Seminário sobre Proteção Indoor e Outdoor (UVB, UVA, Luz Visível, Luz Azul e IR)

CRQ-IV região

Danos dos raios UVB, UVA, HEV, IR e da luz visível nas células

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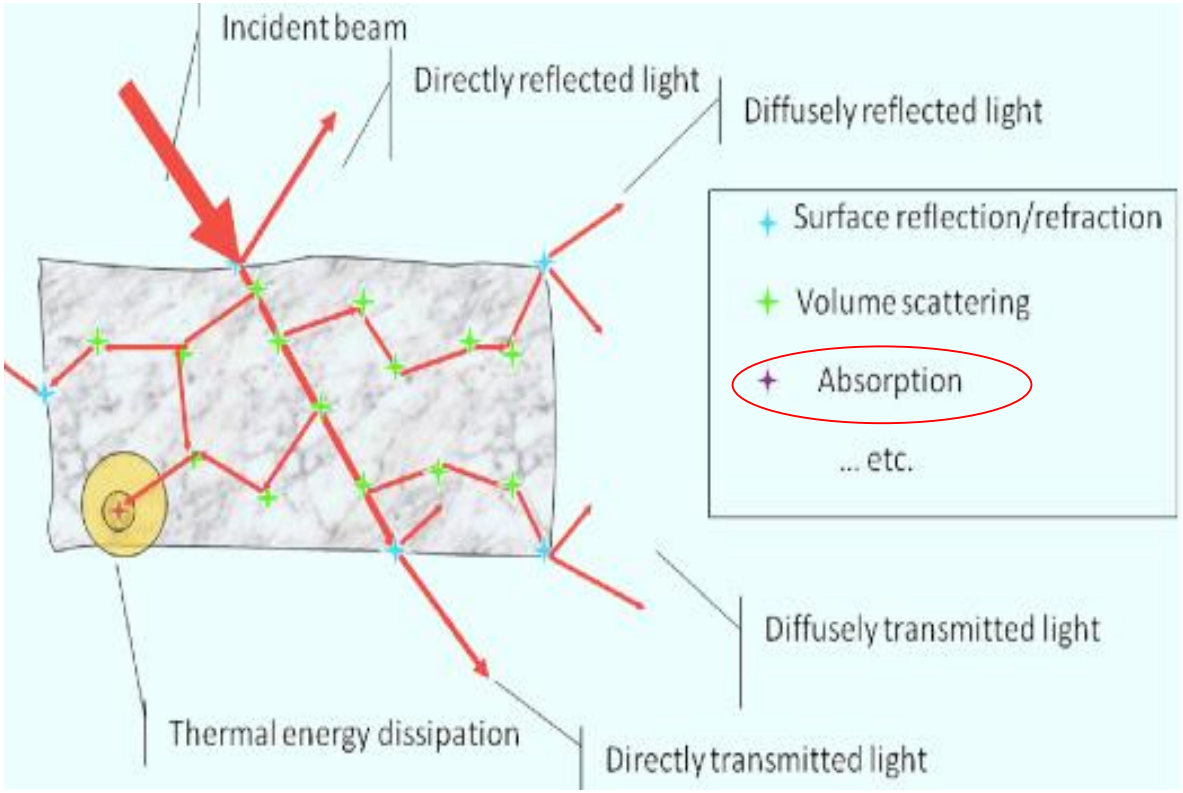
FAPESP

Redoxoma

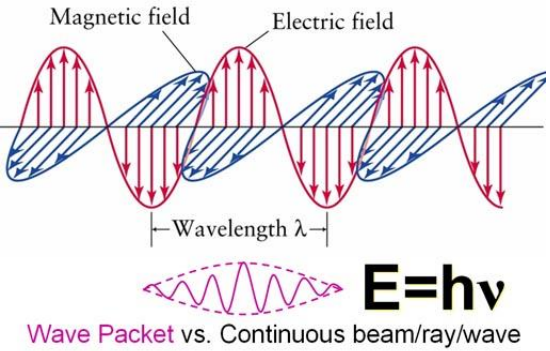
PhotoTech
The USP Research Consortium for Photochemical Technology

UNIVERSIDADE DE SÃO PAULO
SCIENTIA VINCES

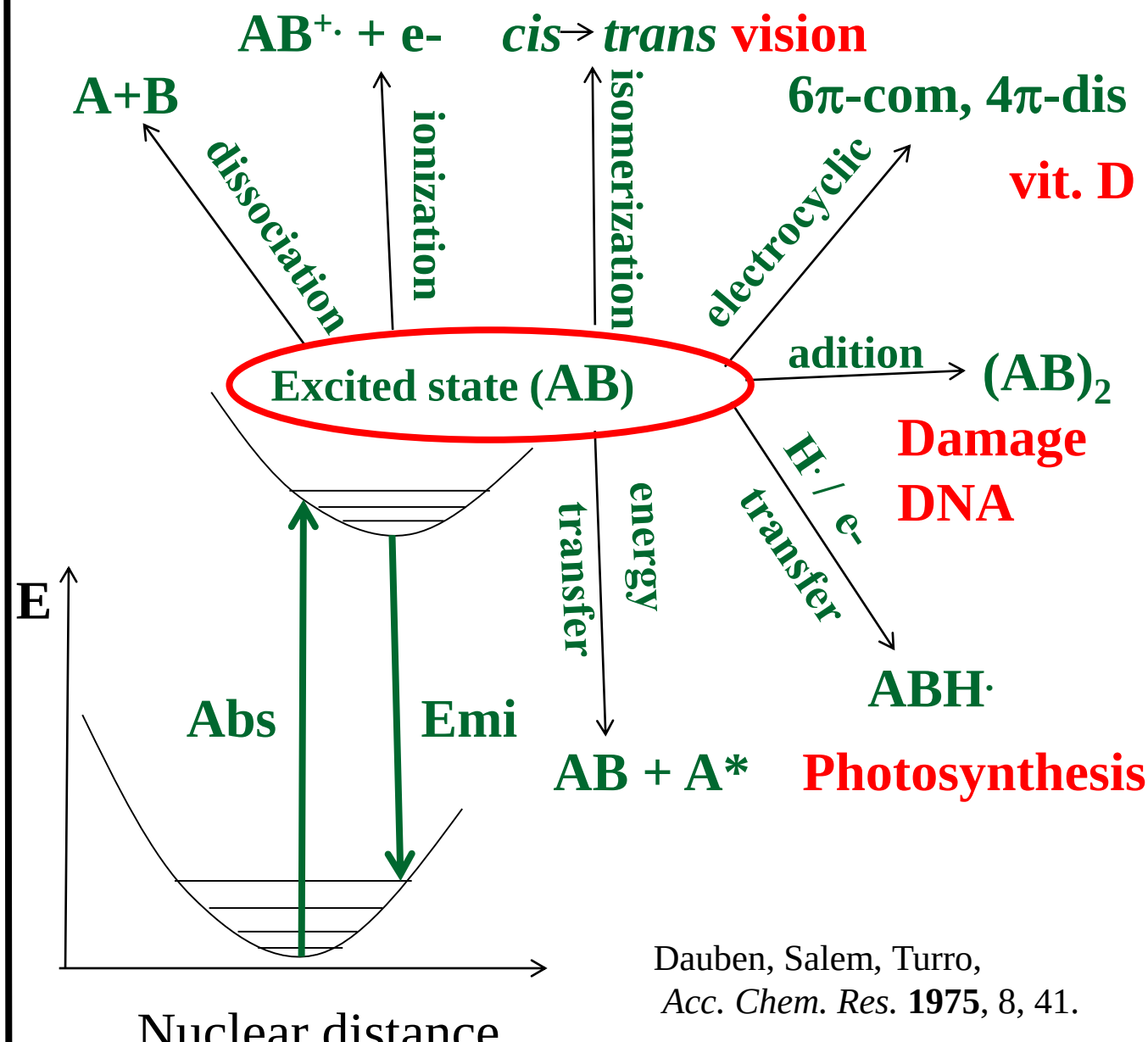
Human Skin and light: in the search for a better sun protection!



Photons



Excited states: ↑reactivity

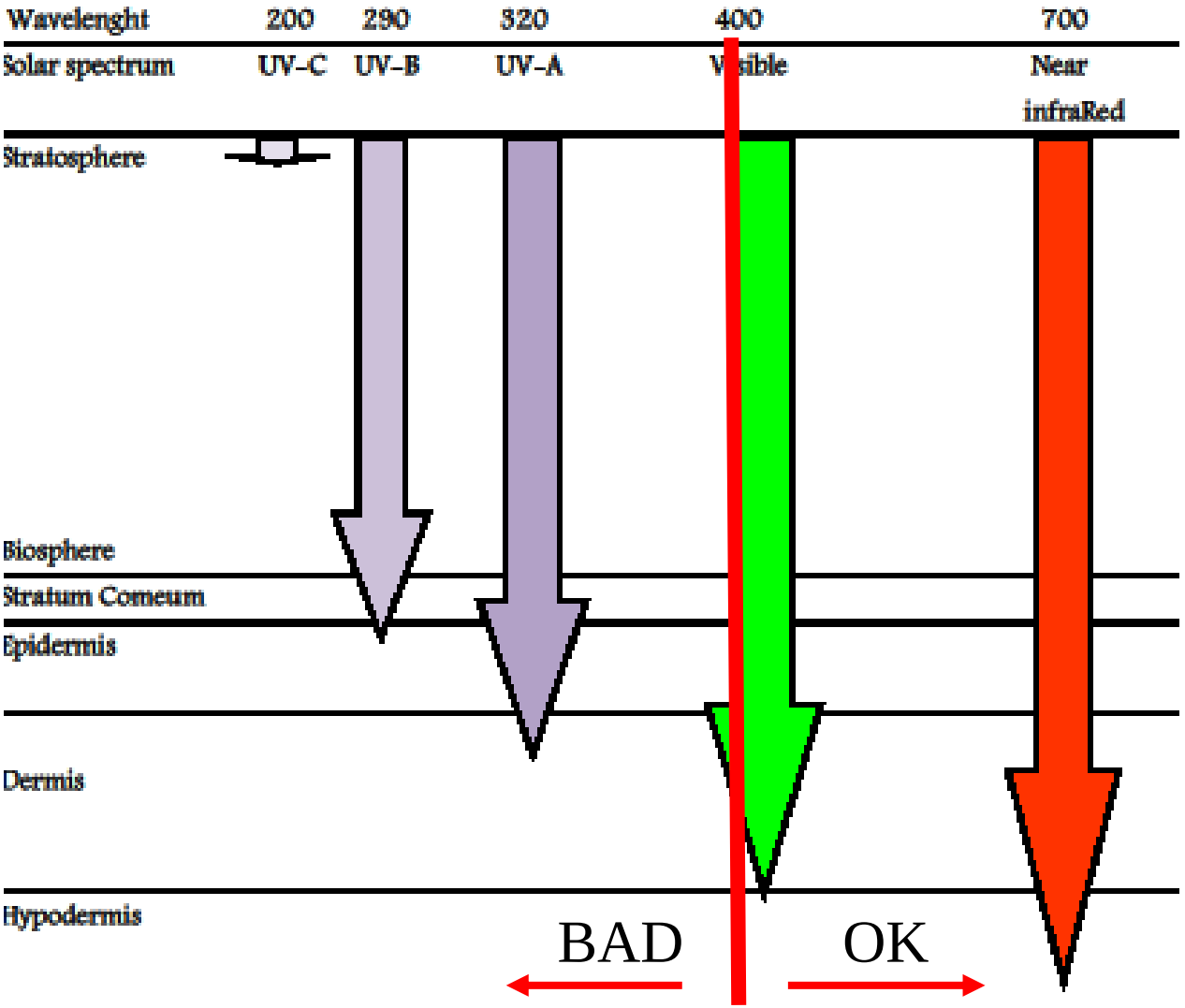


Dauben, Salem, Turro,
Acc. Chem. Res. **1975**, 8, 41.

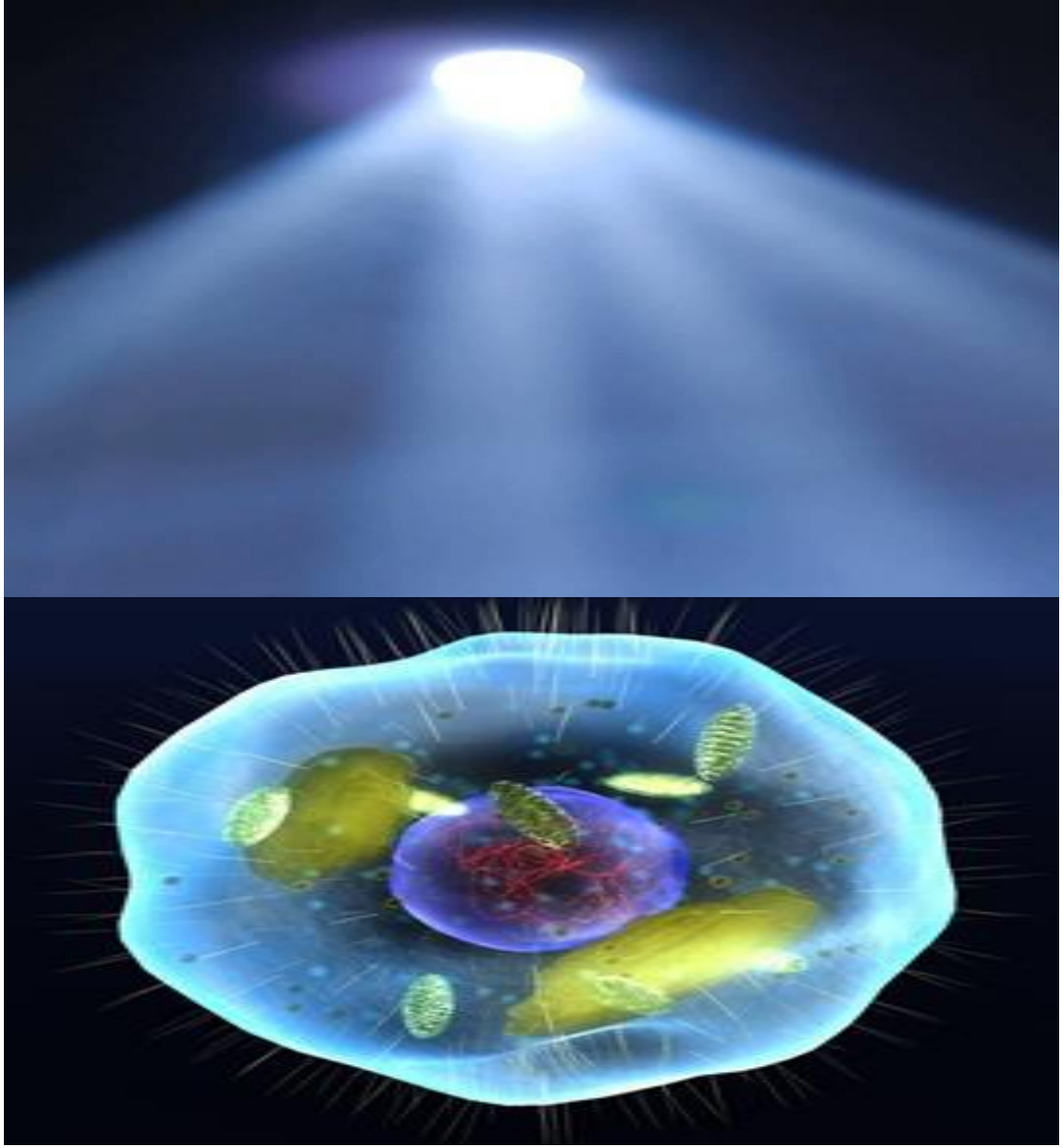
Do we understand the interaction of light with our skin or hair?

1. Which molecules absorb light?

2. How much light is absorbed?



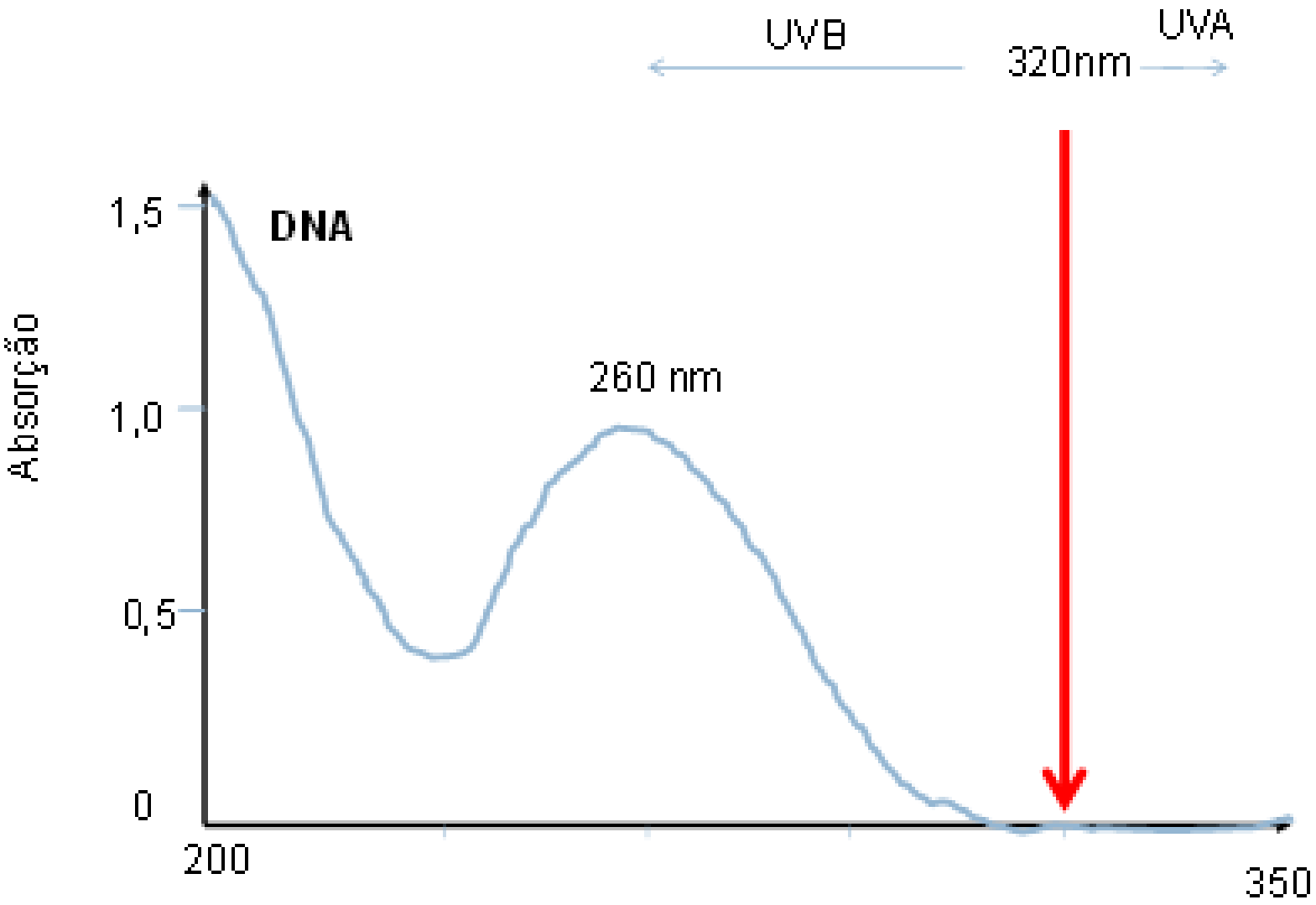
Baptista MS Photochemistry, Photobiology, and Redox Balance in Skin and Hair. Part I & II. *Cosmetiscope*, New York, 17(1, 3), p. 1-11; p. 1-10; 2011.



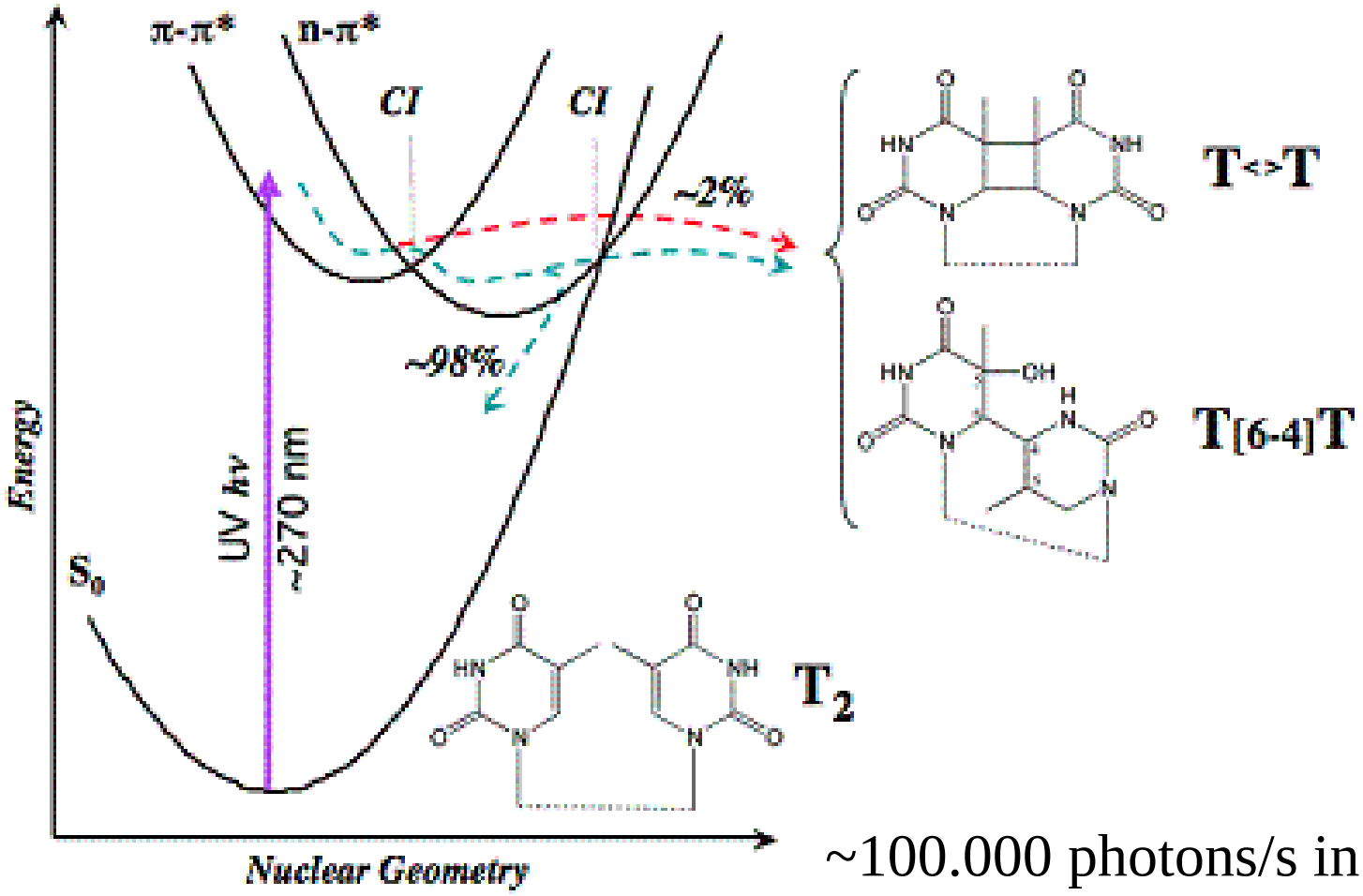
Is this bad for the cell?
How bad?
Why?

UV level


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

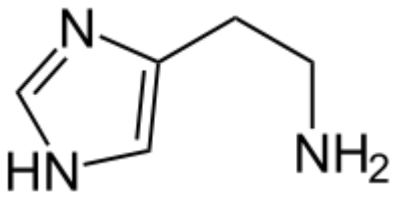

UVB
UVA
320nm
DNA
260 nm
Absorção
1,5
1,0
0,5
0
200
350

Reações no DNA induzidas pela absorção de luz

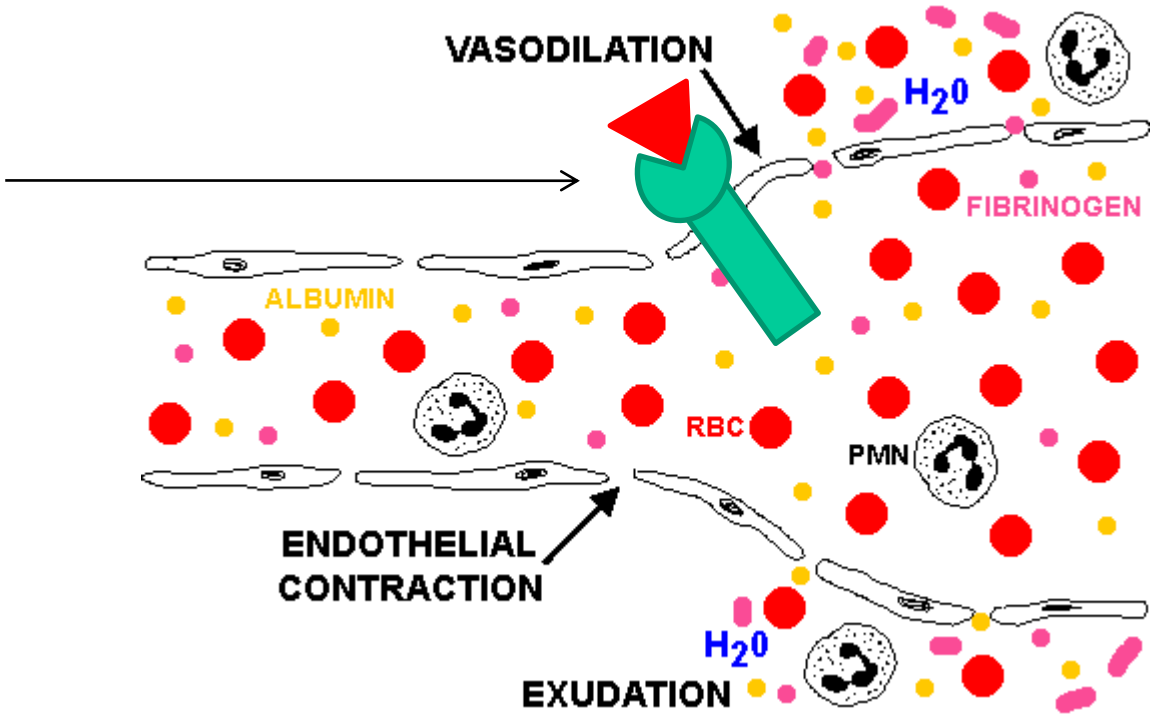


~100.000 photons/s in UV
~20000 photons/s in UVB

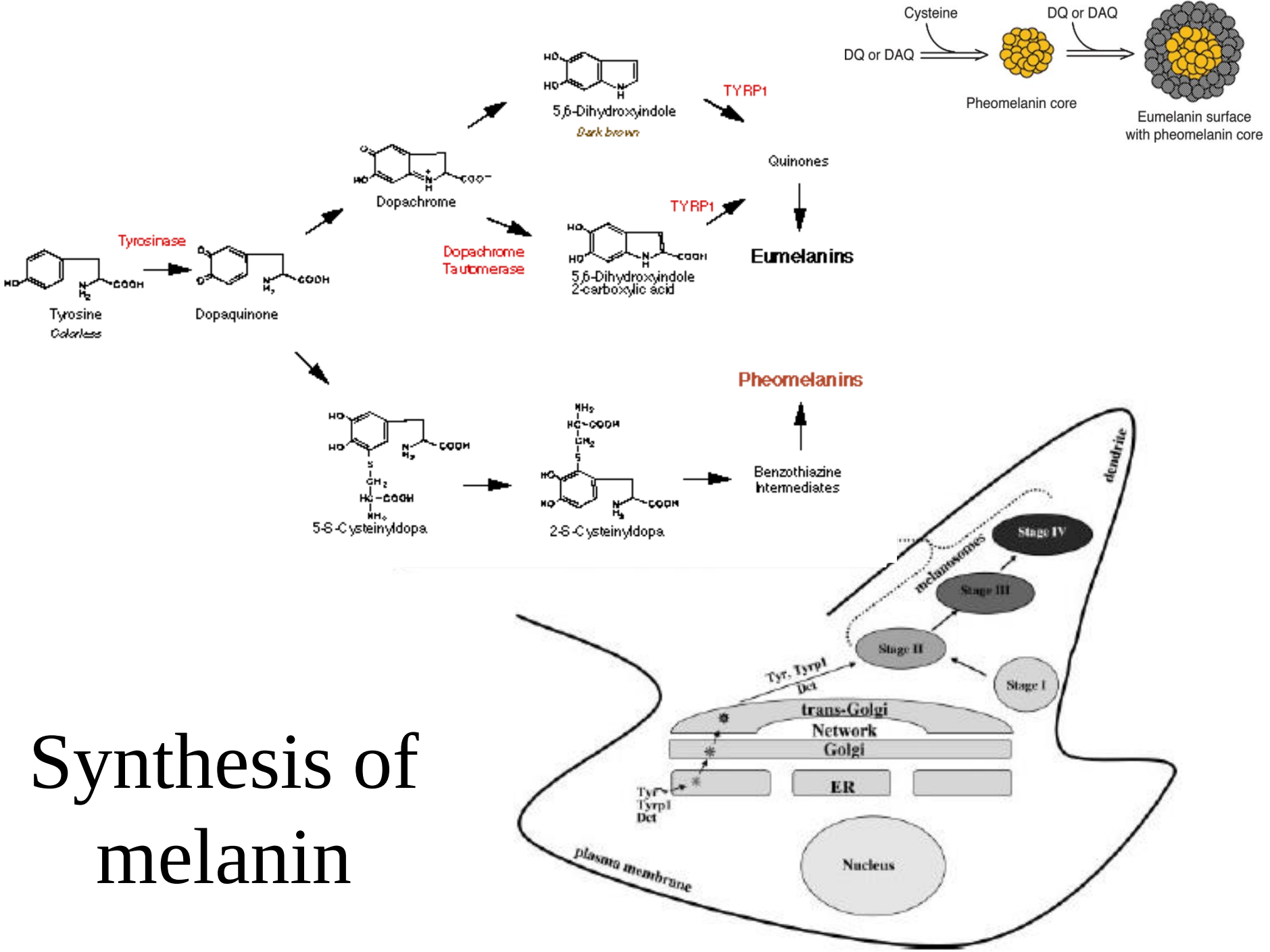
Erythema



Histamine Receptor



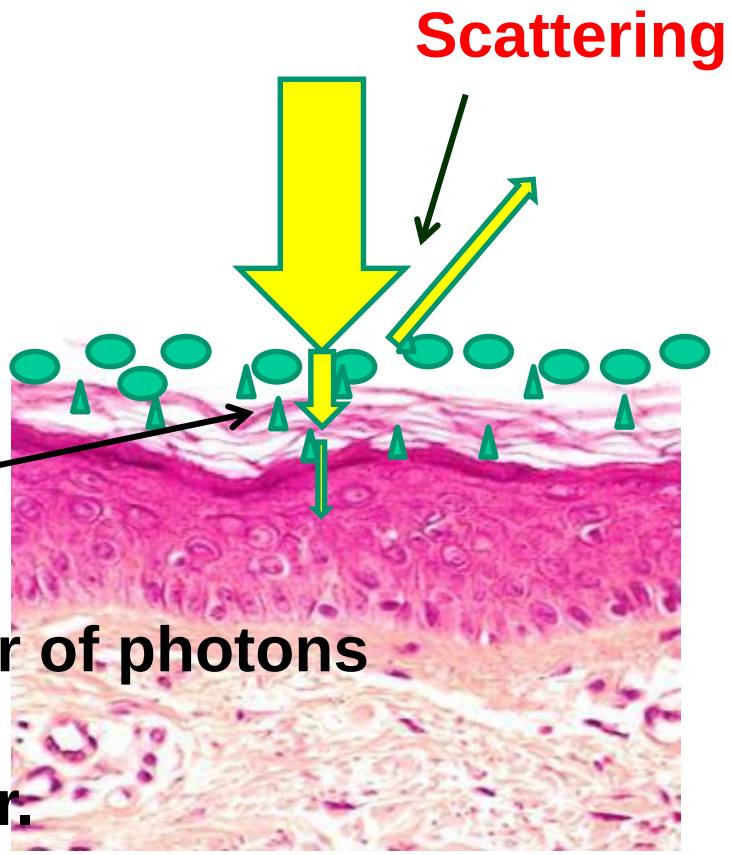
Synthesis of melanin



Avoids burning, photoaging.

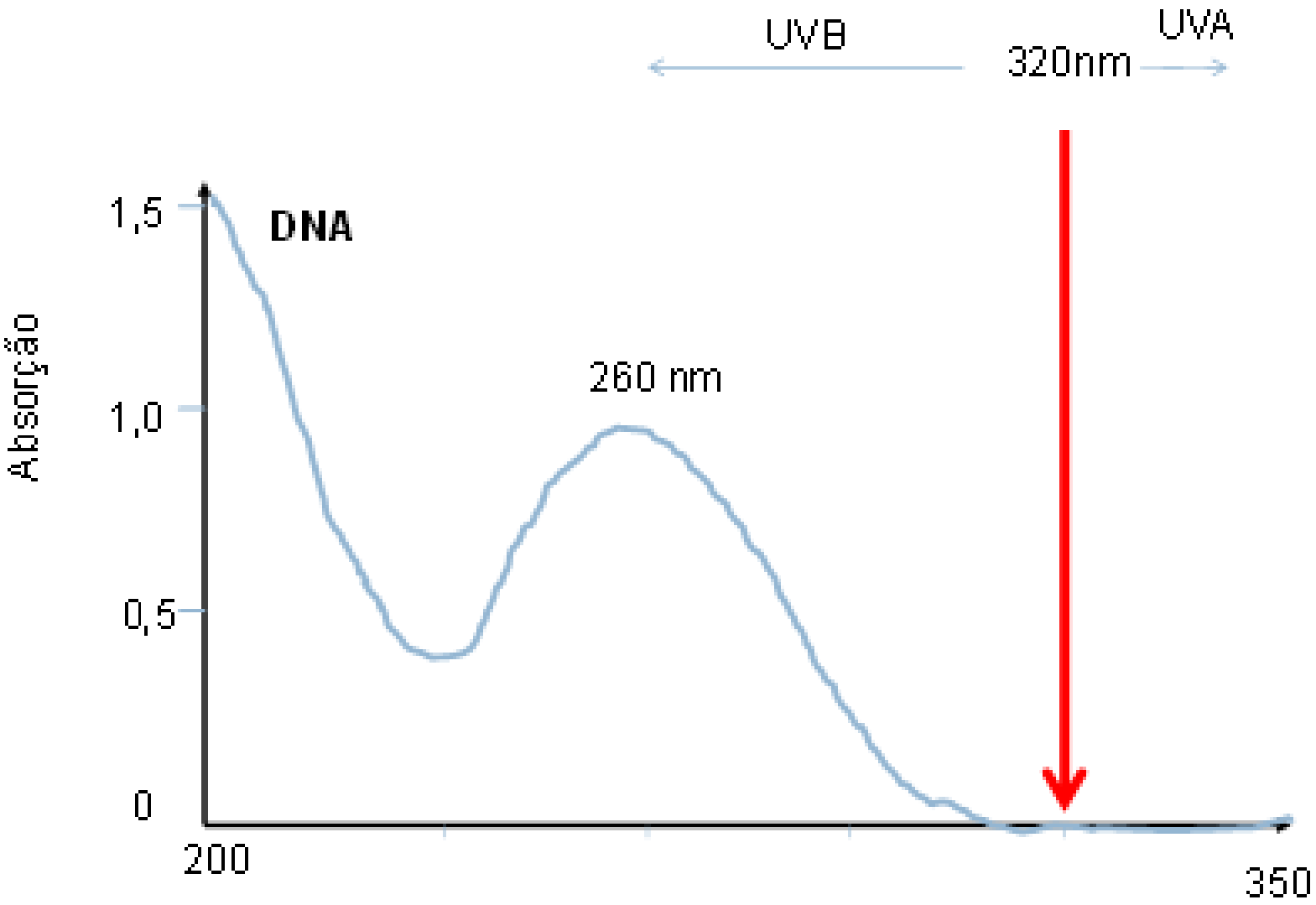
Filtros solares "orgânicos":
Unstable and can cause photosensitivity

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

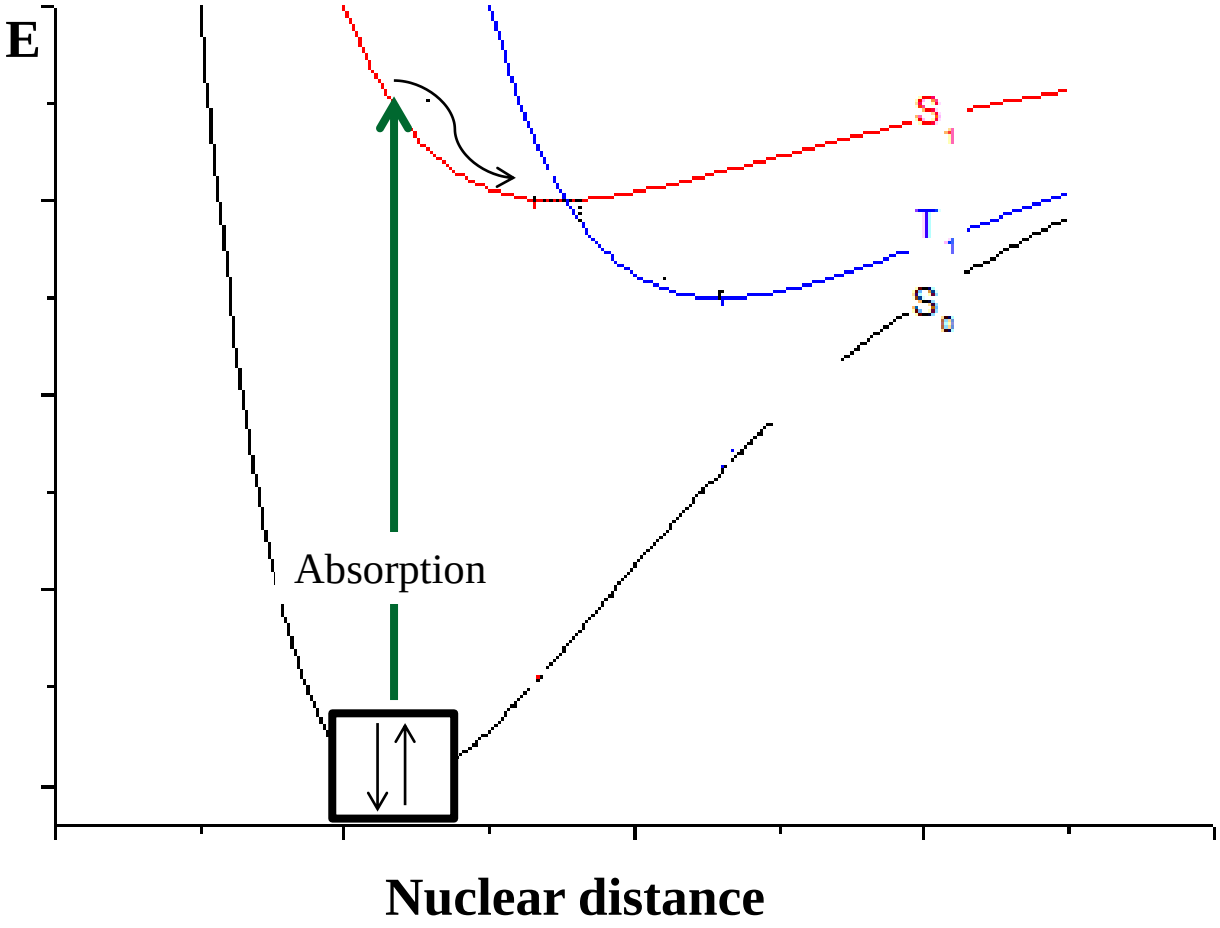


Sun screens: Decrease the number of photons that penetrate :
FPS 10: From 100 photons 10 enter.
FPS 50: From 100 photons 2 enter.

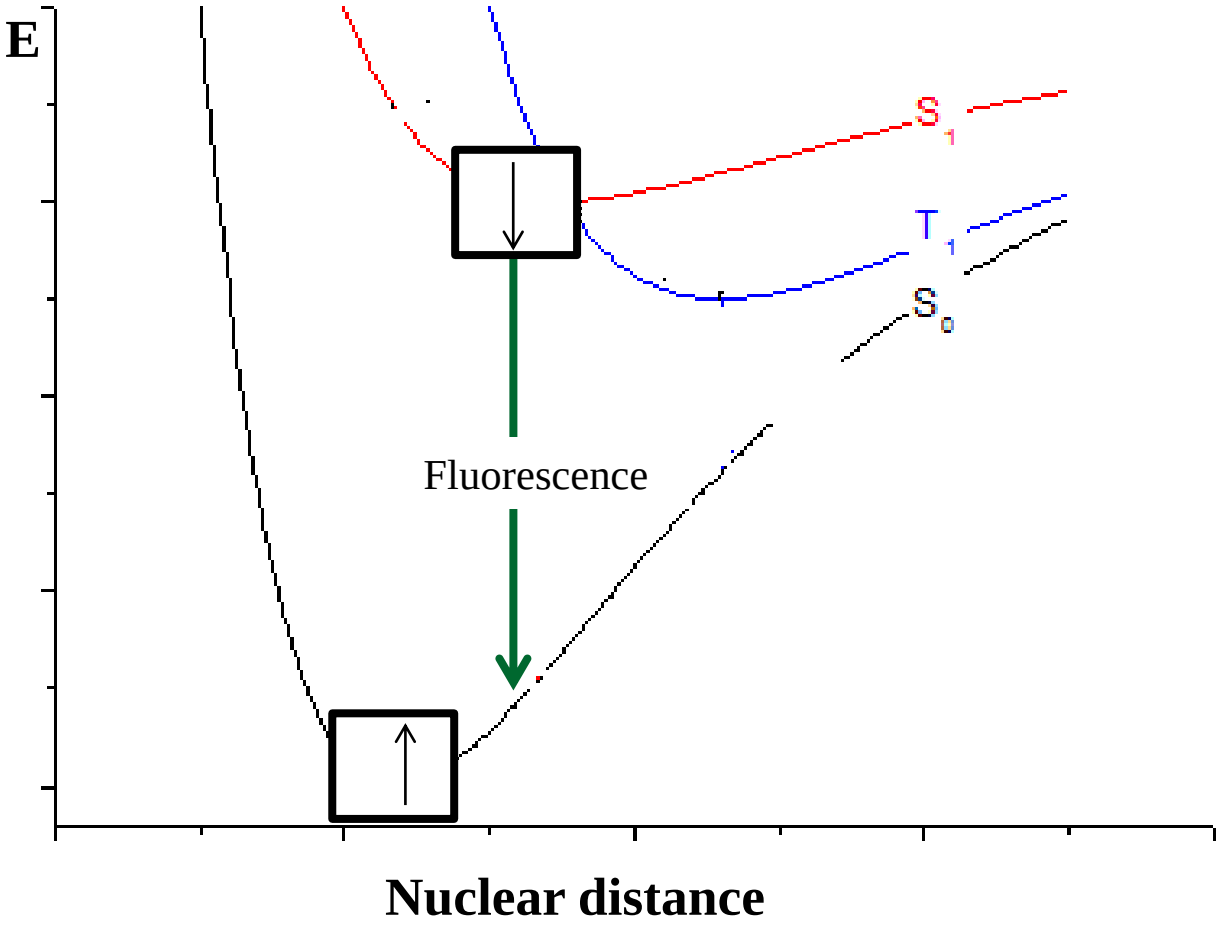
How about UVA, does it affect the skin?


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

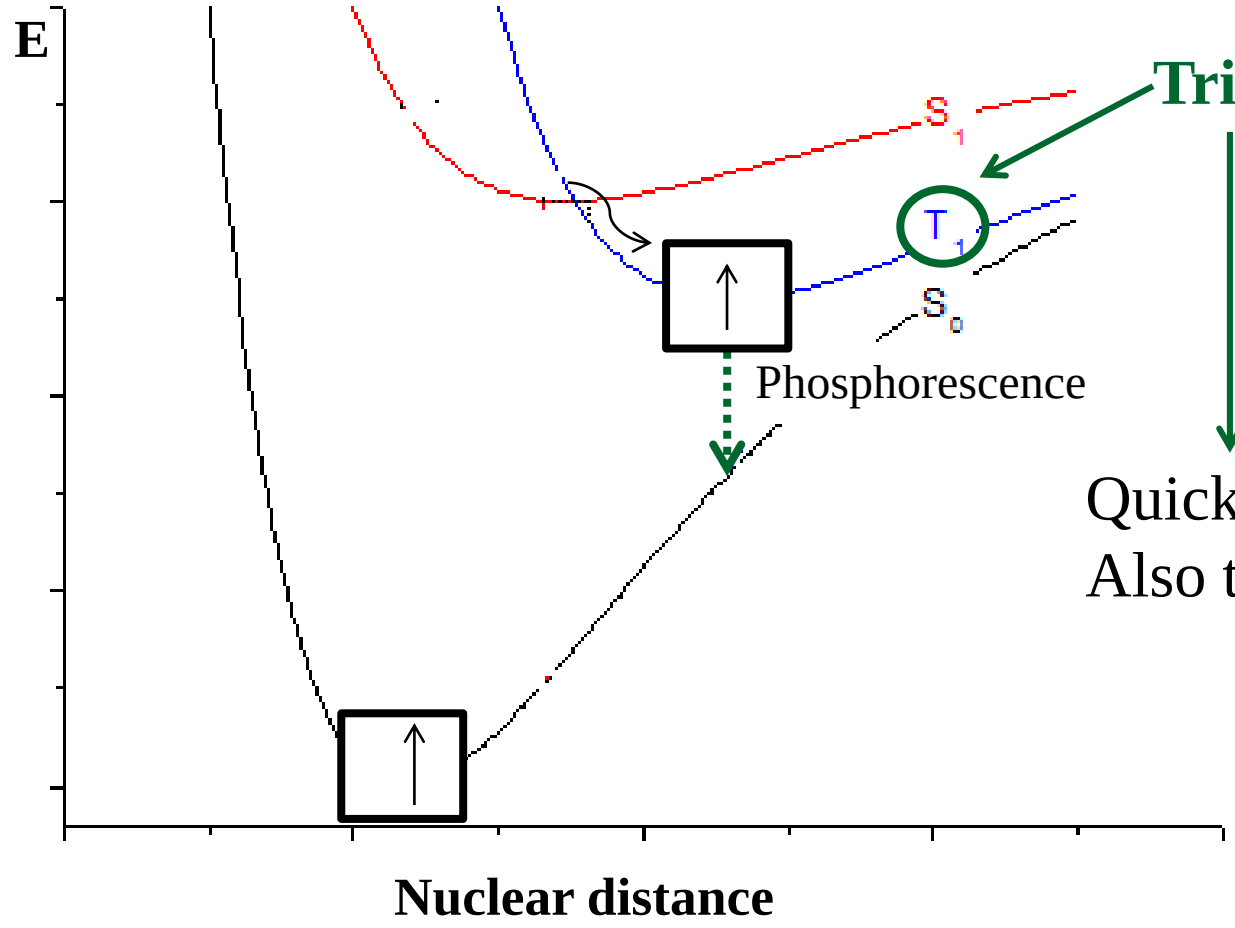
The role of the triplet species in photosensitization processes: absorption



The role of the triplet species photosensitization processes: Singlet state



The role of the triplet species photosensitization processes: Intersystem crossing



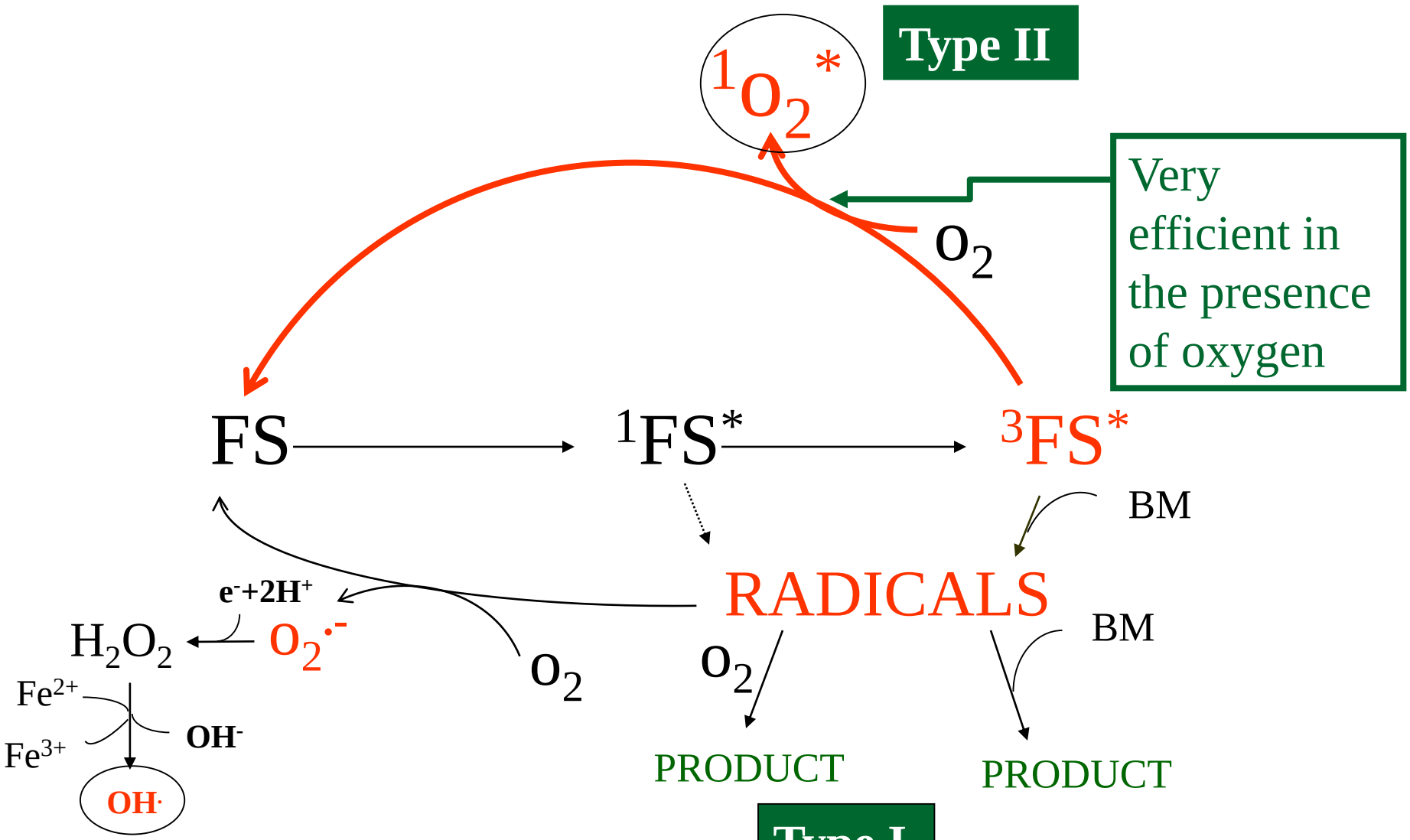
Triplet

Lives longer and is reactive

Quickly reacts with O_2

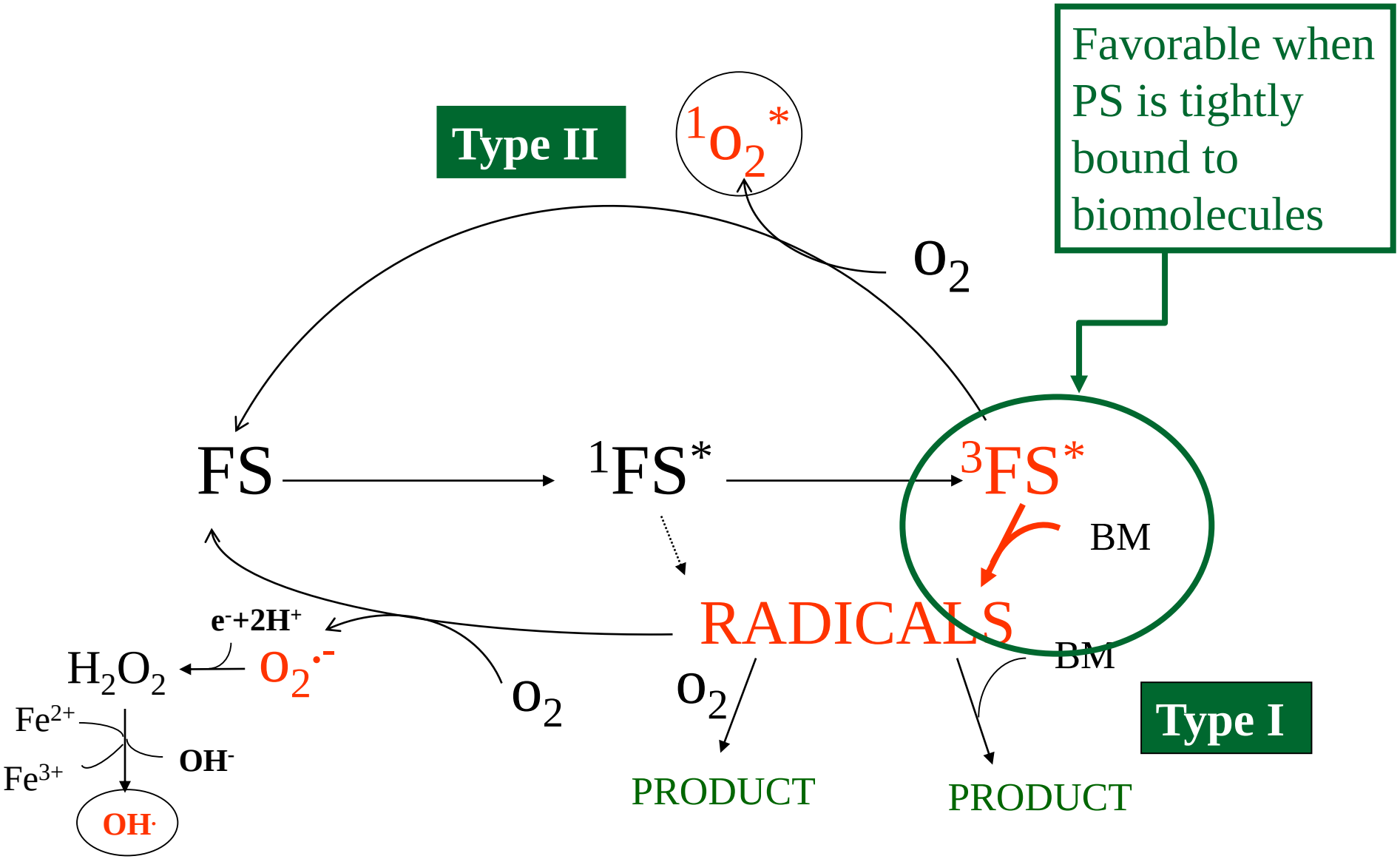
Also transfer of e^-, e^-/H^+, $H\cdot$

Mechanisms of photosensitized oxidations

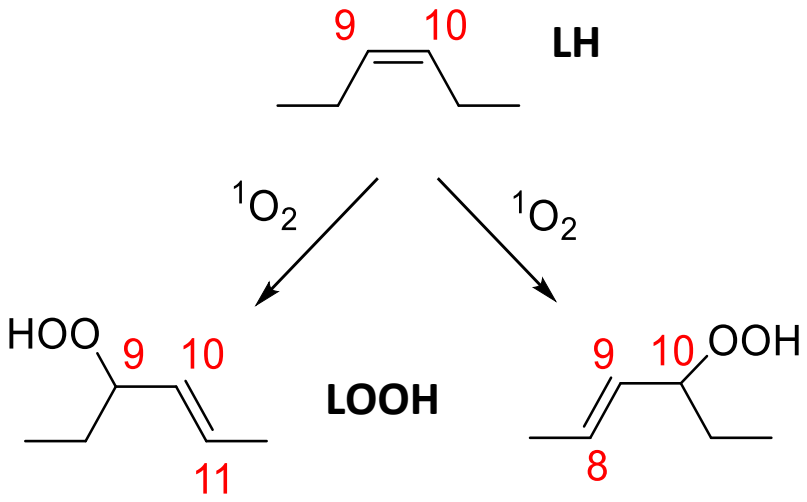


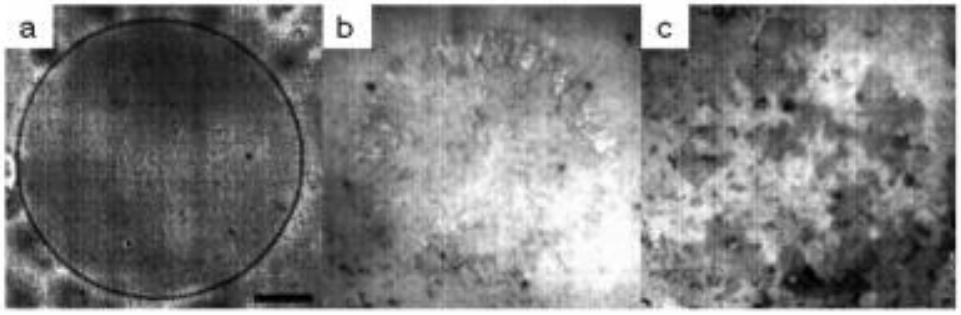
Foote, C.S. *Science* **1968**, *162*, 963.

Mechanisms of photosensitized oxidations



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


9
10
LH
$^1O_2$
$^1O_2$
HOO
9
10
11
LOOH
9
10
OOH
8


a
b
c

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

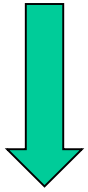
Why in certain situations, the scientific knowledge takes so long to bring benefits to the population?

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.



The basal layer in human squamous tumors harbors more UVA than UVB fingerprint mutations: A role for UVA in human skin carcinogenesis

PNAS 2004 101, 4954-4959.

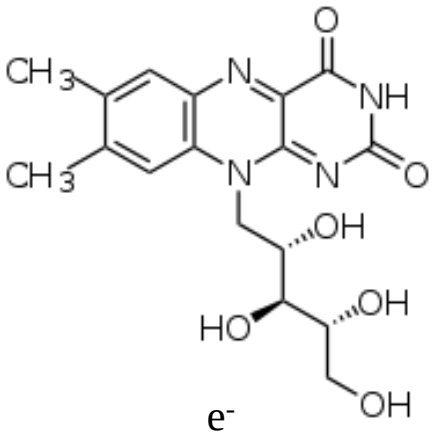
Photochem. Photobiol., 2005, **81**(1), 3–8.

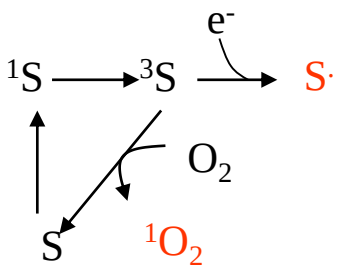
UVA-photosensitization

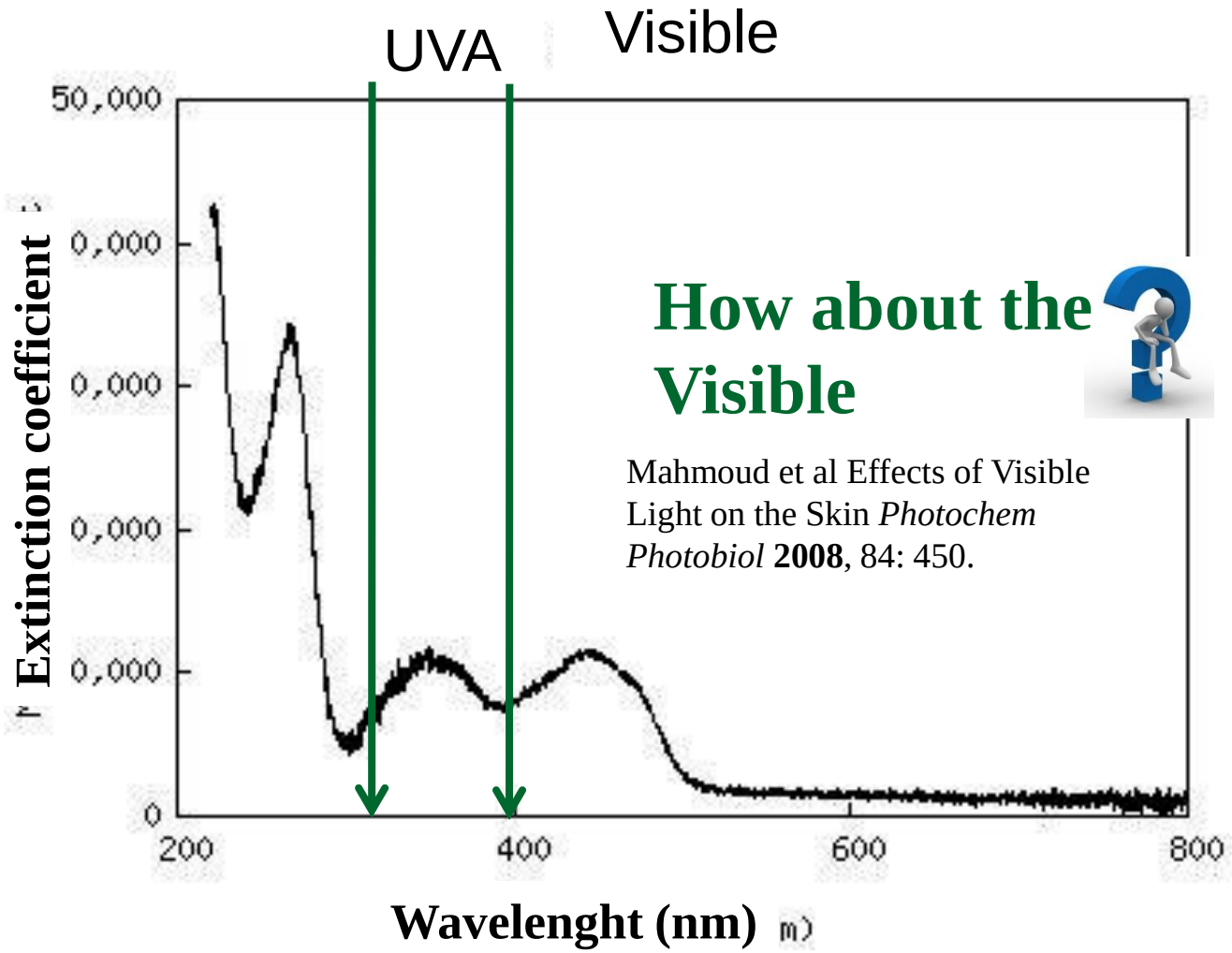
- S Mouret et al, *PNAS* **2006**, 103, 13765.
- Bäumler et al *Biophys J* **2006**, 91, 1452;

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

Flavins



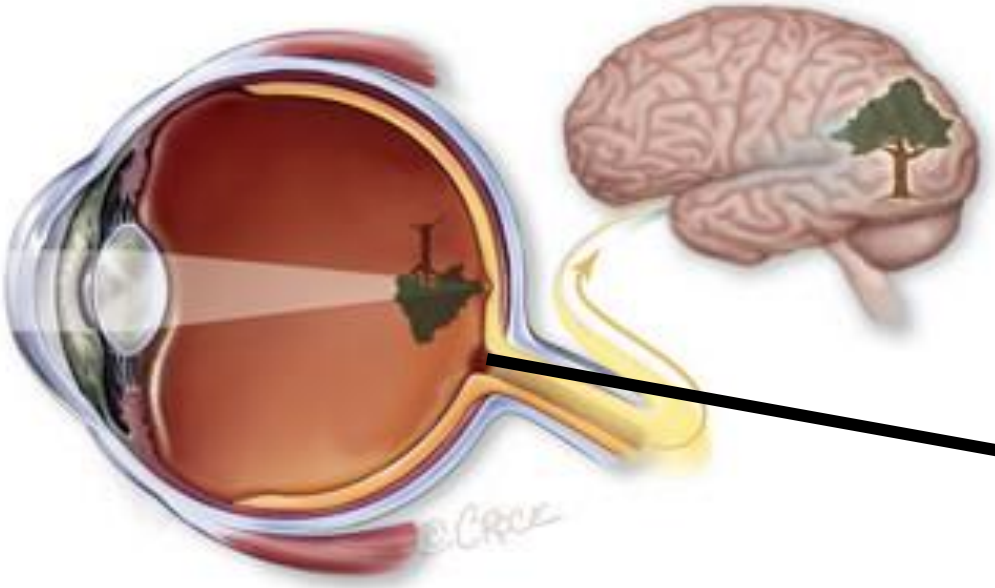


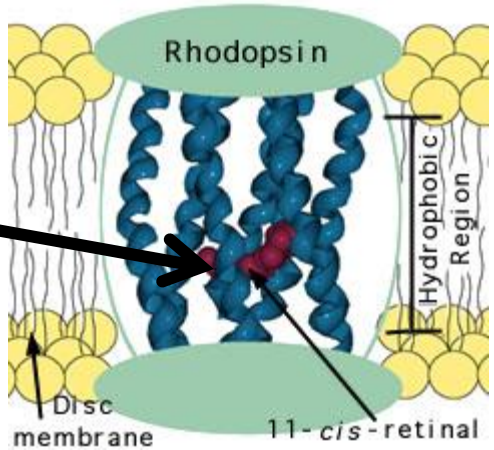


How about the Visible

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

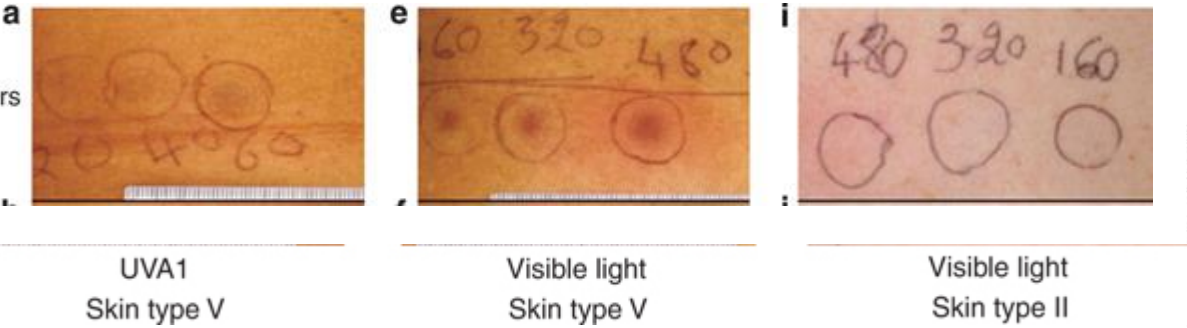
And the visible light

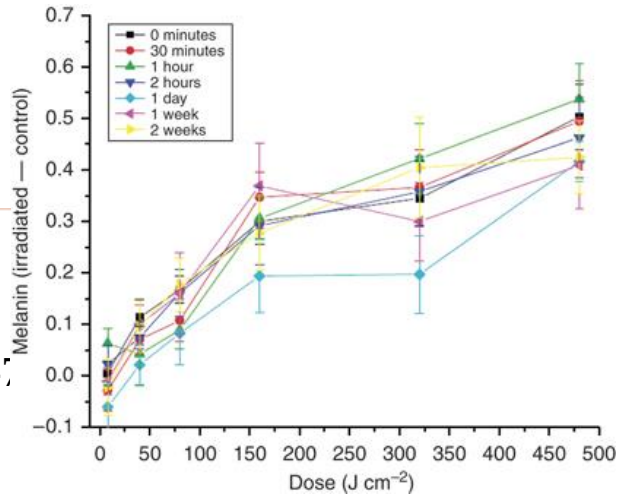




Pigmentation induced by visible light was darker and more sustained than pigmentation induced by UVA in melano competent individuals

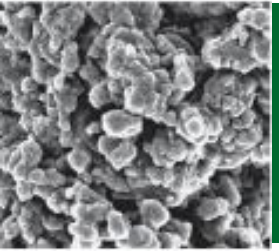
Type II skin does not pigment well, neither with UVA nor with visible!!!



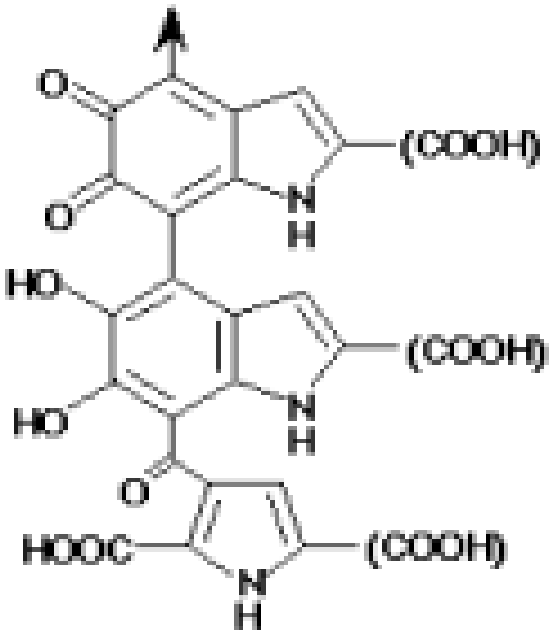


Mahmoud…H.W. Lim et al *J Invest Dermatol* **2010**, 130(8):2092-7
J&J: NJ USA; Dermatology Detroit,Michigan USA

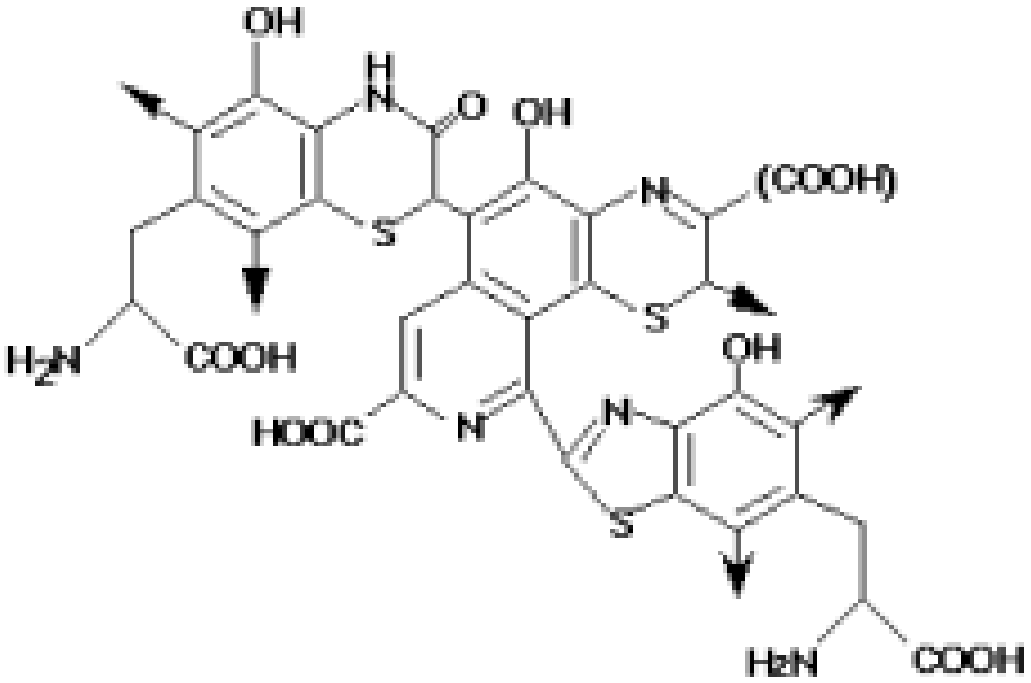
Does melanin cause any harm in the skin that would favor the synthesis of more melanin?



Melanin



Eumelanin

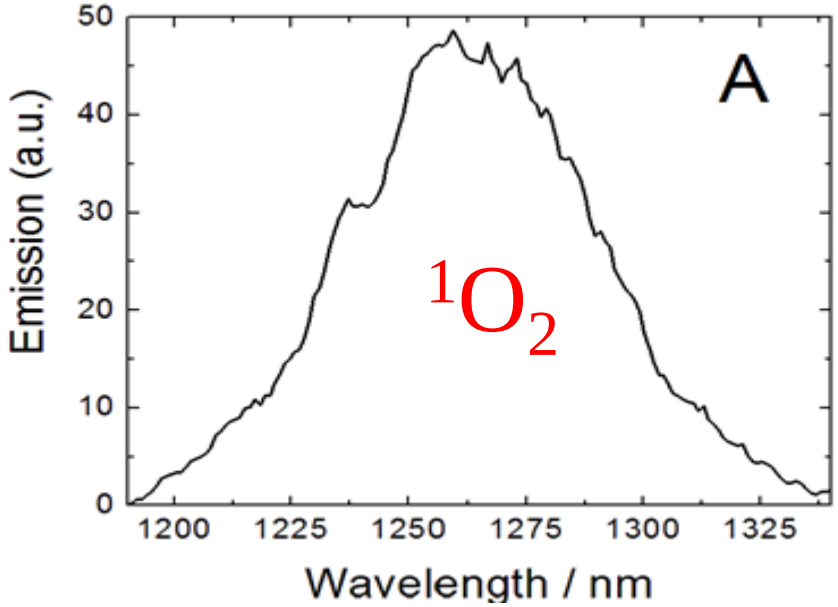


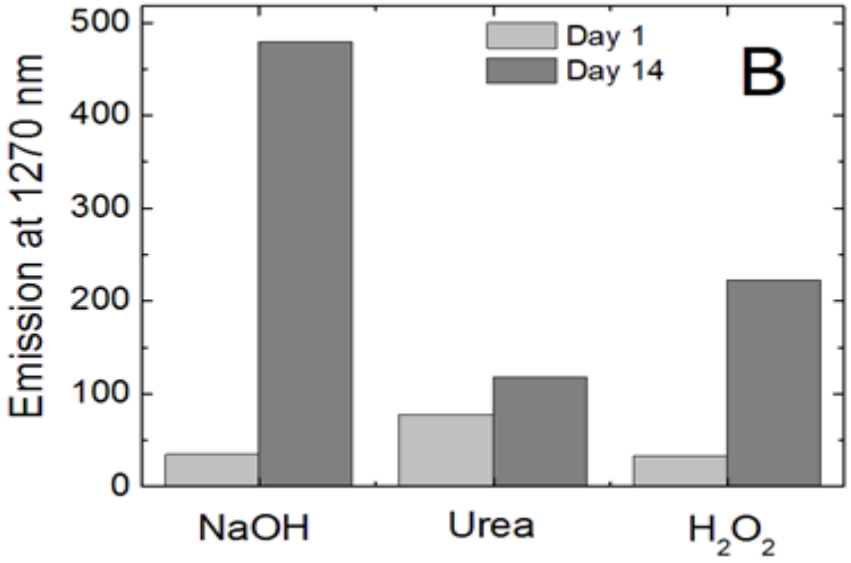
Pheomelanin

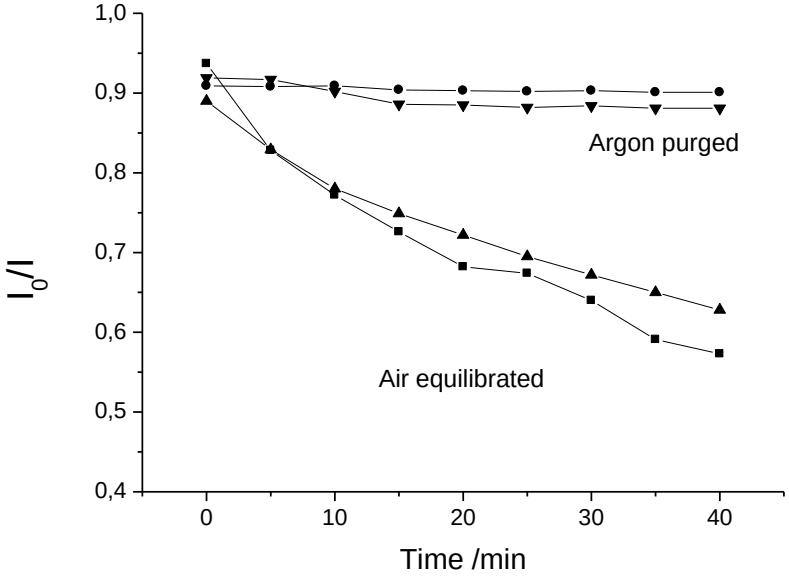
K Wakamatsu and S Ito *Pigment Cell Res* 2002, 15.

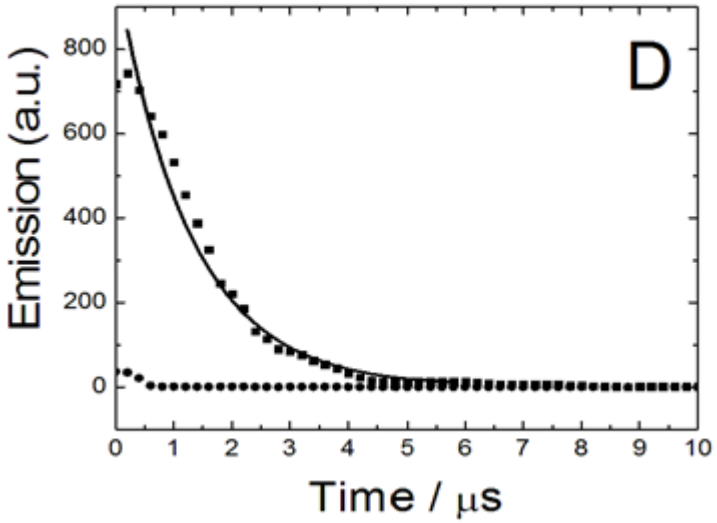
Does melanin generate $^1O_2$

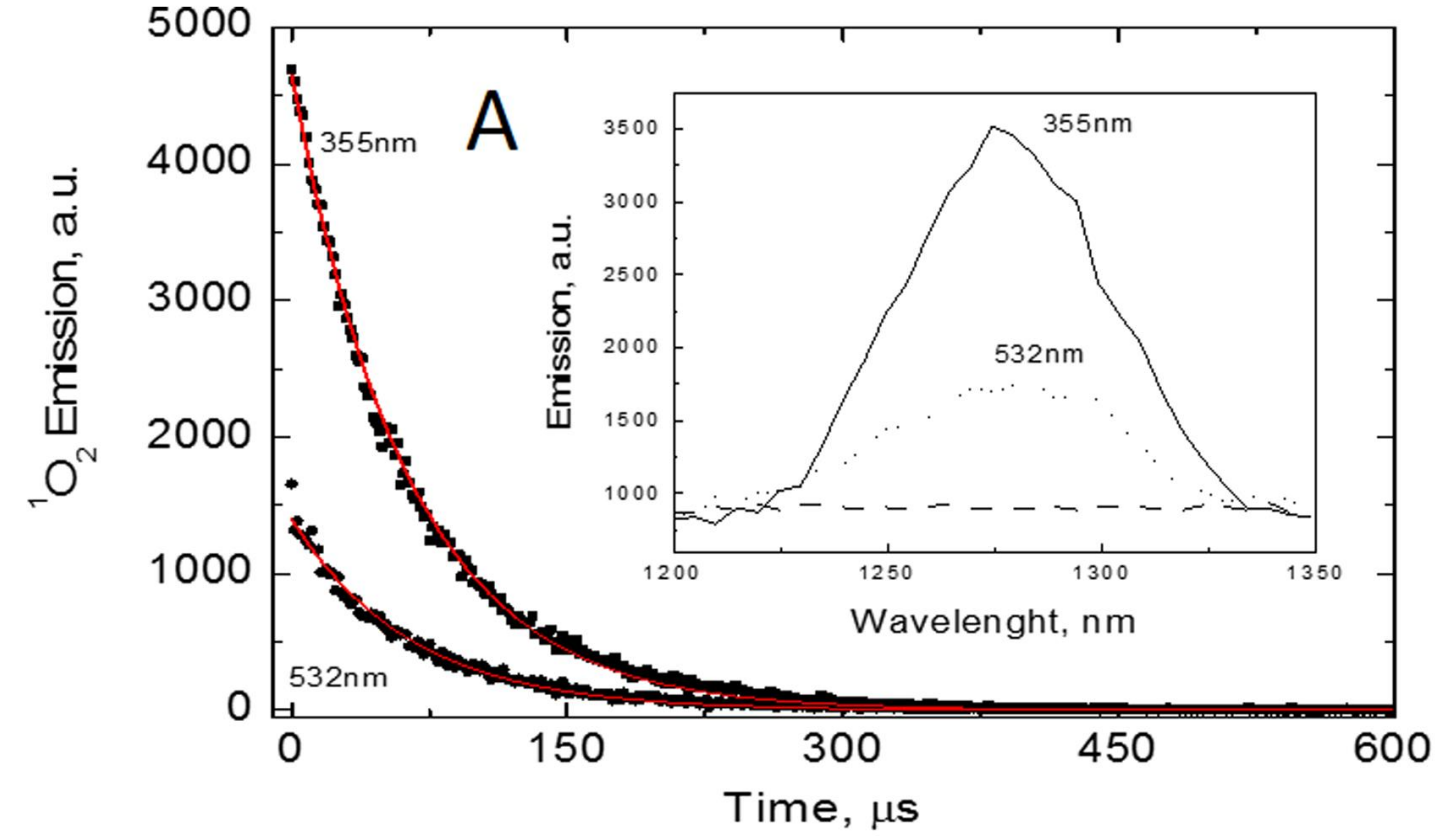
Melanin generates singlet oxygen with irradiation in VISIBLE (532nm)

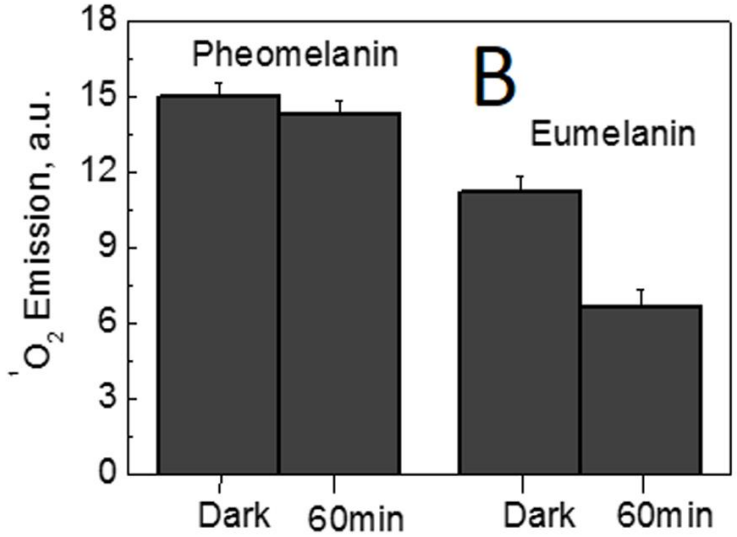


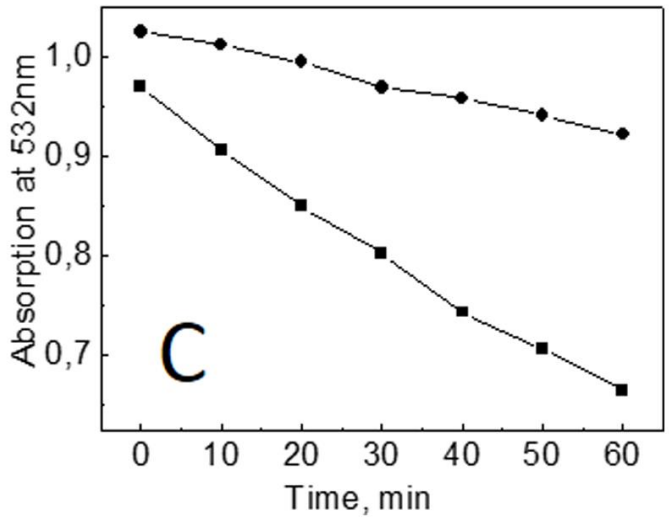




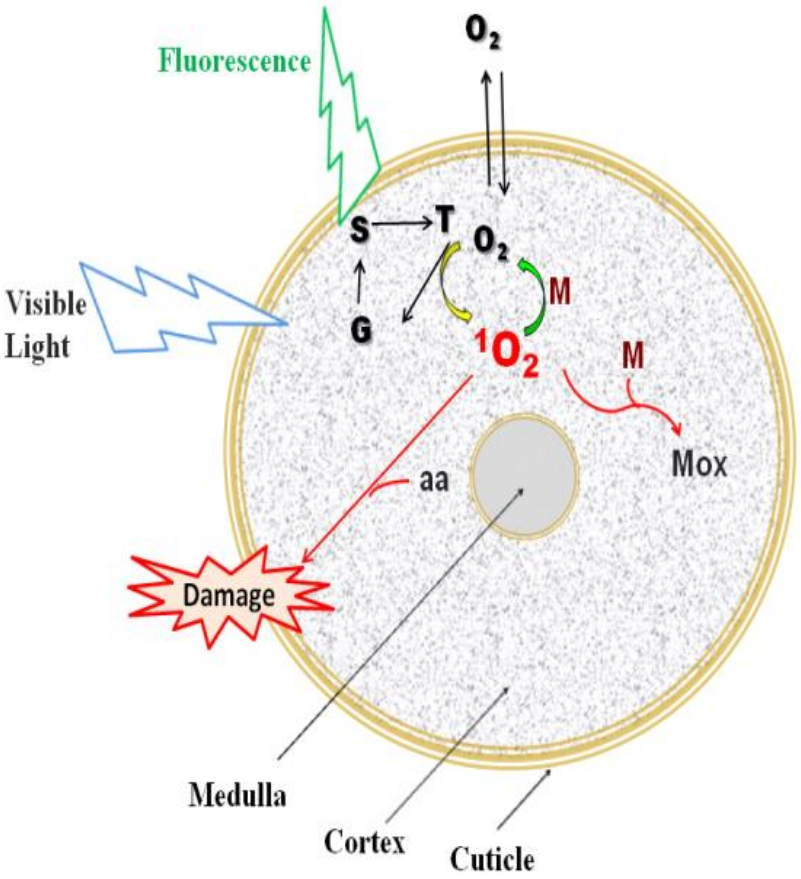


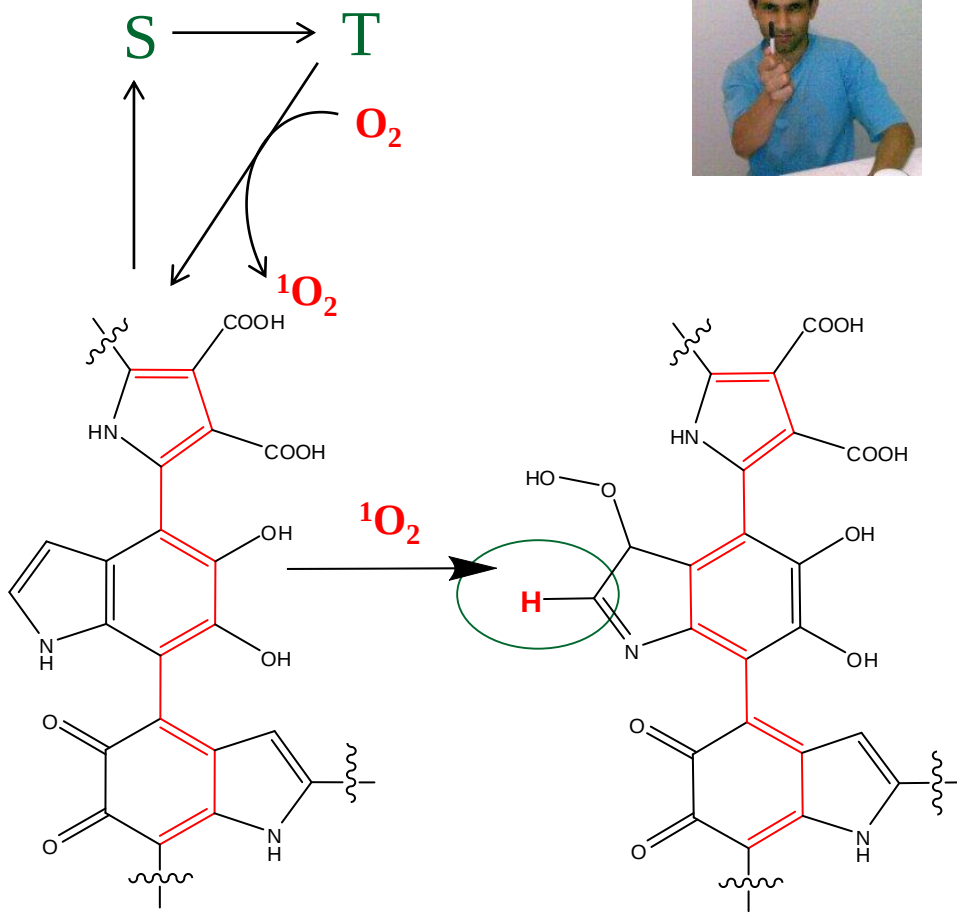

A
355nm
532nm
$^1O_2$ Emission, a.u.
Time, μs
Emission, a.u.
Wavelenght, nm


B
Pheomelanin
Eumelanin
$^1O_2$ Emission, a.u.
Dark
60min
Dark
60min


C
Absorption at 532nm
Time, min

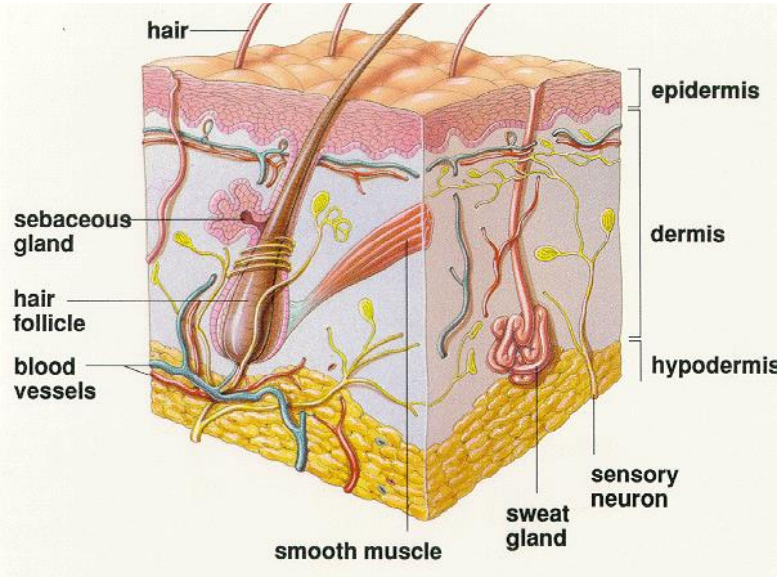
Photolysis of melanin with visible light (400-700nm) is much faster in the presence of oxygen. Photolysis product showed the presence of a hydroperoxide at C3 of indol





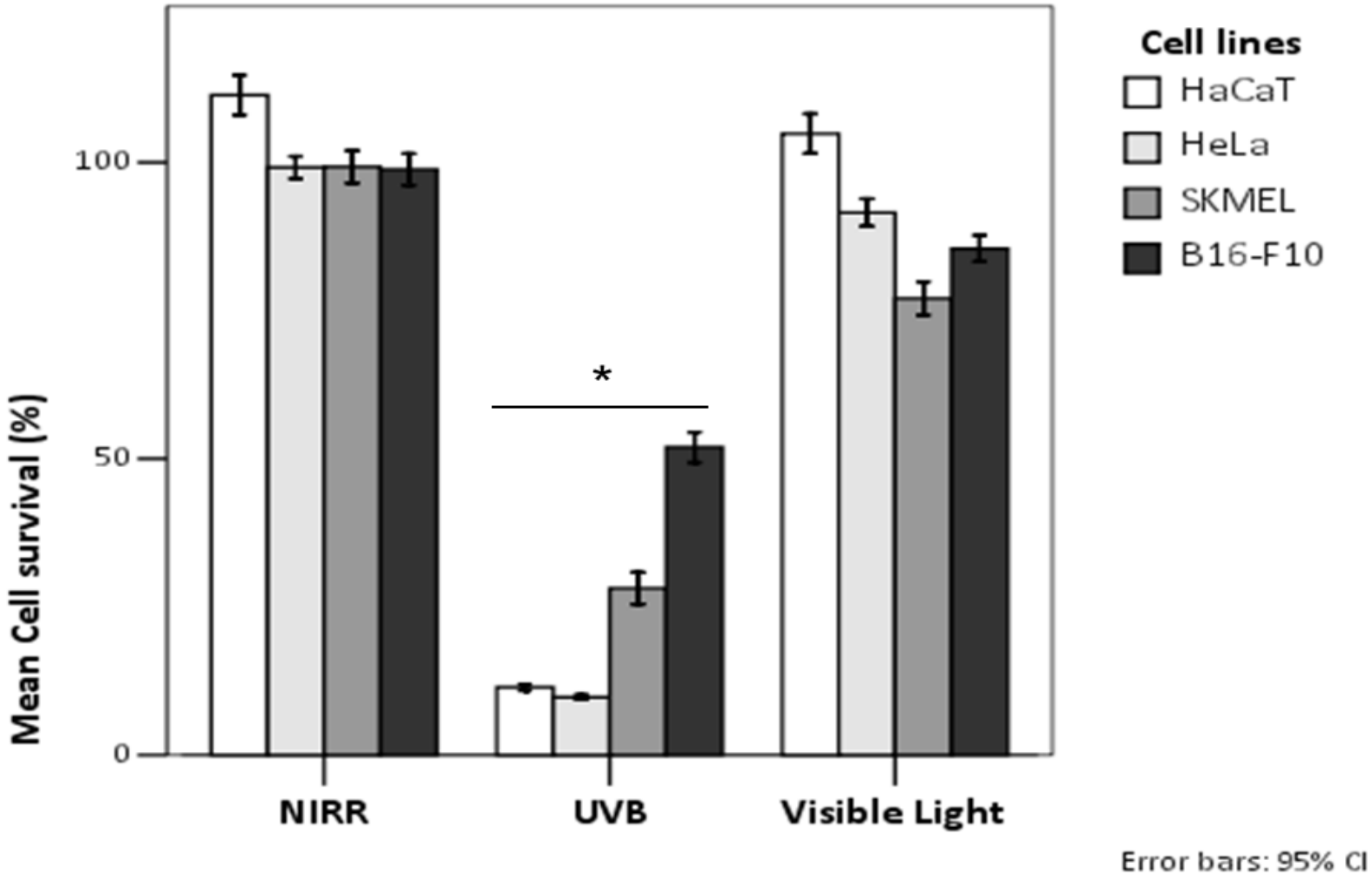
Chiarelli-Neto et al *Free Radic Biol Med* **2011**, 51, 1195

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

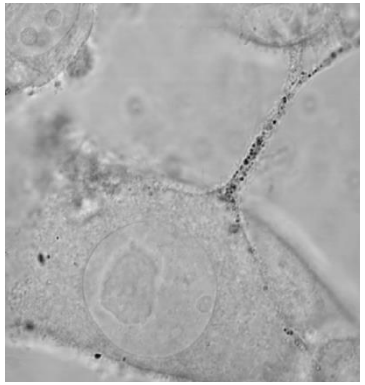
METHODS

B16F10 Cells

Tyr +NH_4Cl

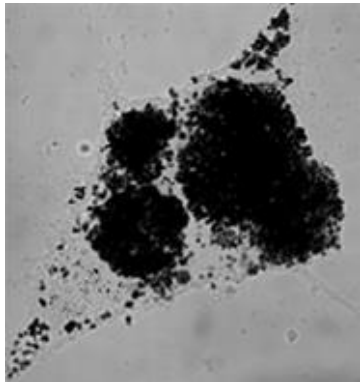
48h

$(Melanin)_n$



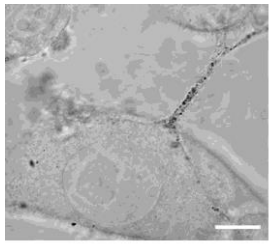
B16F10

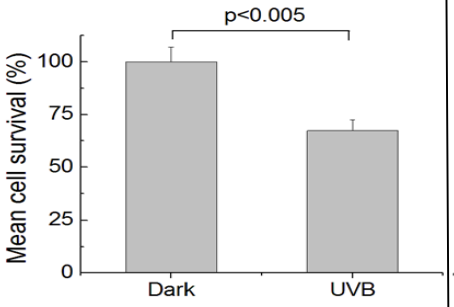
48h

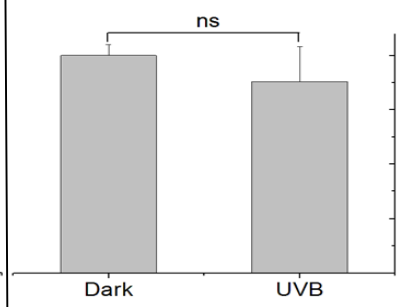


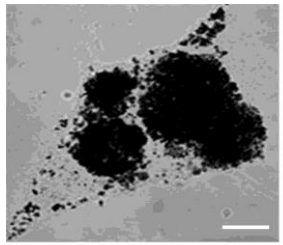
B16F10

A

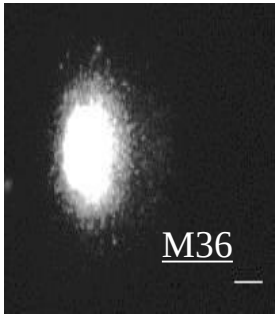


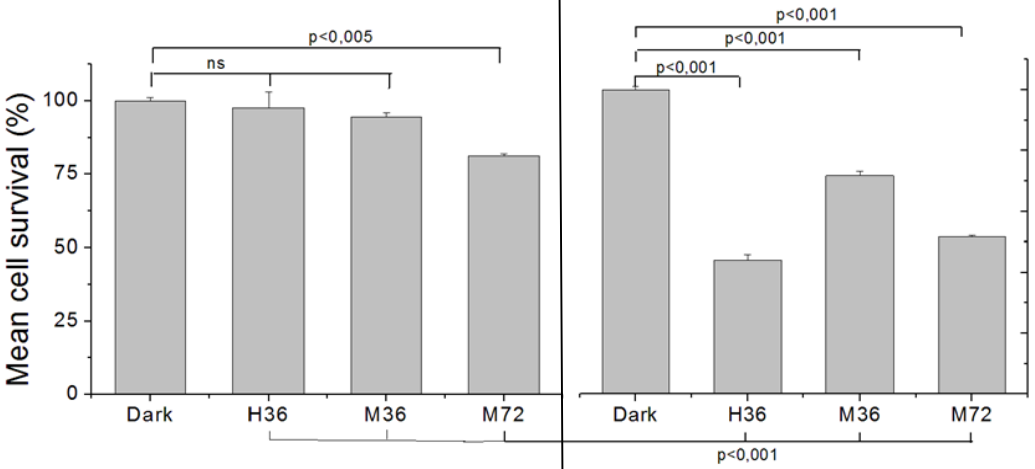

p<0.005
Mean cell survival (%)
100
75
50
25
0
Dark
UVB

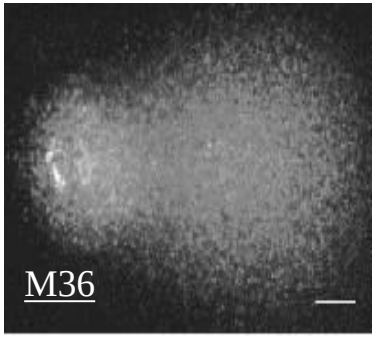

ns
Dark
UVB



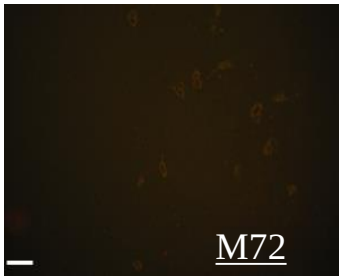
B

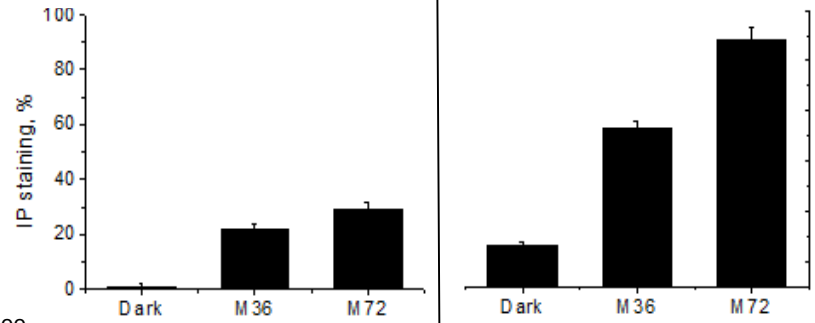

M36

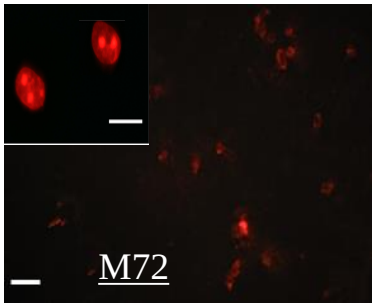

p<0,005
ns
Mean cell survival (%)
100
75
50
25
0
Dark
H36
M36
M72
p<0,001
p<0,001
p<0,001
p<0,001
Dark
H36
M36
M72


M36

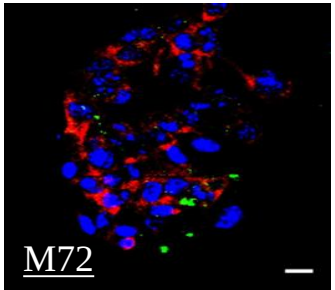
C

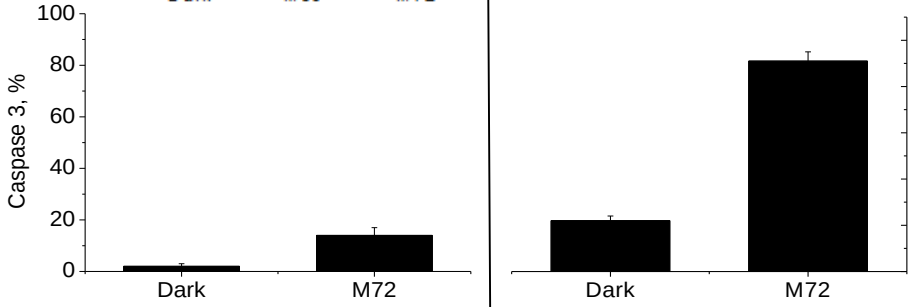

M72

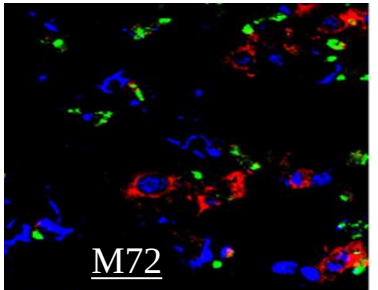

IP staining, %
100
80
60
40
20
0
Dark
M36
M72
Dark
M36
M72


M72

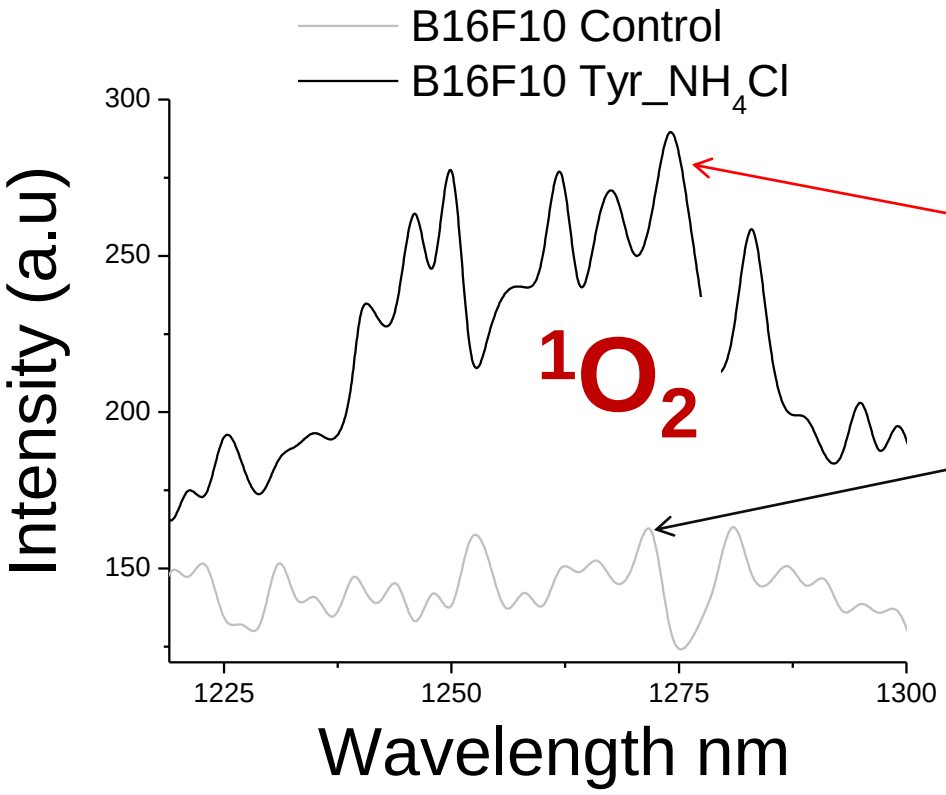
D

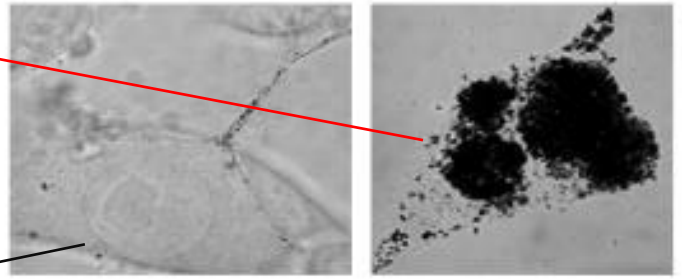

M72


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


M72

$^1O_2$ IN OVER PIGMENTATED B16F10 CELLS



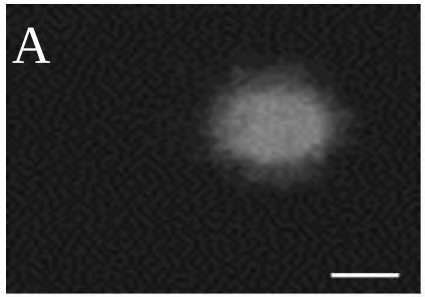


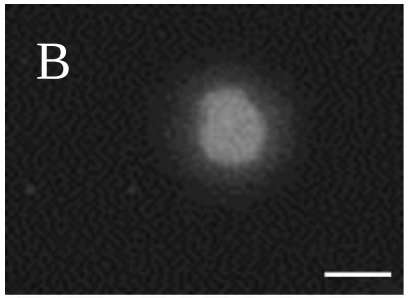
B16F10 B16F10

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

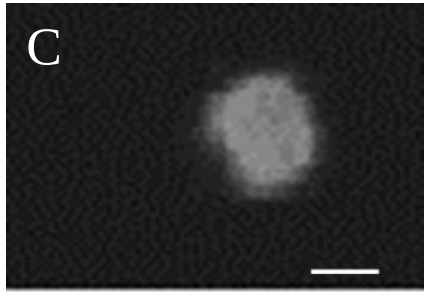
There is $^1O_2$ generation under visible light in cells over pigmentated. The over pigmentation causes damages in cells.

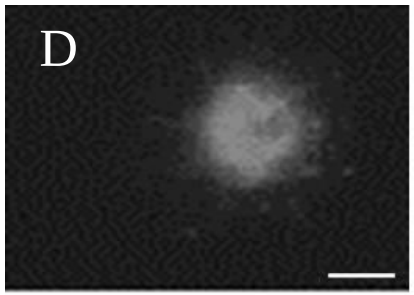
Melanin **CT** +++++



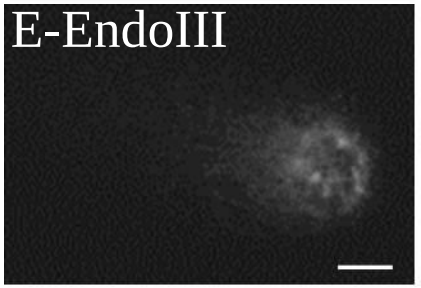


0

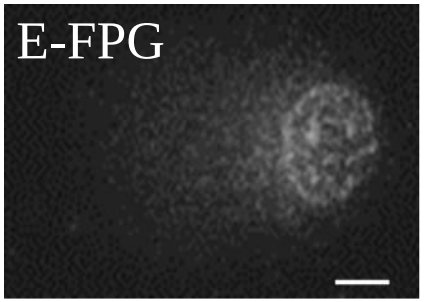




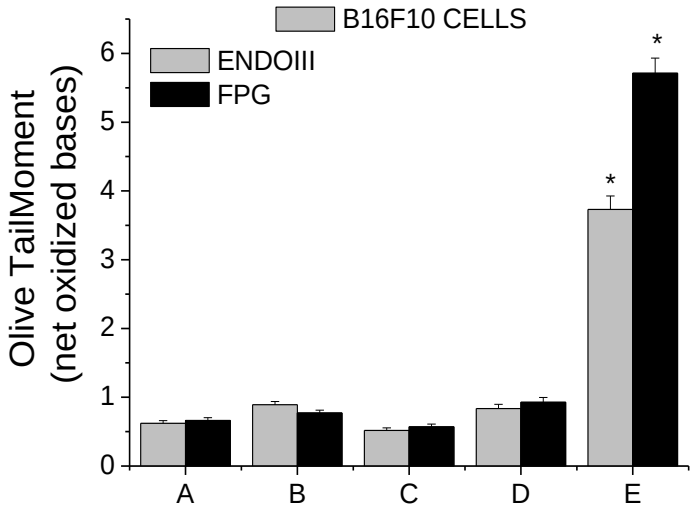
6J



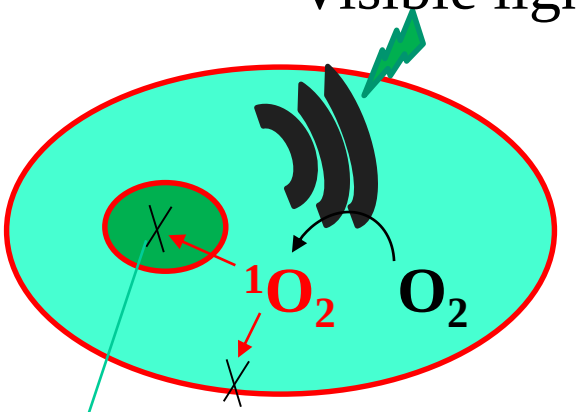
6J

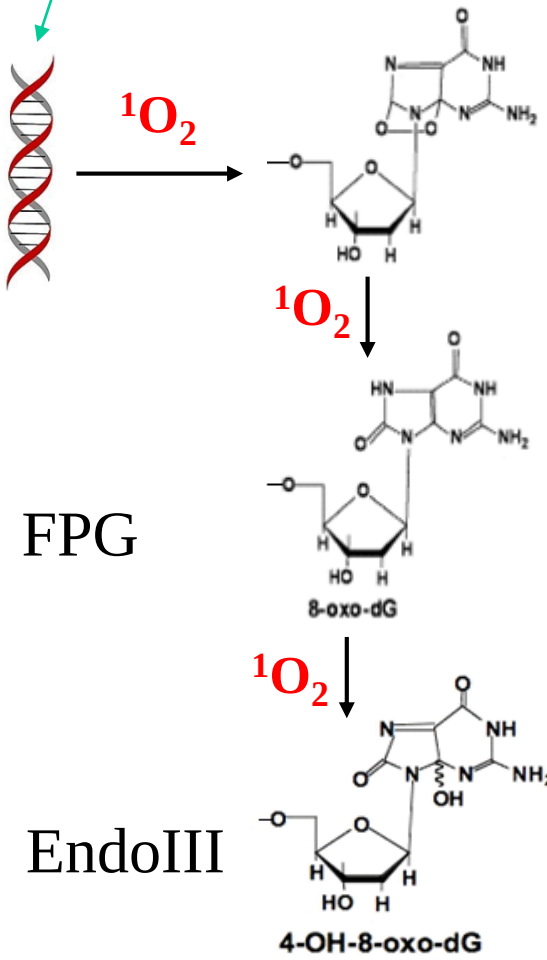


6J



Visible light--singlet oxygen--DNA damage

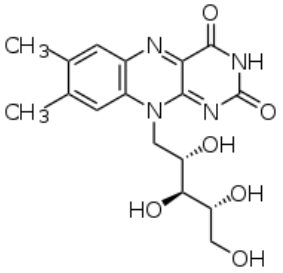


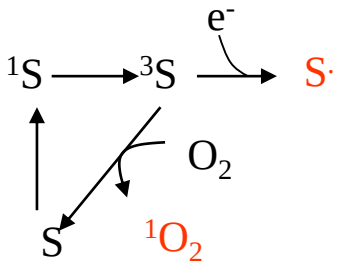


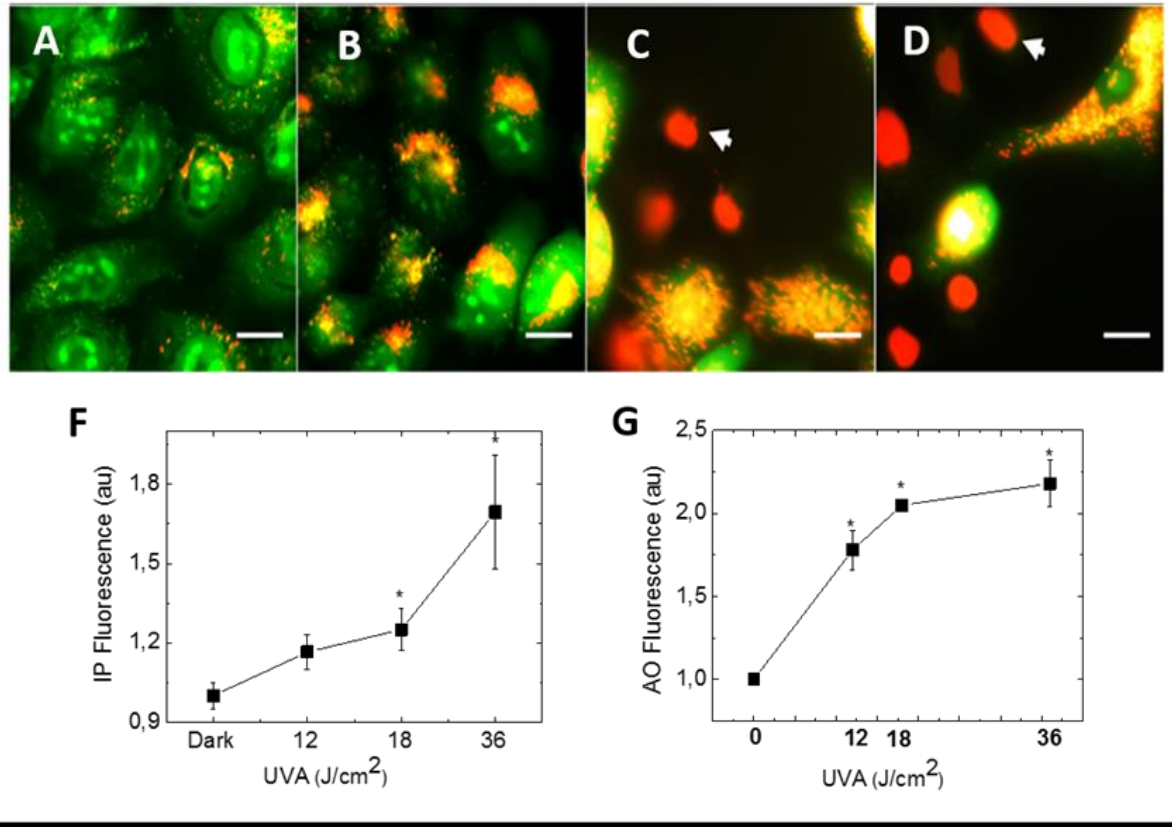
Does visible Light affects other cells?

Which molecule would absorb light?

UVA

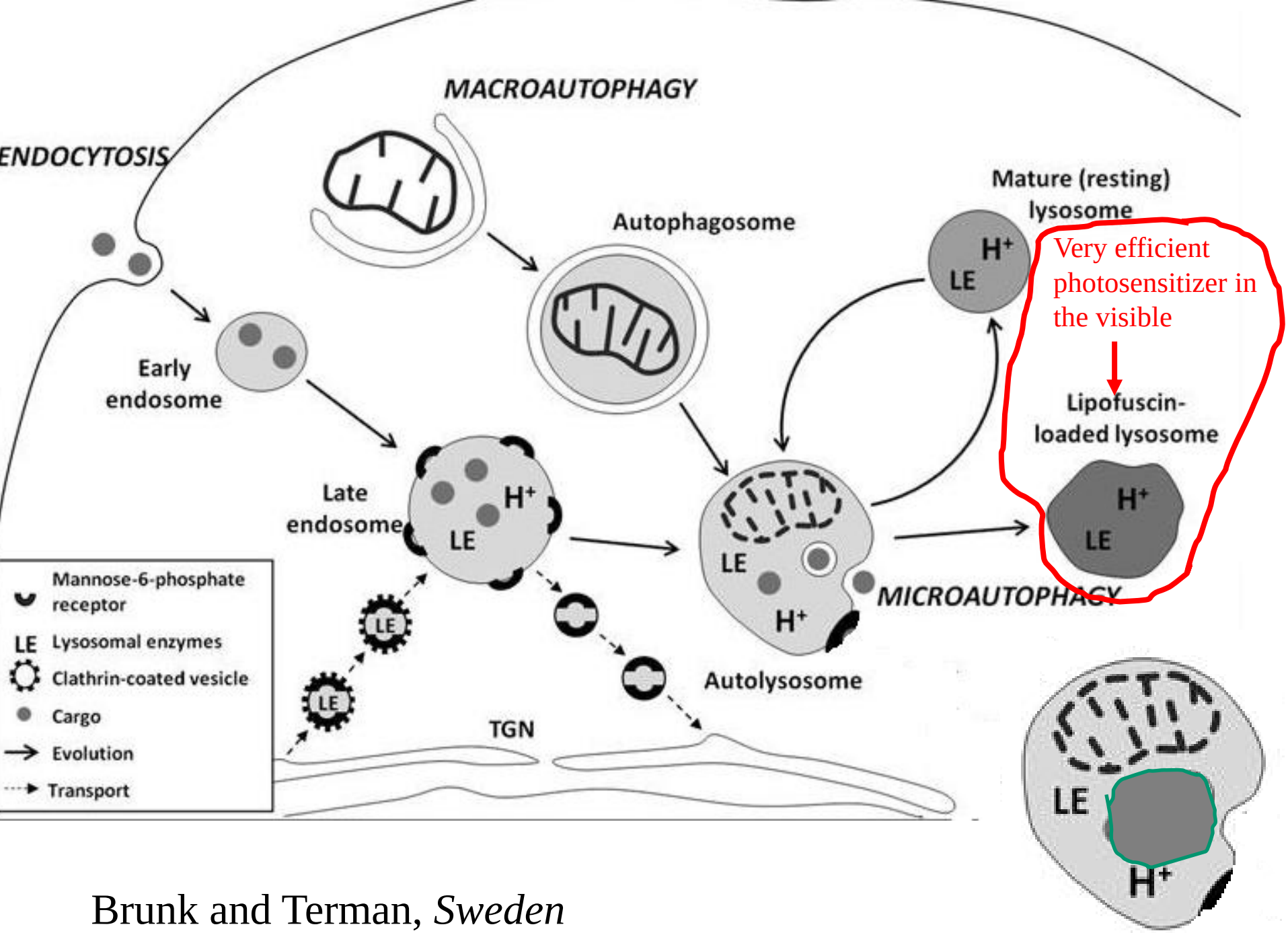






UVA causes inhibition of autophagic flux!

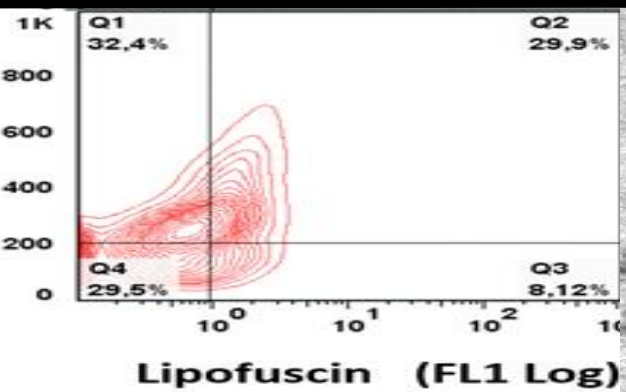
Lamore & Wondrak *PPS* **2012**,11,163

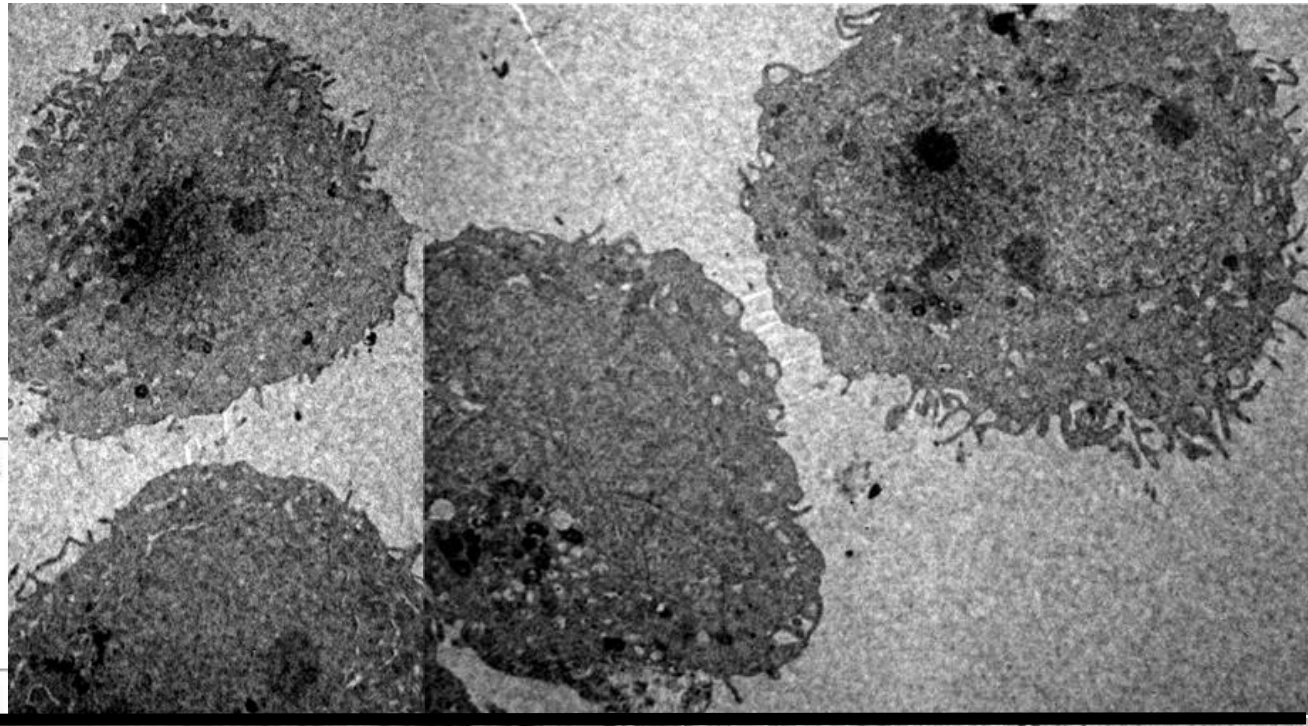


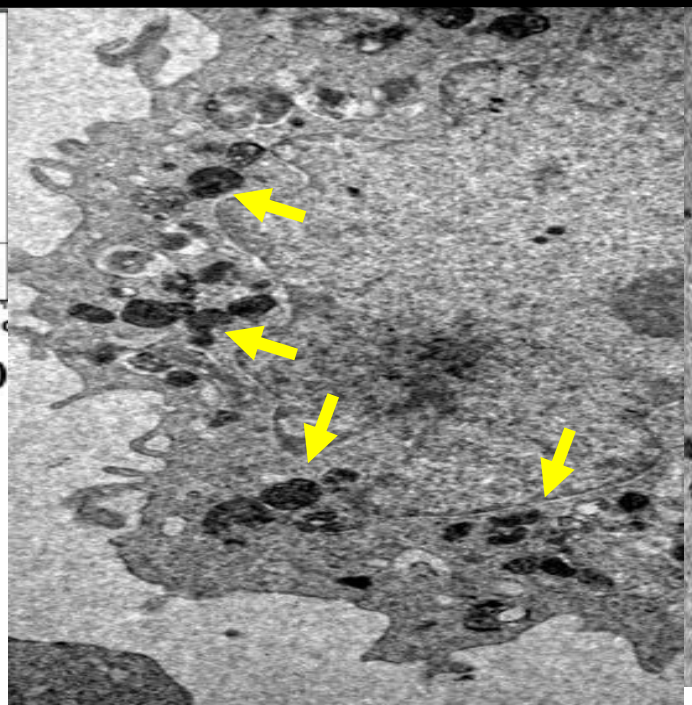
Brunk and Terman, *Sweden*

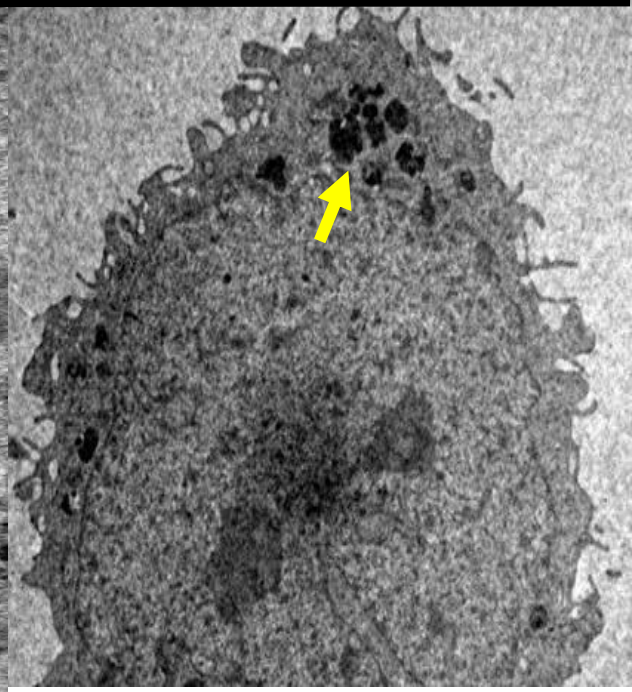

1K
800
600
400
200
Q1
33,2%
Q2
5,00%
Q4
Q3

Dark


1K
800
600
400
200
0
Q1
32,4%
Q2
29,9%
Q4
29,5%
Q3
8,12%
10^0
10^1
10^2
Lipofuscin (FL1 Log)

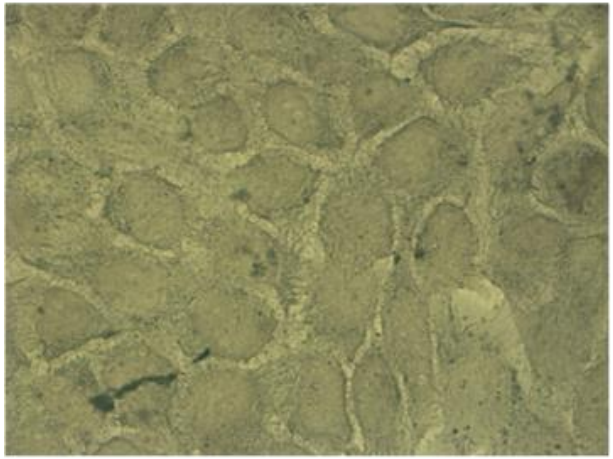




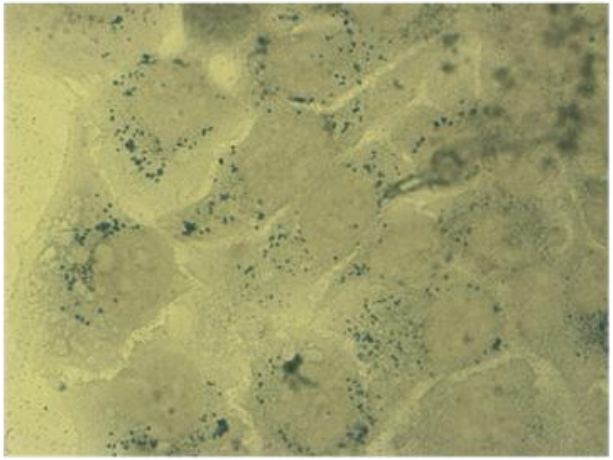


Lipofuscin x wavelenght

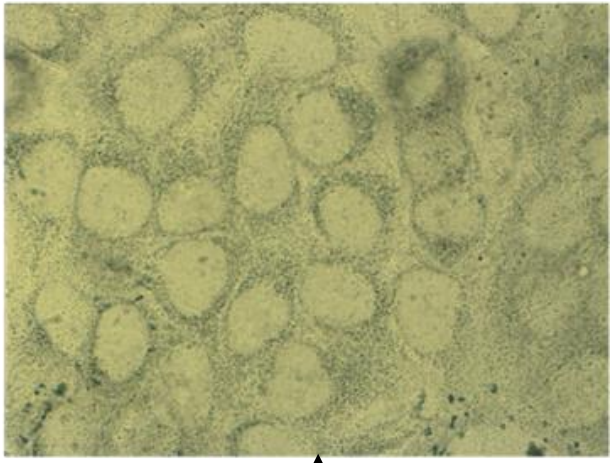
Dark



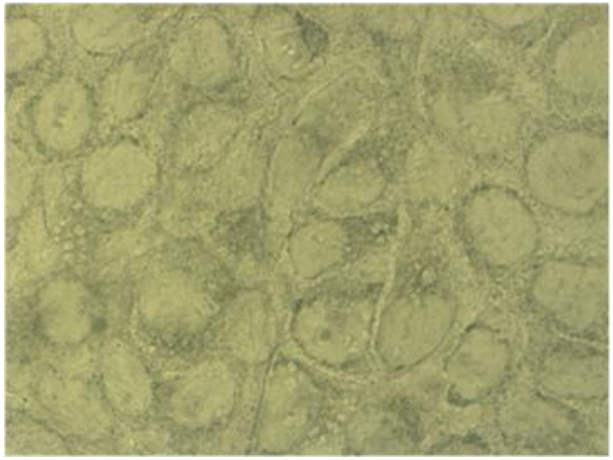
408 nm



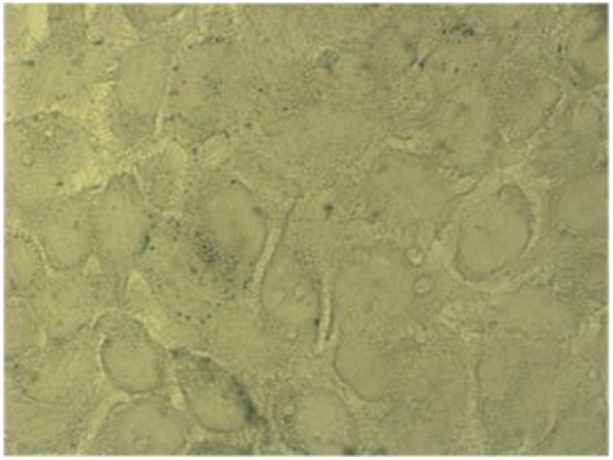
466 nm

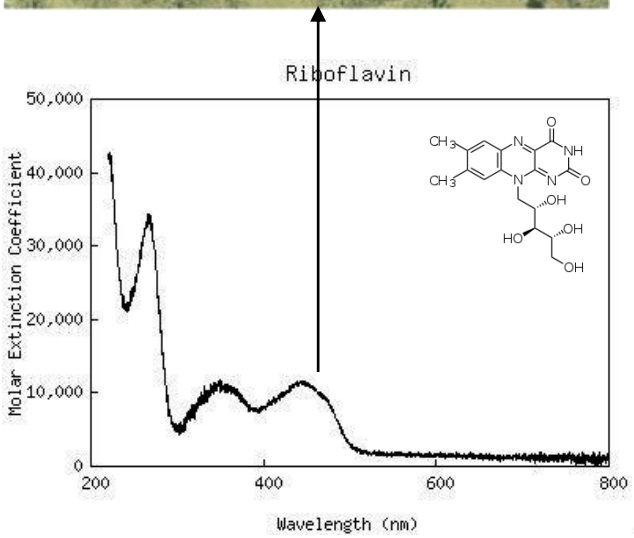


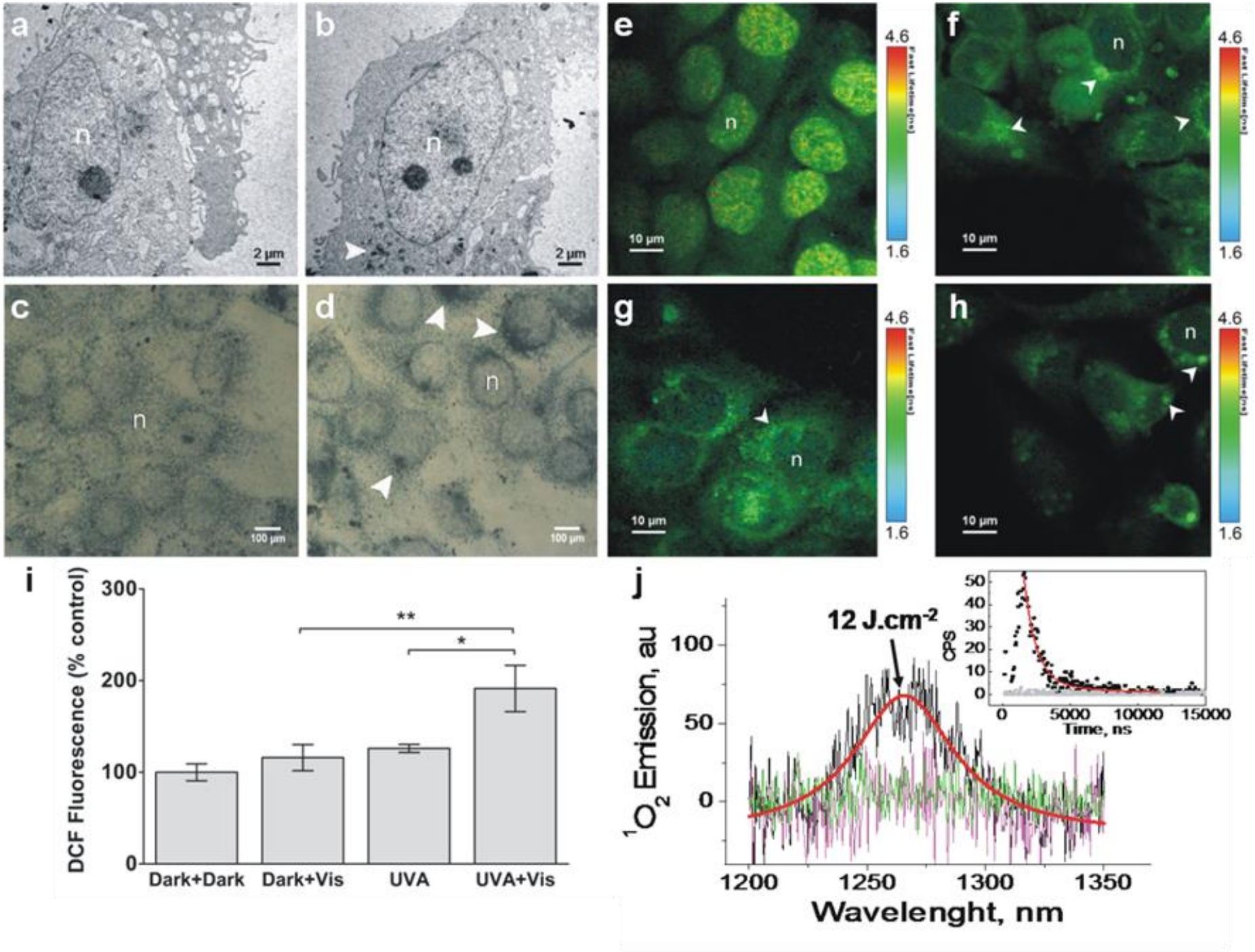
522 nm

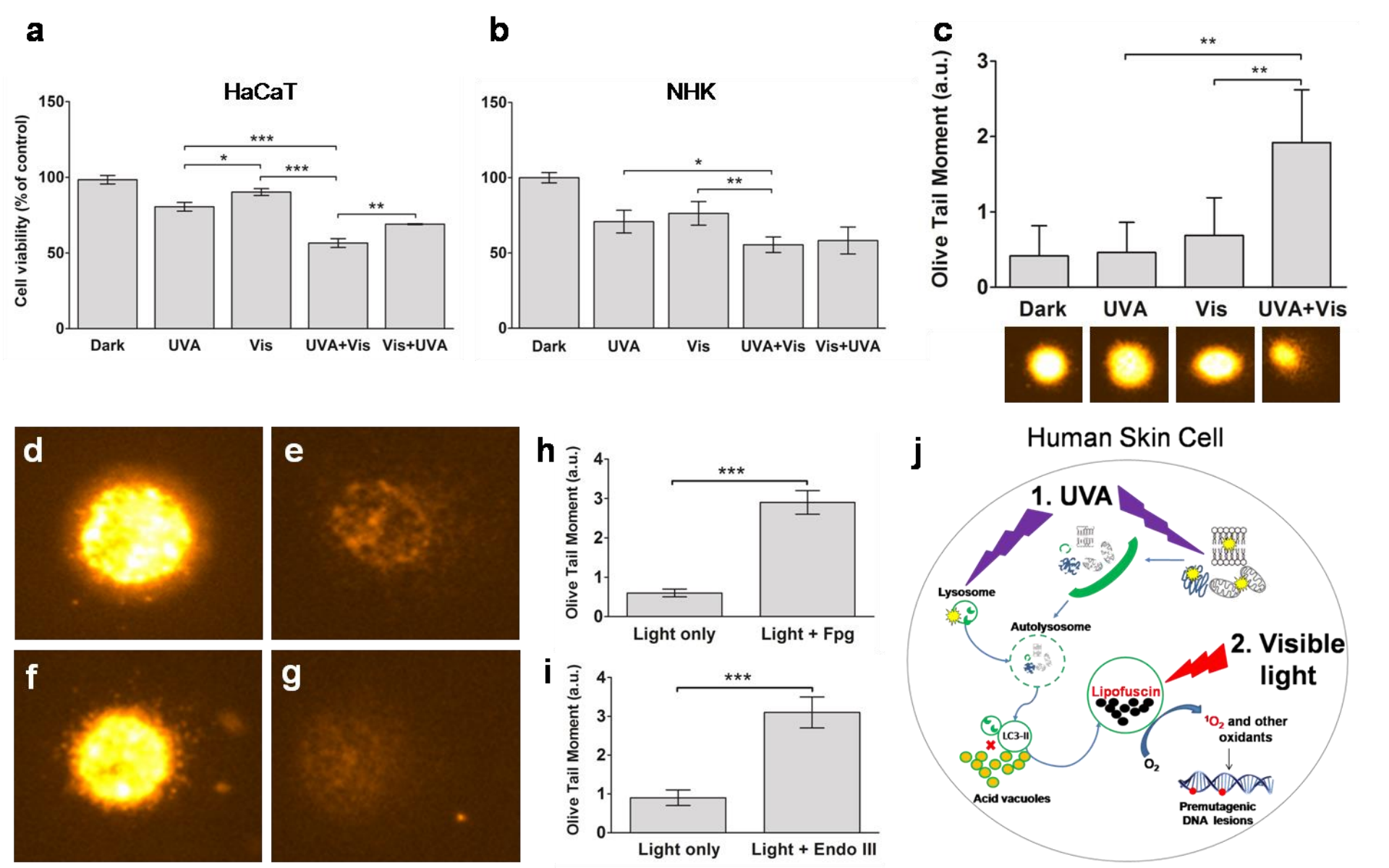


650 nm

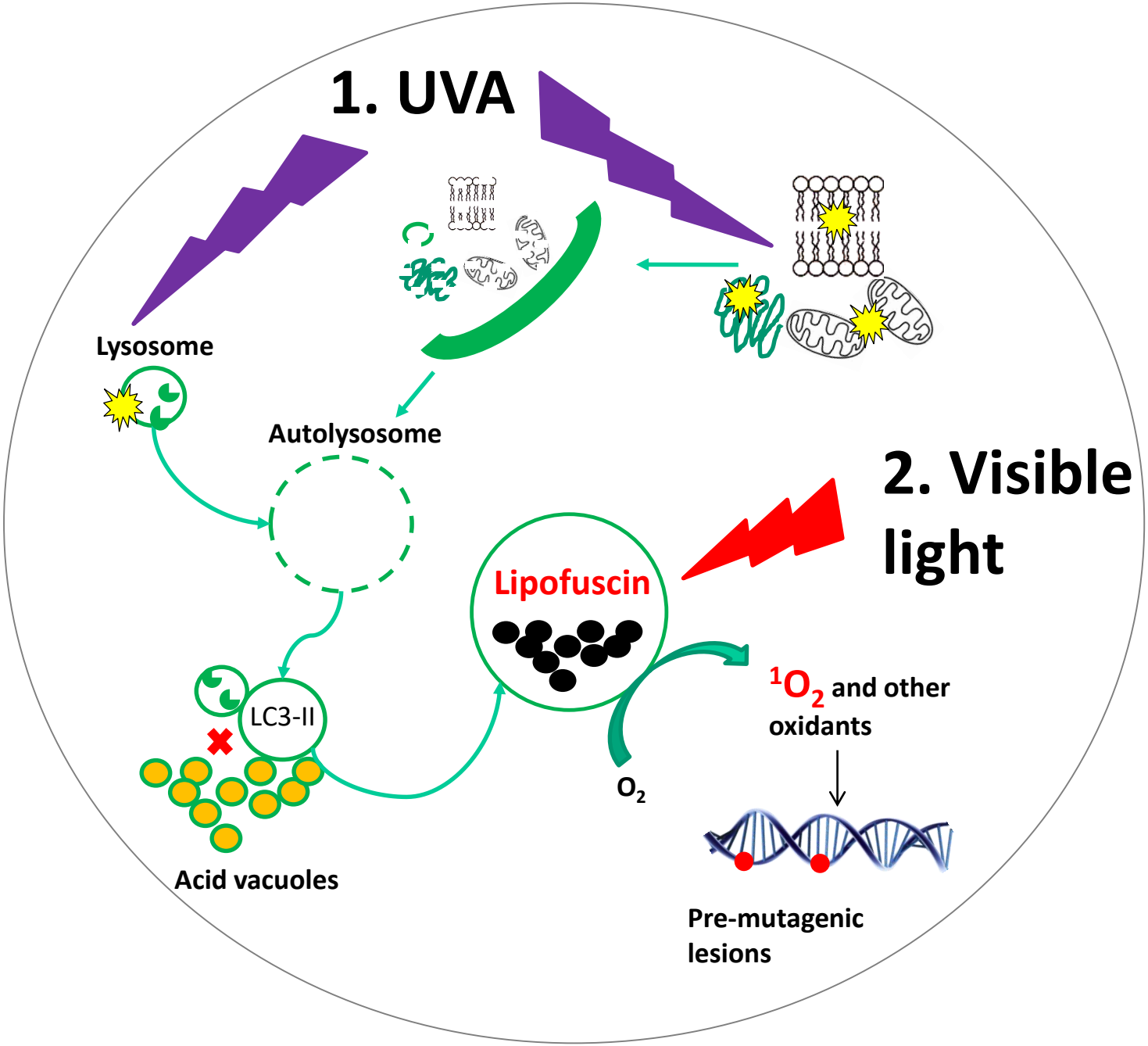





a
b
c
d
e
f
g
h
i
j
n
2 µm
100 µm
10 µm
4.6
1.6
DCF Fluorescence (% control)
300
200
100
0
**
*
Dark+Dark
Dark+Vis
UVA
UVA+Vis
12 J.cm-2
1O2 Emission, au
Wavelenght, nm
1200
1250
1300
1350
CPS
Time, ns
5000
10000
15000



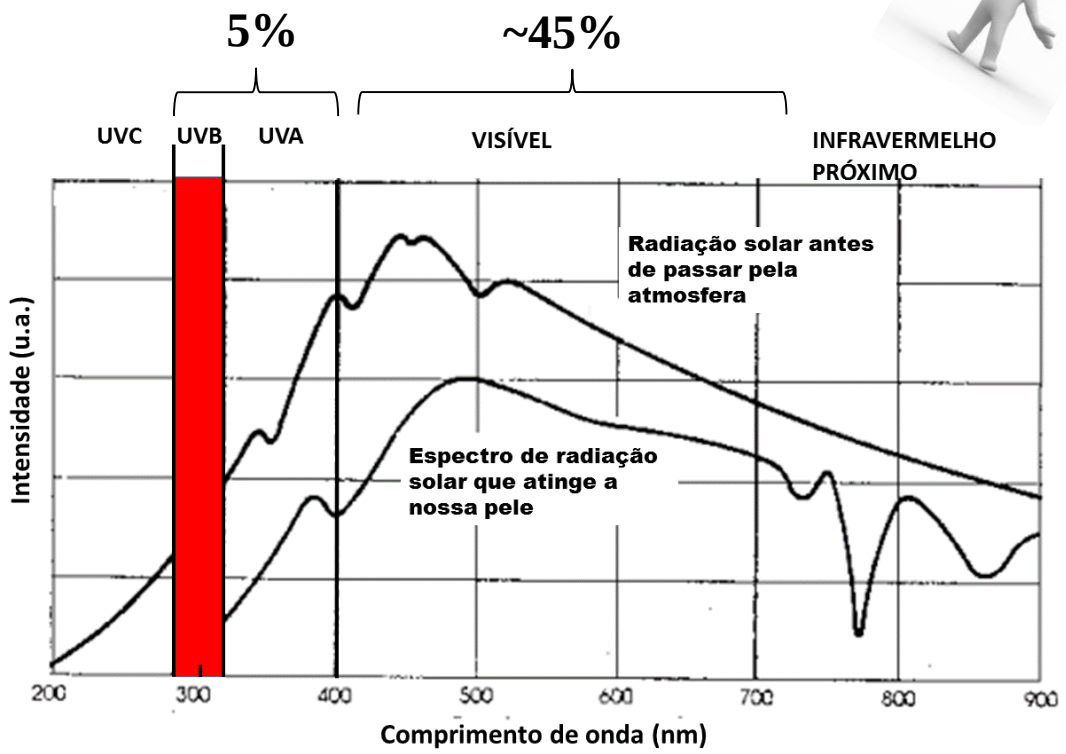
Tonolli et al Lipofuscin generated by UVA turns keratinocytes photosensitive to visible light *Journal of Investigative Dermatology* 2017



The Journal of investigative dermatology 137 (11), 2447

Visible light should be considered in skin protection strategies against photoinduced damage. It is certainly involved in photoaging and in other skin diseases.

Conclusion

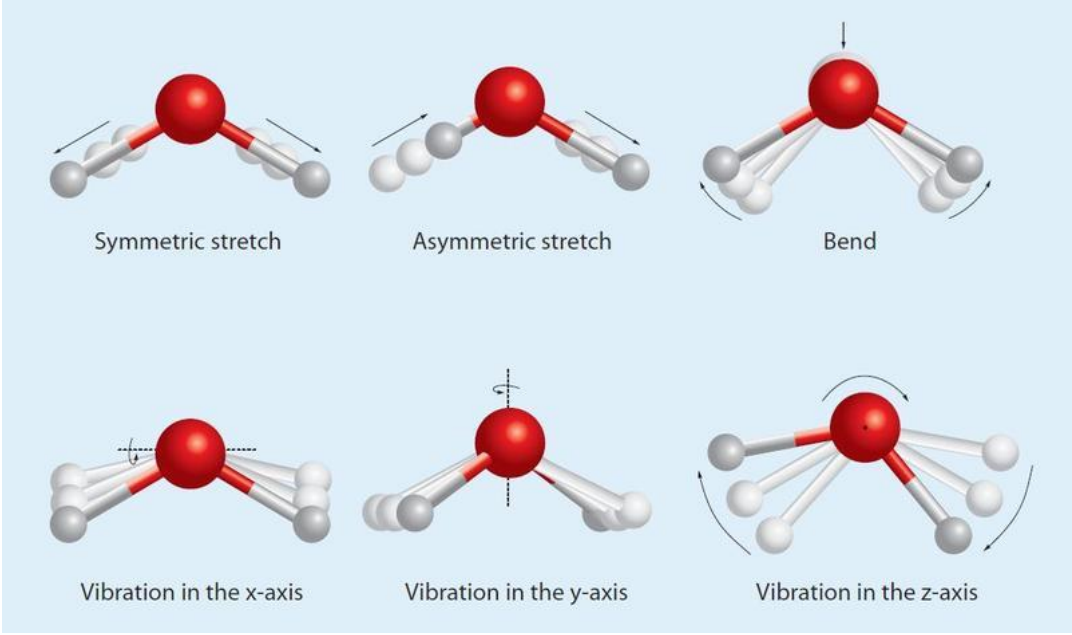


We hope to stimulate the development of more robust sun protection agents!

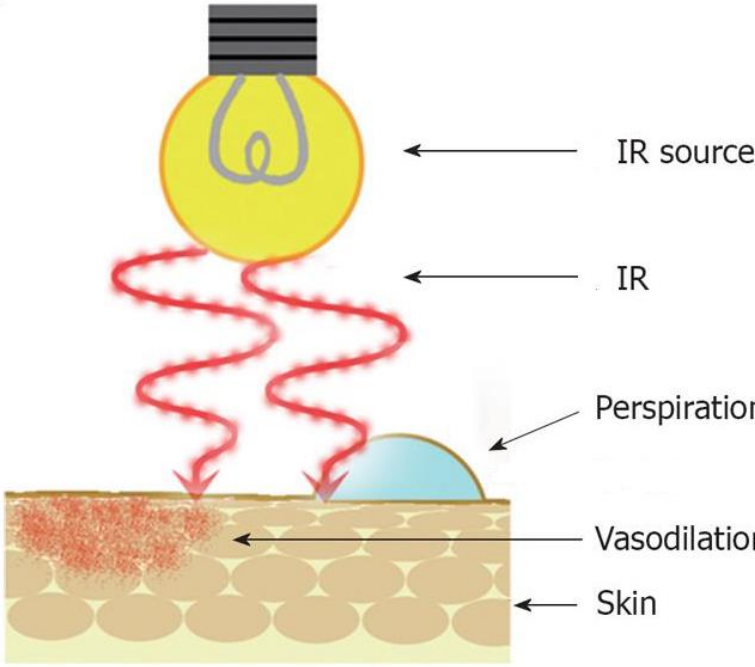
Infra-red

↓

Heat



inflammatory cellular infiltration, disrupts the dermal extracellular matrix by inducing matrix metalloproteinases, and alters dermal structural proteins, thereby adding to premature skin aging.



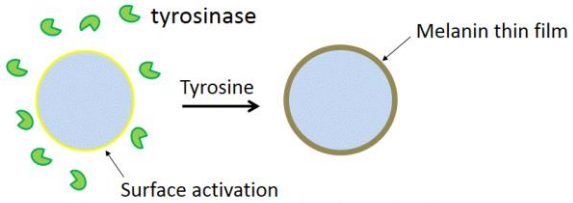
Developing strategies to protect skin against visible light

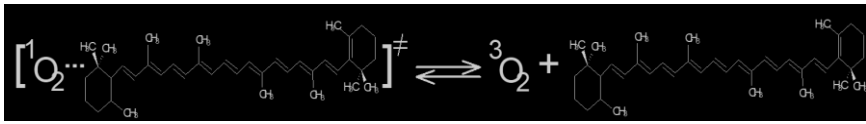
Visible light

Filters

Membrane protection

Anti-oxidant

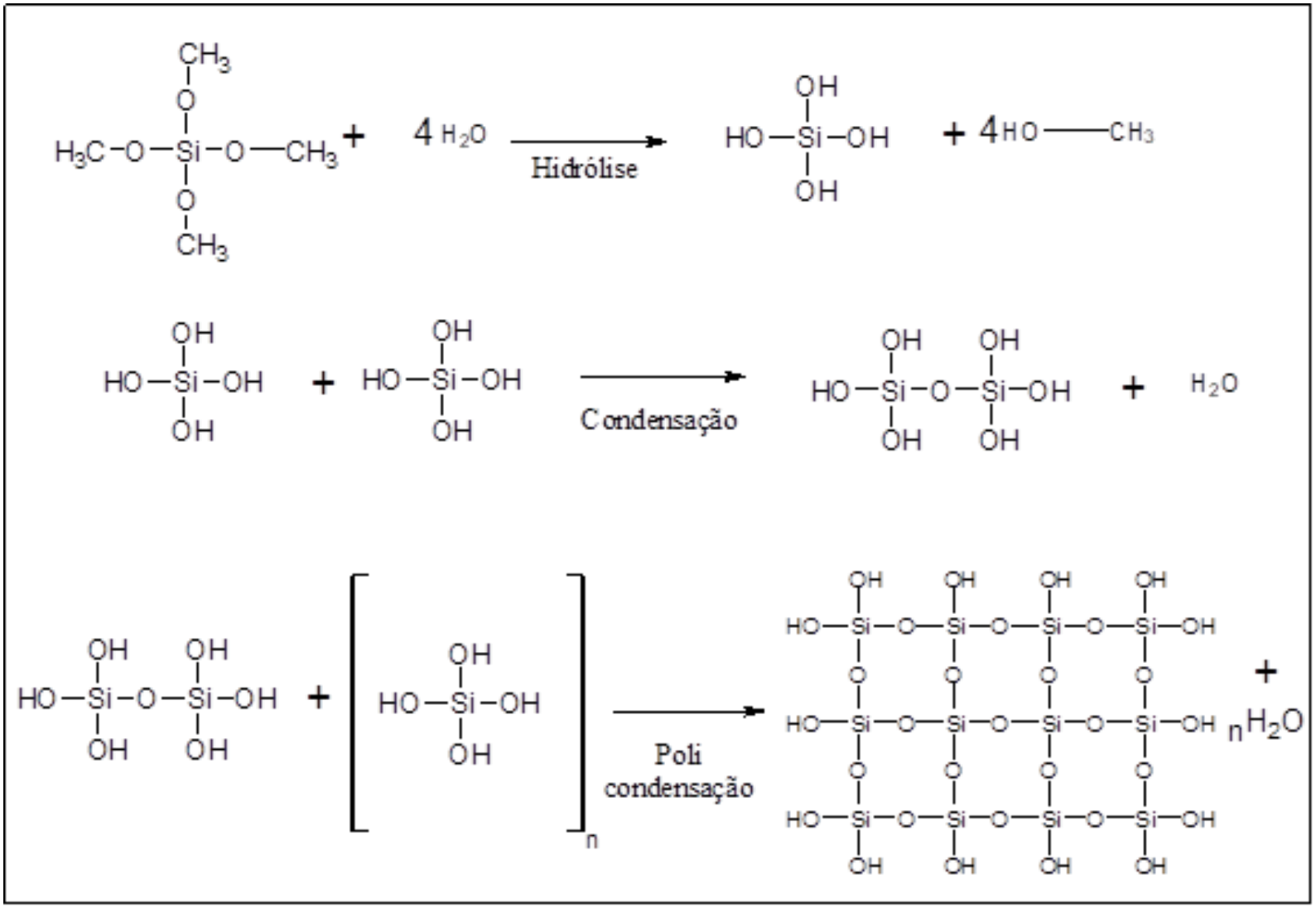




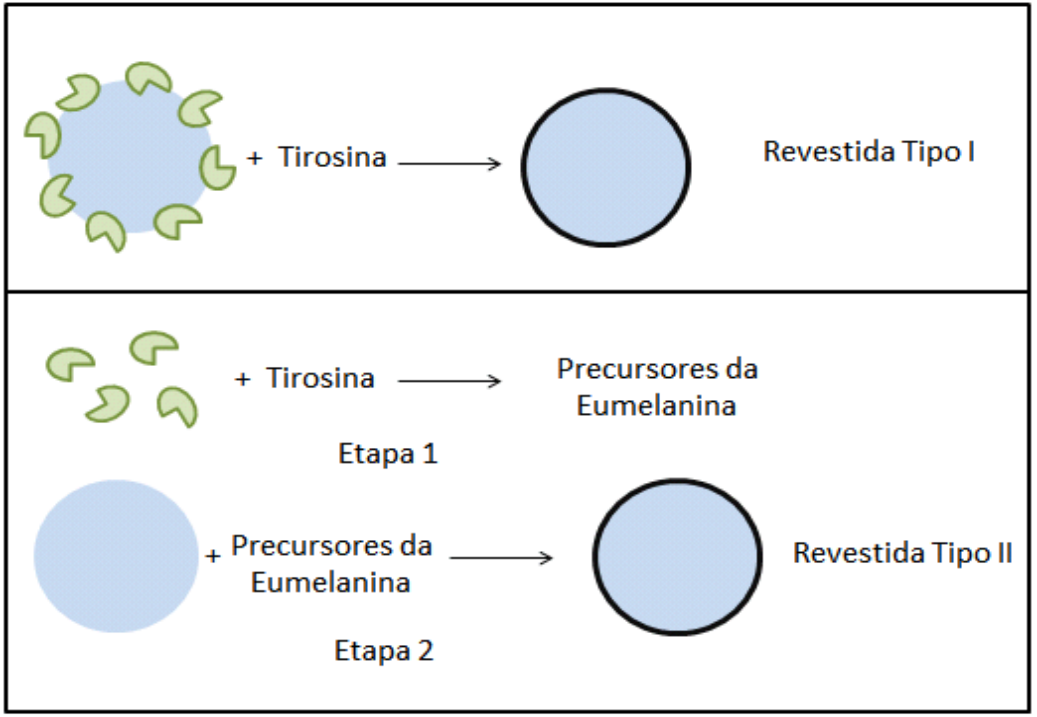
Patente de invenção: BR 10 2016 024262 2

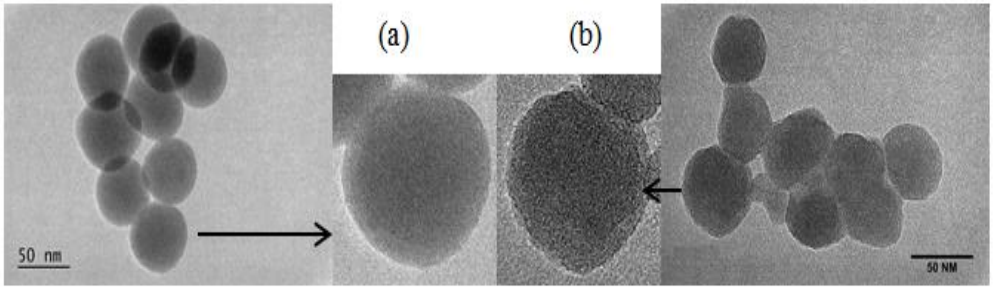
Rodrigues et al, *PPS*, 2016, 15 (3), 334-350;
Mertins et al *Biochimica et Biophysica Acta* 1848 (2015) 2180–2187

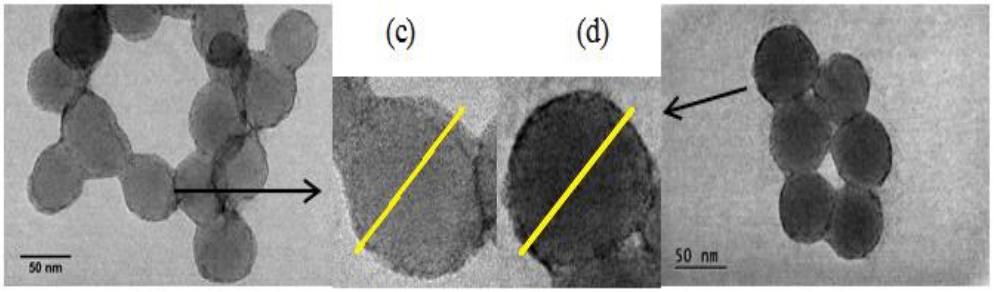
Silica nanoparticles

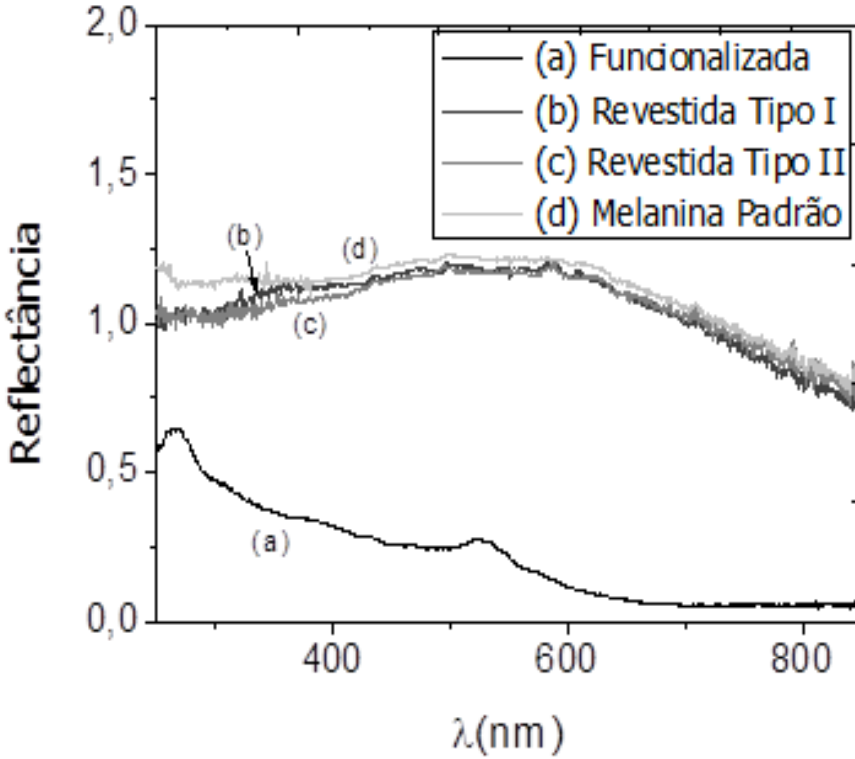


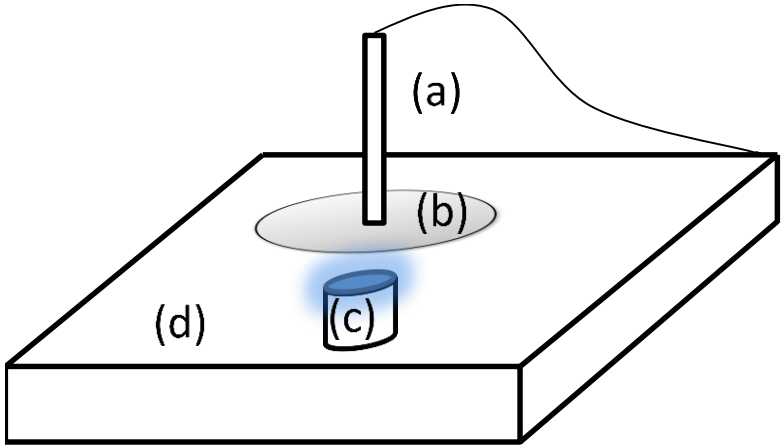
Nanofilm of melanin



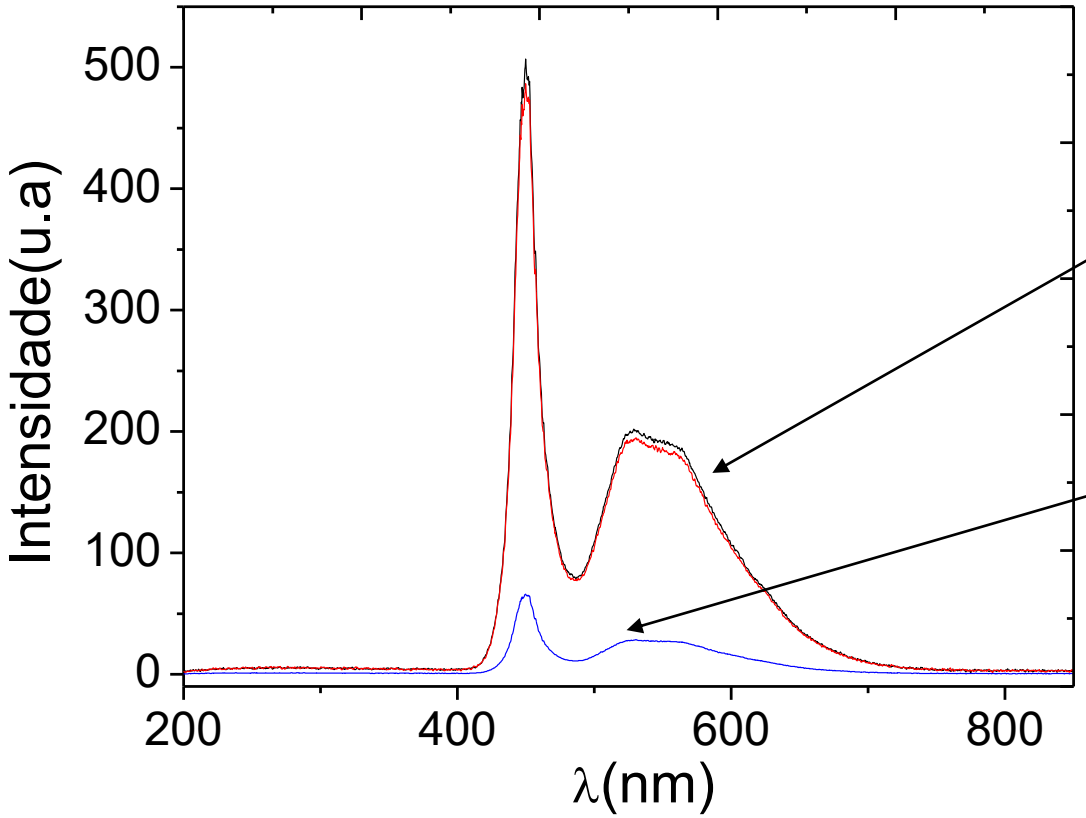








(a) Detector
(b) Amostra
(c) Fonte
(d) Suporte plano

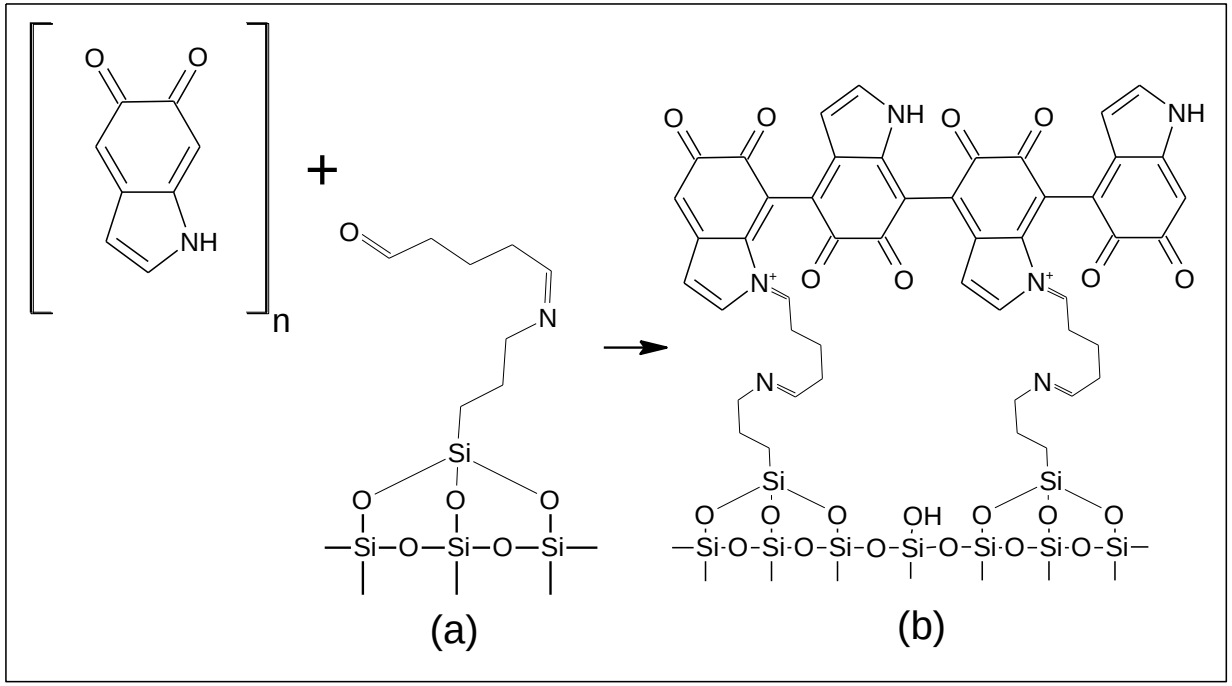


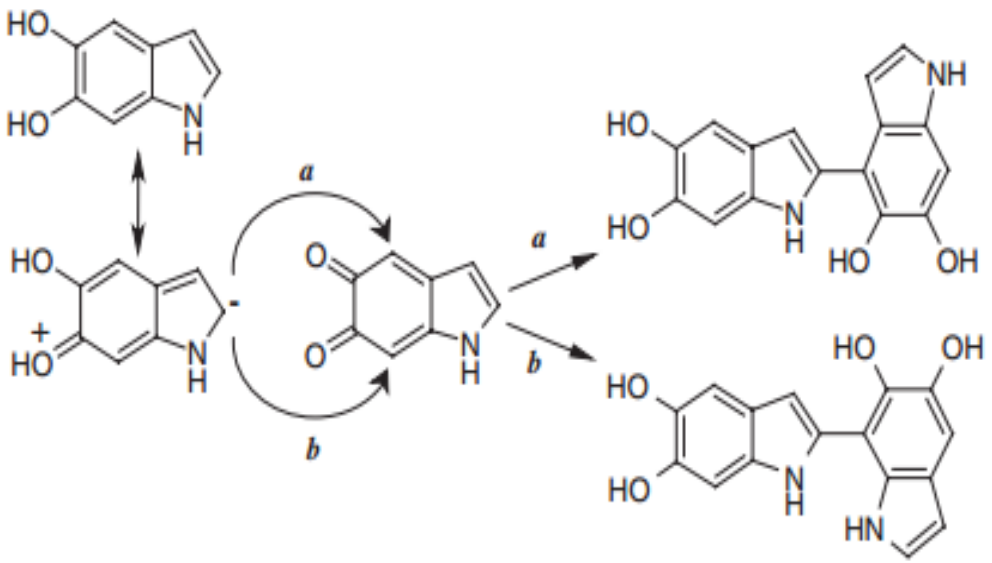
Light transmission trough filter and with sílica nanoparticles only

Light transmission By film made of ~2 mg melanina coated nanoparticles

Melanin-coated sílica nanoparticles have acceptable collors, and protect against the effects of visible light

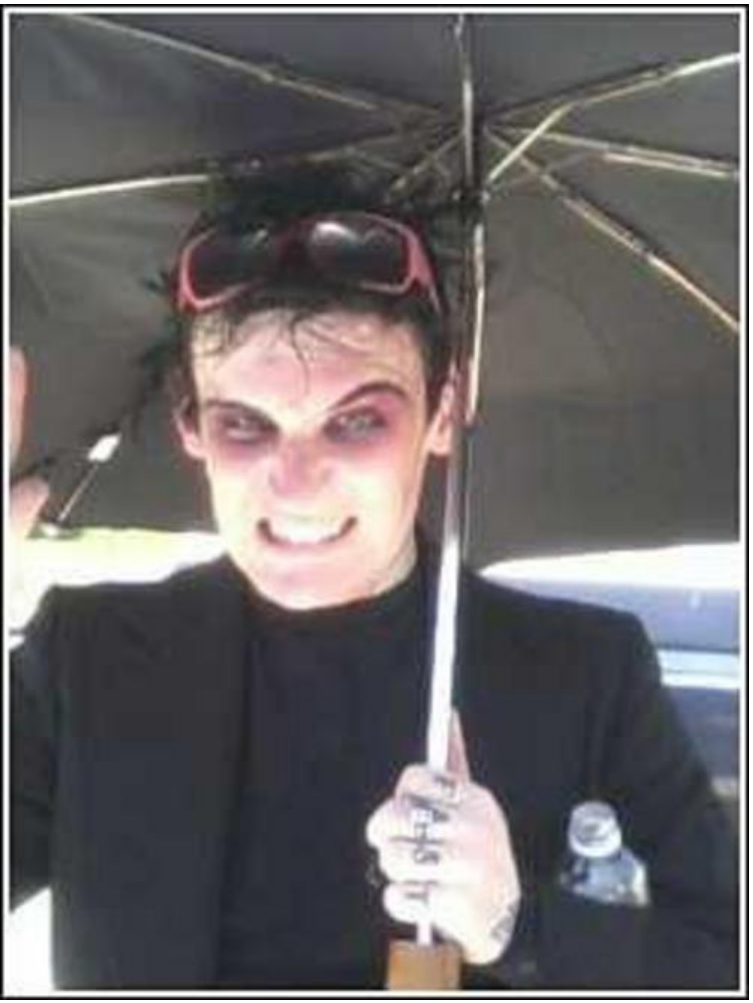
Mechanism of formation of melanin films





Patent: BR 10 2016 024262 2

Can/should we avoid completly sun exposition?



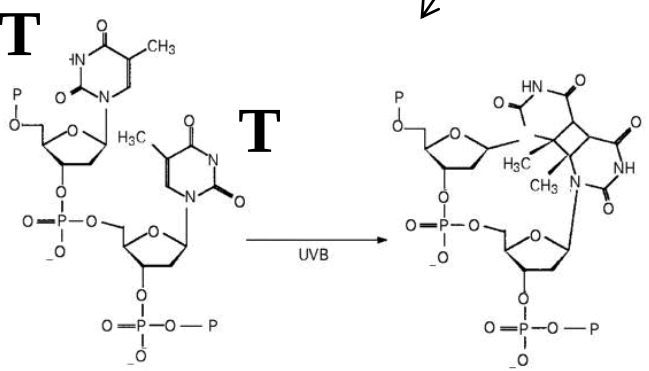
The U.S. economic burden due to vitamin D insufficiency from inadequate exposure to solar UVB irradiance… was estimated at $40-56 billion, whereas the economic burden, for excess UV irradiance was estimated at $6-7 Billion (2004).

William B. Grant, Cedric F. Garland, and Michael F. Holick *Photochemistry and Photobiology 2005, 1276.*

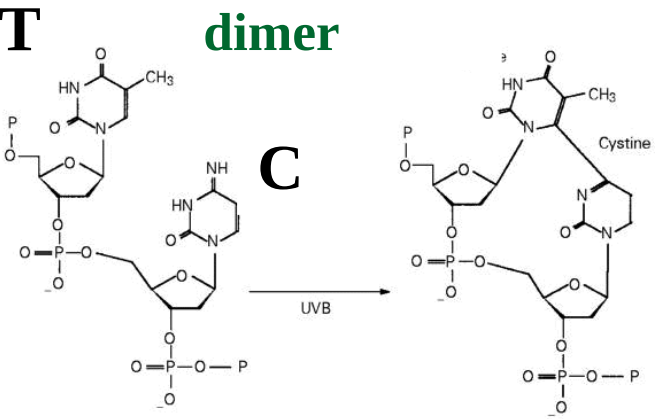
UVB (290-320nm)

Cycloaddition x **electrocyclic conrotatory** 6π

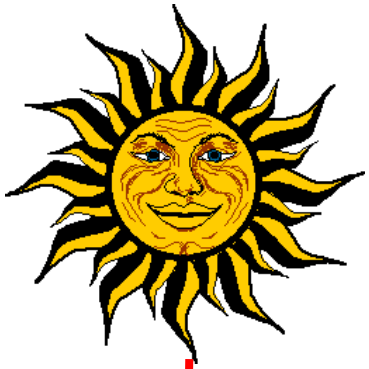
The contradiction in photobiology

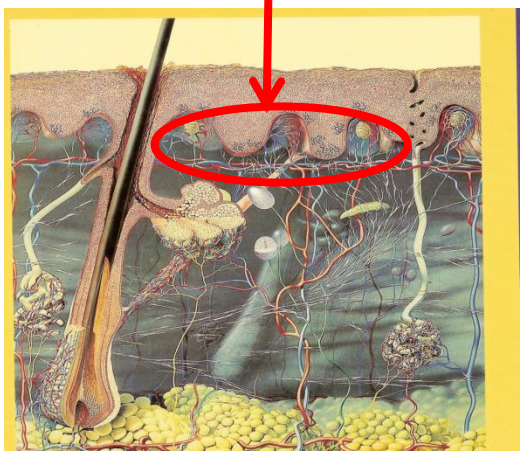


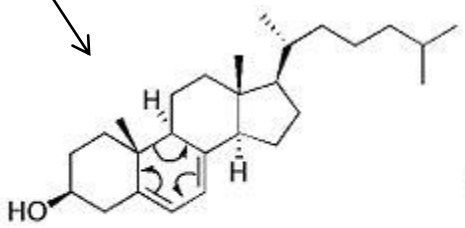
Cyclobutyl-pyrimidine dimer



Photoproduct 6-4

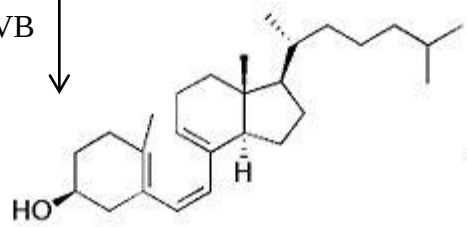






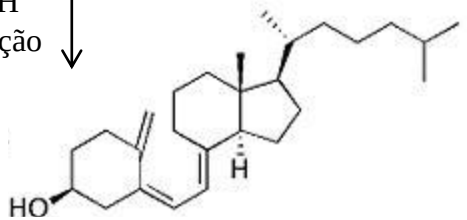
7-dehidrocolesterol

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.

Utilização de filtro solar evita a produção de vitamina D

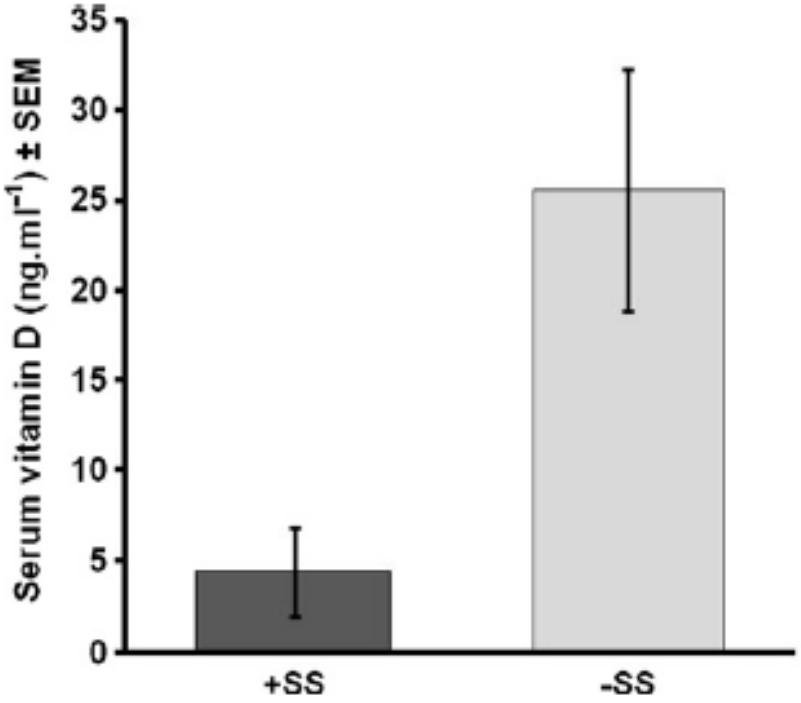


Fig. 4. Effect of sunscreen use on circulating vitamin D status in a laboratory study after a single whole body exposure to 1MED UVB. From Matsuoka et al. [62]. Multiply by 2.599 to convert ng ml^{-1} to nmol l^{-1} (see baseline data in Table 3).

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