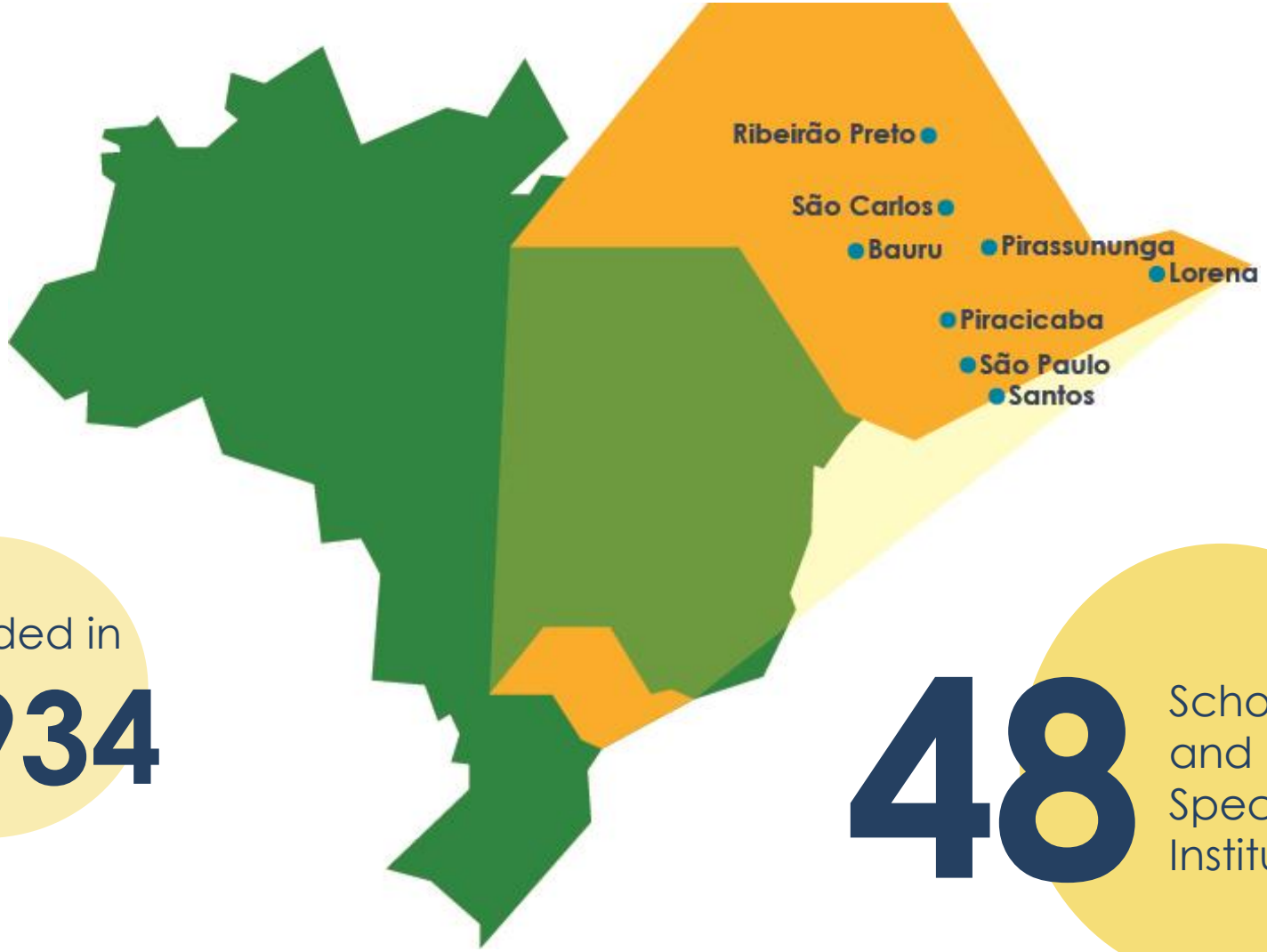


City of São Paulo

12% of brazilian´s gross national product


USP is
COMPREHENSIVE
& EXTENSIVE
90 000 students (60k undergraduate and 30k graduate)
academic areas and include 240 undergraduate
programs and 239 graduate programs
USP occupies a total area of approximately
76 km², of which 1.7 km²
corresponds to University buildings


USP is
PRODUCTIVE
The university scientific productivity
corresponds to at least 30% of all
that is produced in Brazil



Founded in

1934

48 Schools and Specialized Institutes

Municipalities in the State of São Paulo with USP Campi (Red)

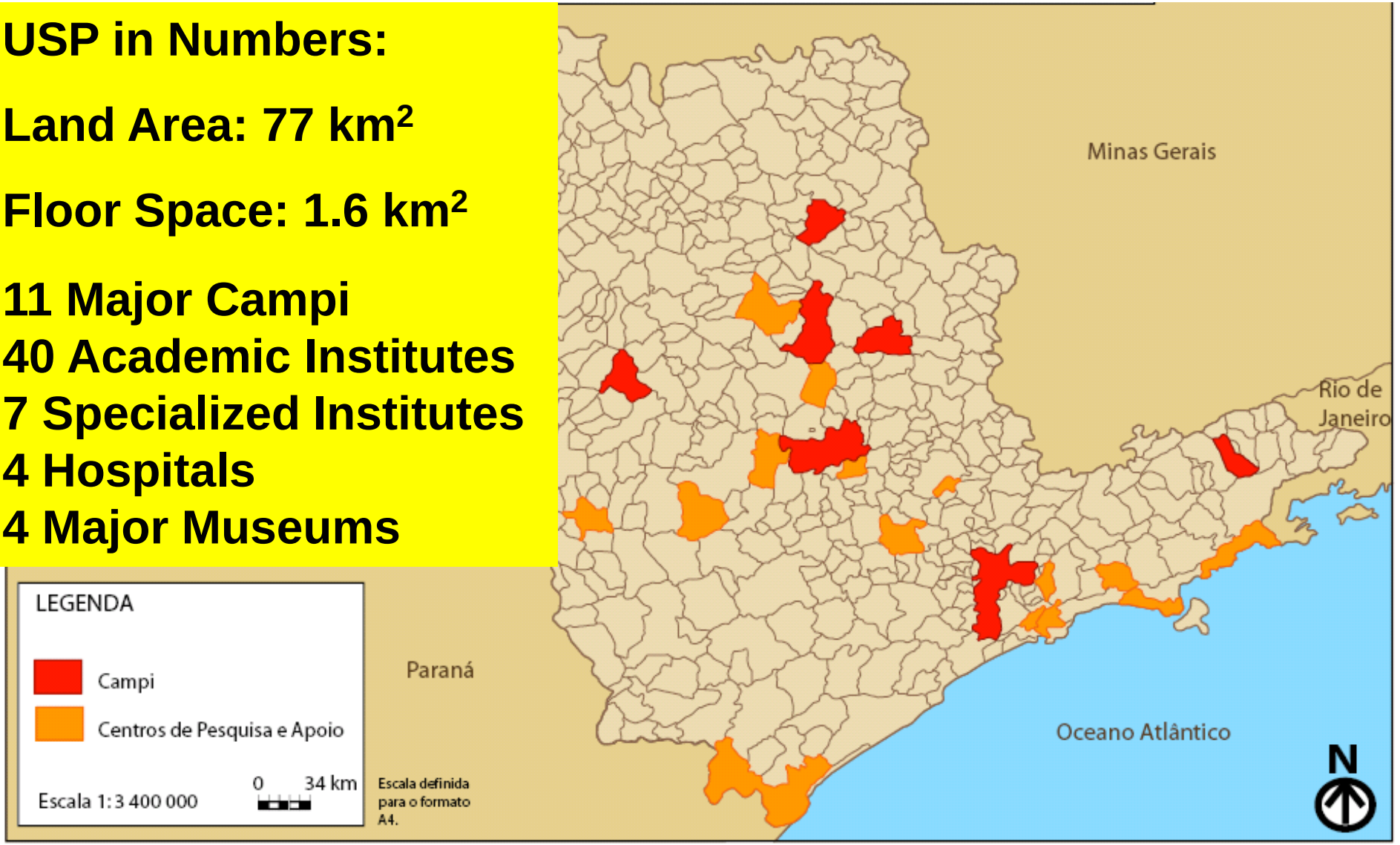
or USP Research Centers, Stations or Museums (Orange)

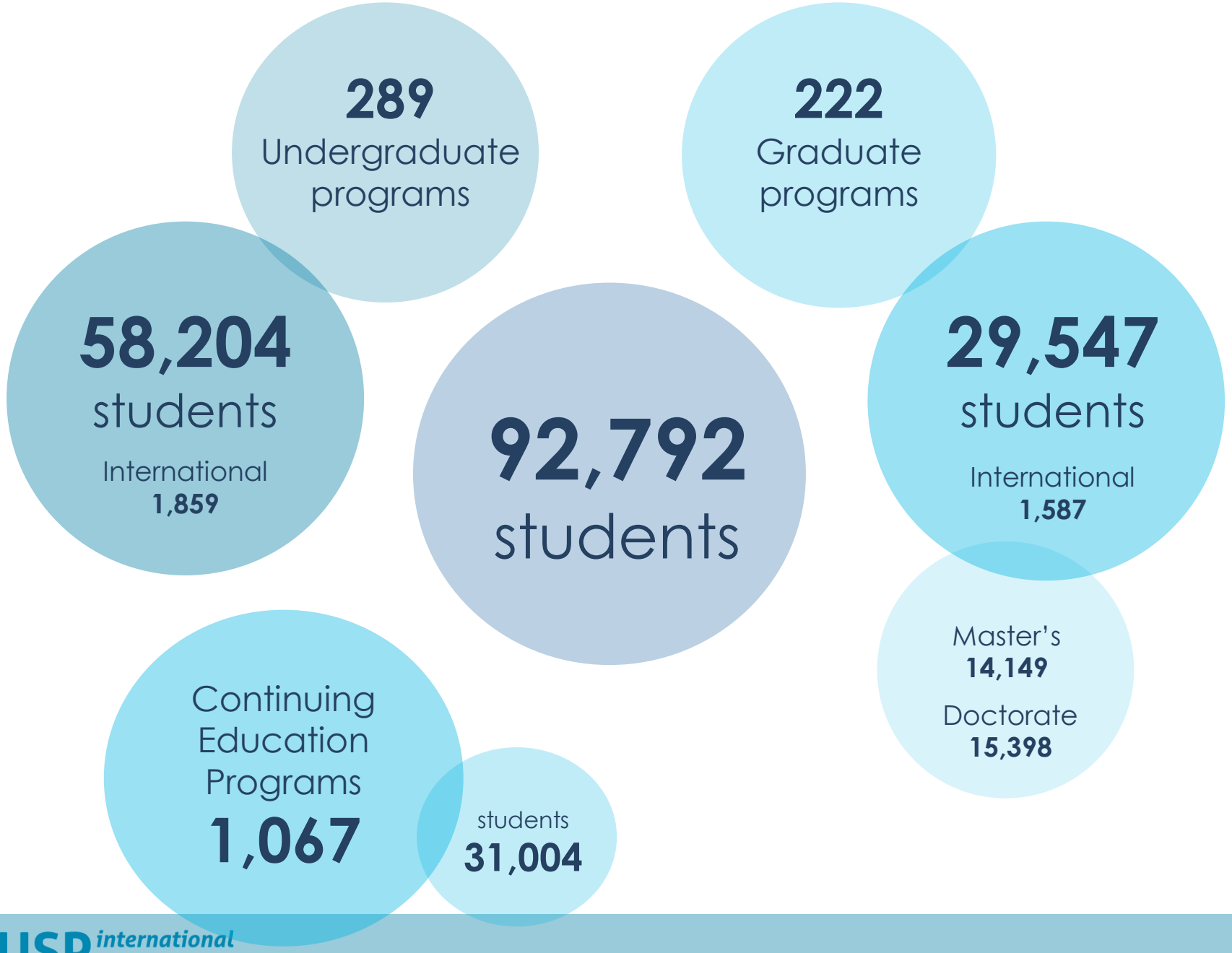
USP in Numbers:

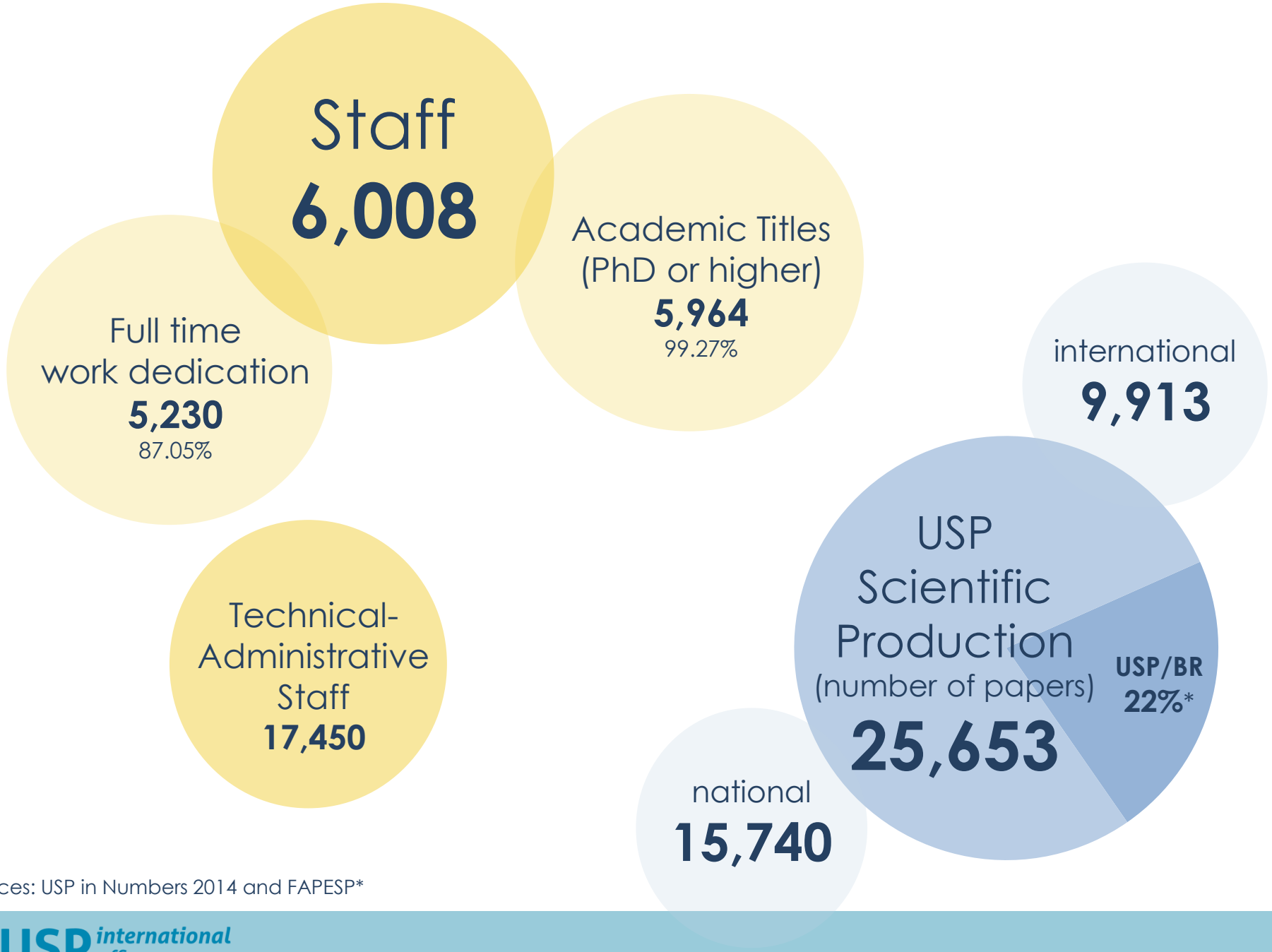
Land Area: 77 km²

Floor Space: 1.6 km²

11 Major Campi
40 Academic Institutes
7 Specialized Institutes
4 Hospitals
4 Major Museums




289
Undergraduate programs
222
Graduate programs
58,204
students
International
1,859
92,792
students
29,547
students
International
1,587
Master's
14,149
Doctorate
15,398
Continuing Education Programs
1,067
students
31,004



Sources: USP in Numbers 2014 and FAPESP*

USP Ranking

77

U.S. News Best Global Universities

10

The Times Higher Education BRICS & Emerging Economies Rankings 2015

201-225

The Times Higher Education 2014

132

QS World University Rankings 2014

QS WORLD UNIVERSITY RANKINGS by subject

ranked in 29/30 subjects

Top 50 in 7
Top 100 in 25

ARWU (Shangai) 2014

101-150

National Taiwan University Ranking 2014

62

51-100 Engineering & Technology

27 Agriculture & Forestry

Institute of Chemistry in numbers

Universidade de São Paulo
Instituto de Química

130 professors

83 (chem) + 47 (biochem)

555 undergrad students

400 grad students

250 (chem) + 150 (biochem)

32 M.Sc. / year

52 Ph.D. / year

3.5 students / supervisor

max.: 10

1442 Ph.D. / 1970 – 2012

1058 M.Sc. / 1970 – 2012

Rosangela Itri-IFUSP

ICS-France-M3 group

FAPESP, USP

CNPq, NAP-Phototech, FarmaService, CAPES, CNRS, UJF, Cofecub, Fulbright, EU