TOPICAL APPLICATION OF NITRIC OXIDE-RELEASING NANOPARTICLES COMBINED WITH UV IRRADIATION ENHANCE NITROGEN OXIDES STORES IN HUMAN EPIDERMIS

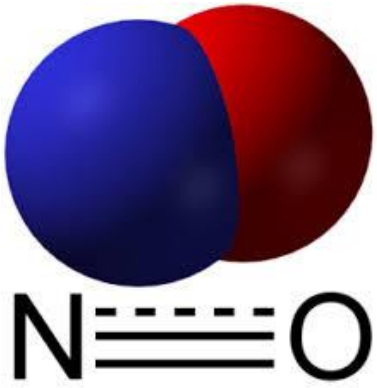
Milena Trevisan Pelegrino

M. T. Pelegrino[1,2], R. Weller[3], X. Chen[3], J. S. Bernardes[4] e A. B. Seabra[1,2]

[1]Universidade Federal de São Paulo; [2]Universidade Federal do ABC;
[3]University of Edinburgh; [4] Laboratório Nacional de Nanotecnologia

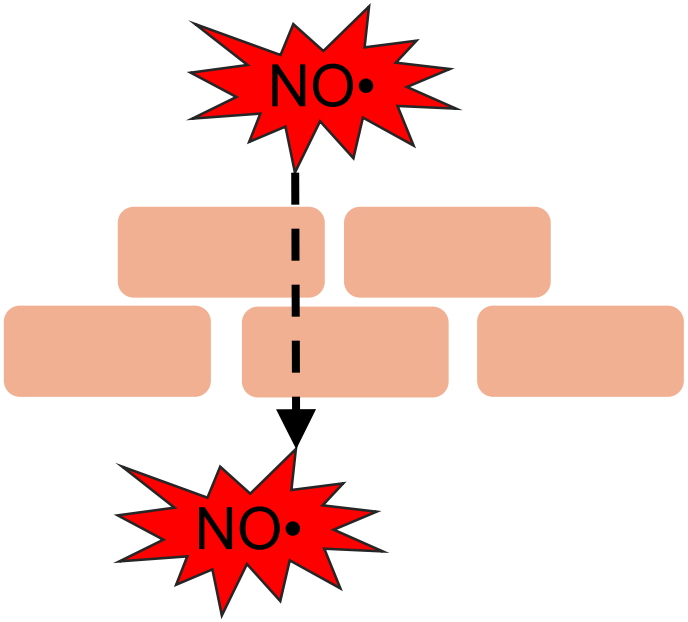
Nitric Oxide (NO)

- Small molecule
- Instable
- Lipophilic behavior



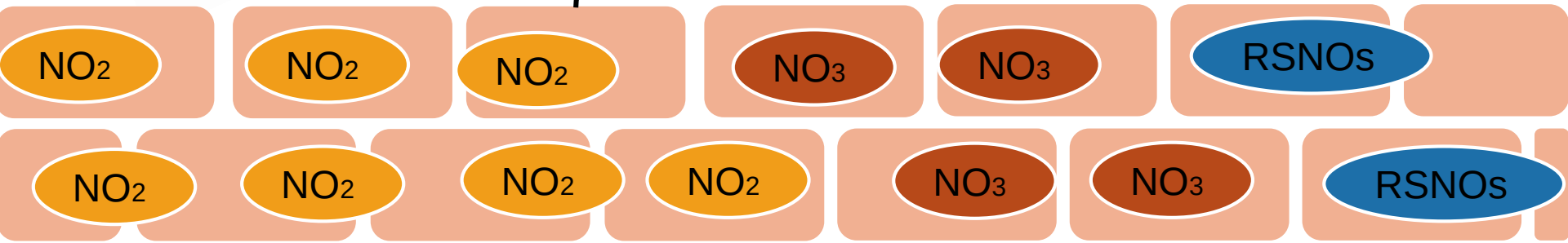
Physiologic processes

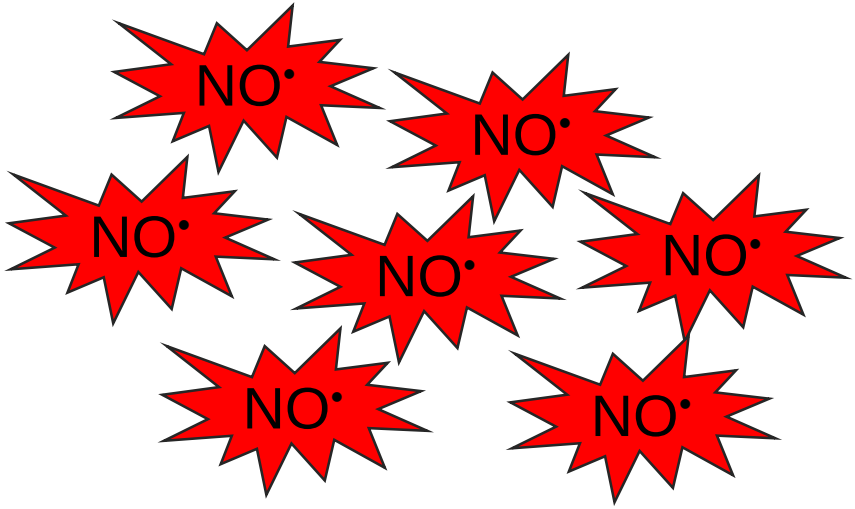
- Vasodilation
- Wound healing
- Immune response
- Antioxidant and anticancer activities

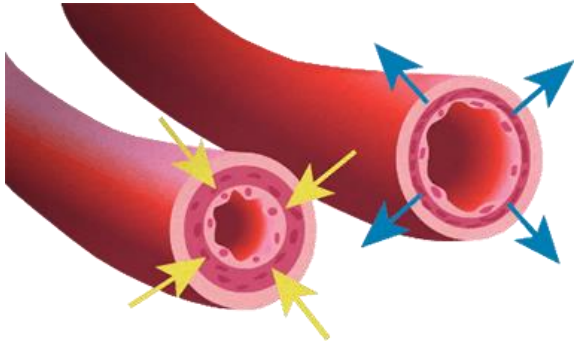


(CARDOZO *et al.*, 2014; SEABRA, et al., 2015)

NO storage in human skin







Mechanism capable of modulating the NO bioactivity in the bloodstream

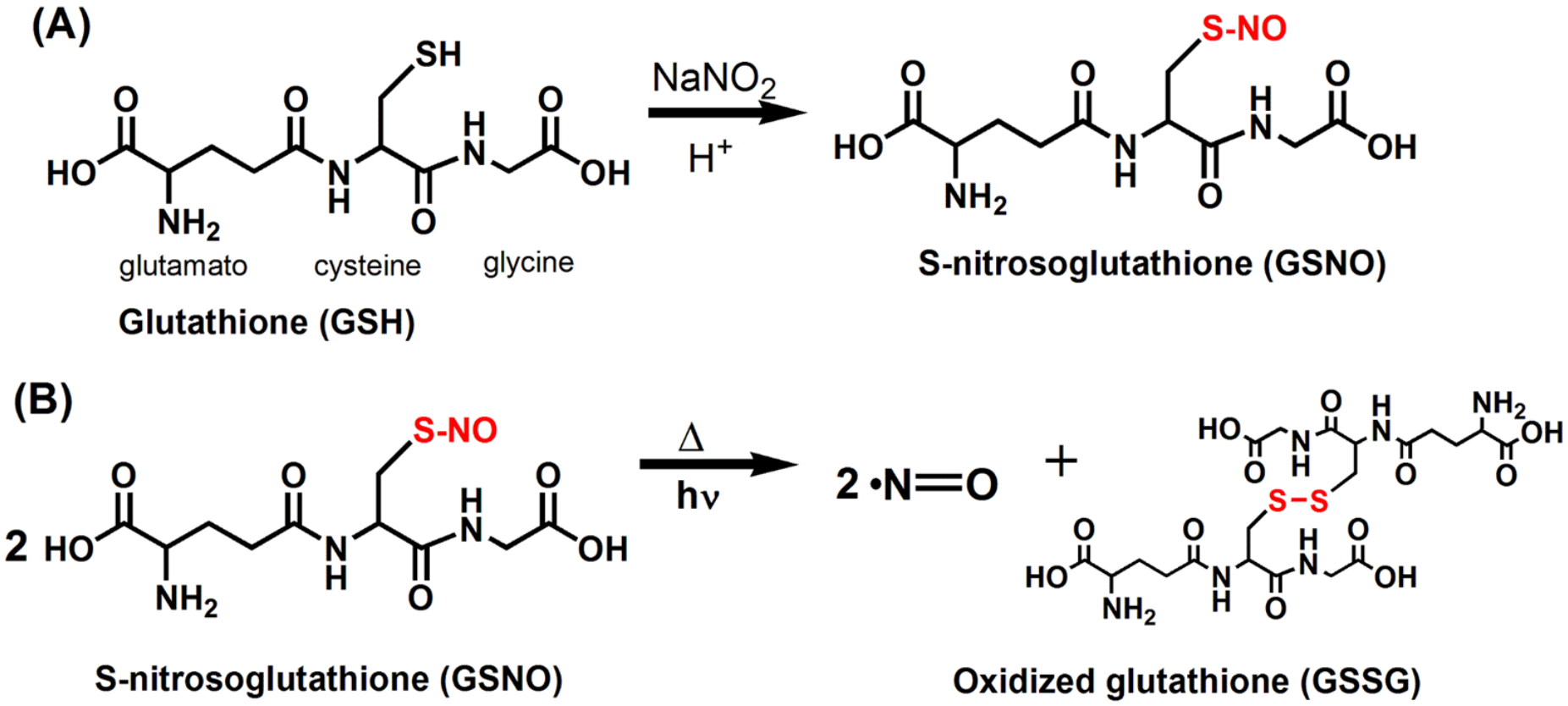
(MALINSKI et al., 1993; MOWBRAY et al., 2008, 2009)

Leading cause of global mortality

The decrease of the blood pressure *ca.* of 10 mmHg → Decrease the risk for stroke by 56% and for coronary heart disease by 37%

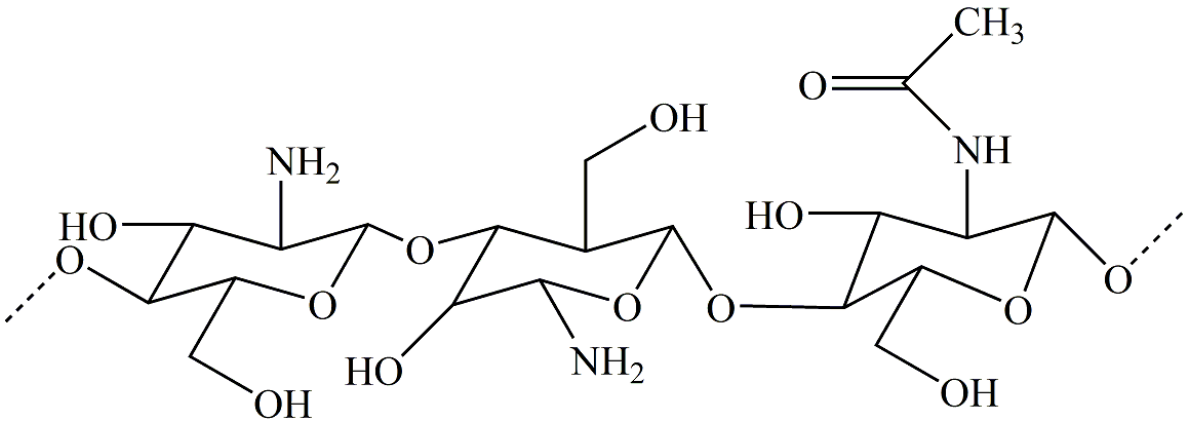
(SANTULLI, 2013; GO et al., 2014; WELLER et al. 2016)

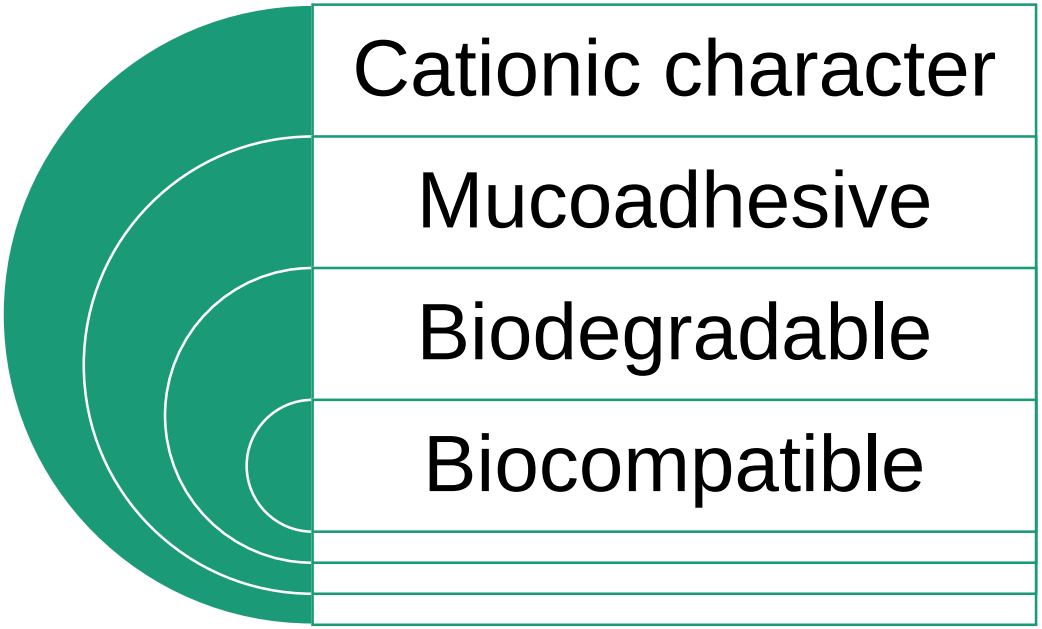
Nitrosothiols (RSNOs)



(BRANDT et al., 2014; LIU et al., 2014; WANG, 2012)

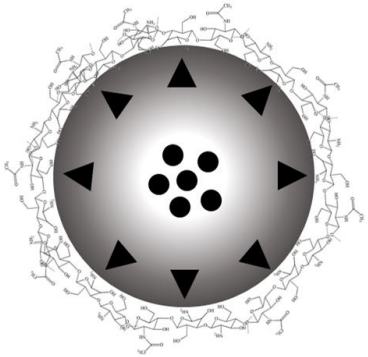
Chitosan nanoparticle



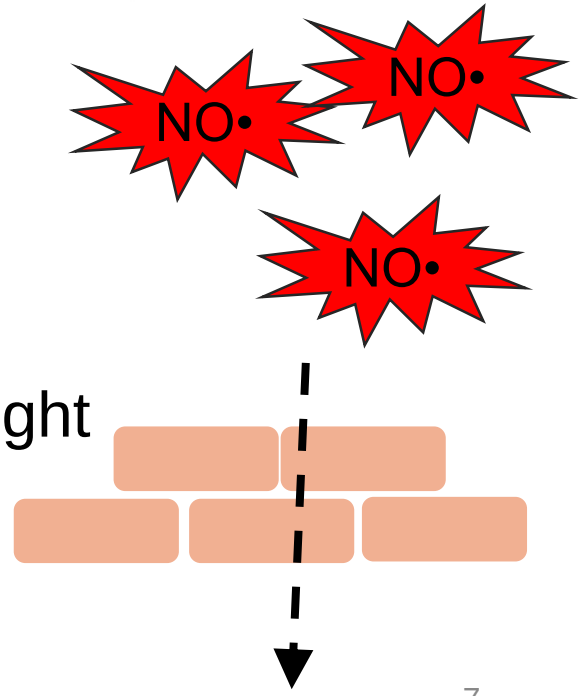


Goal

- Synthesize and characterize NO-releasing chitosan nanoparticles;

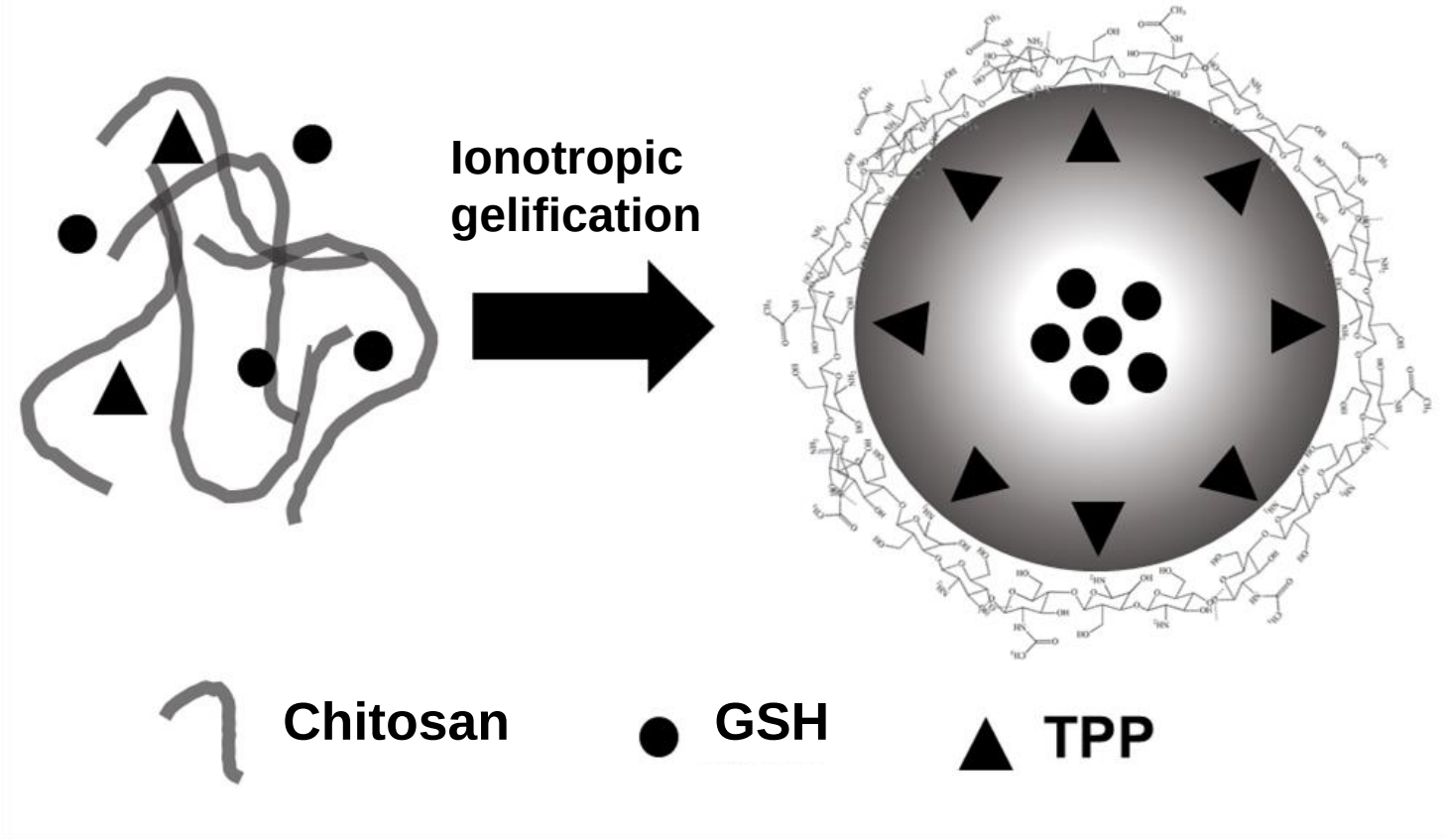


- Evaluate the kinetics profiles of NO release from chitosan nanoparticles using *in vitro* (Franz Cell) and *ex vivo* methods;



- Investigate the influence of the ultraviolet light in the NO release from chitosan nanoparticles.

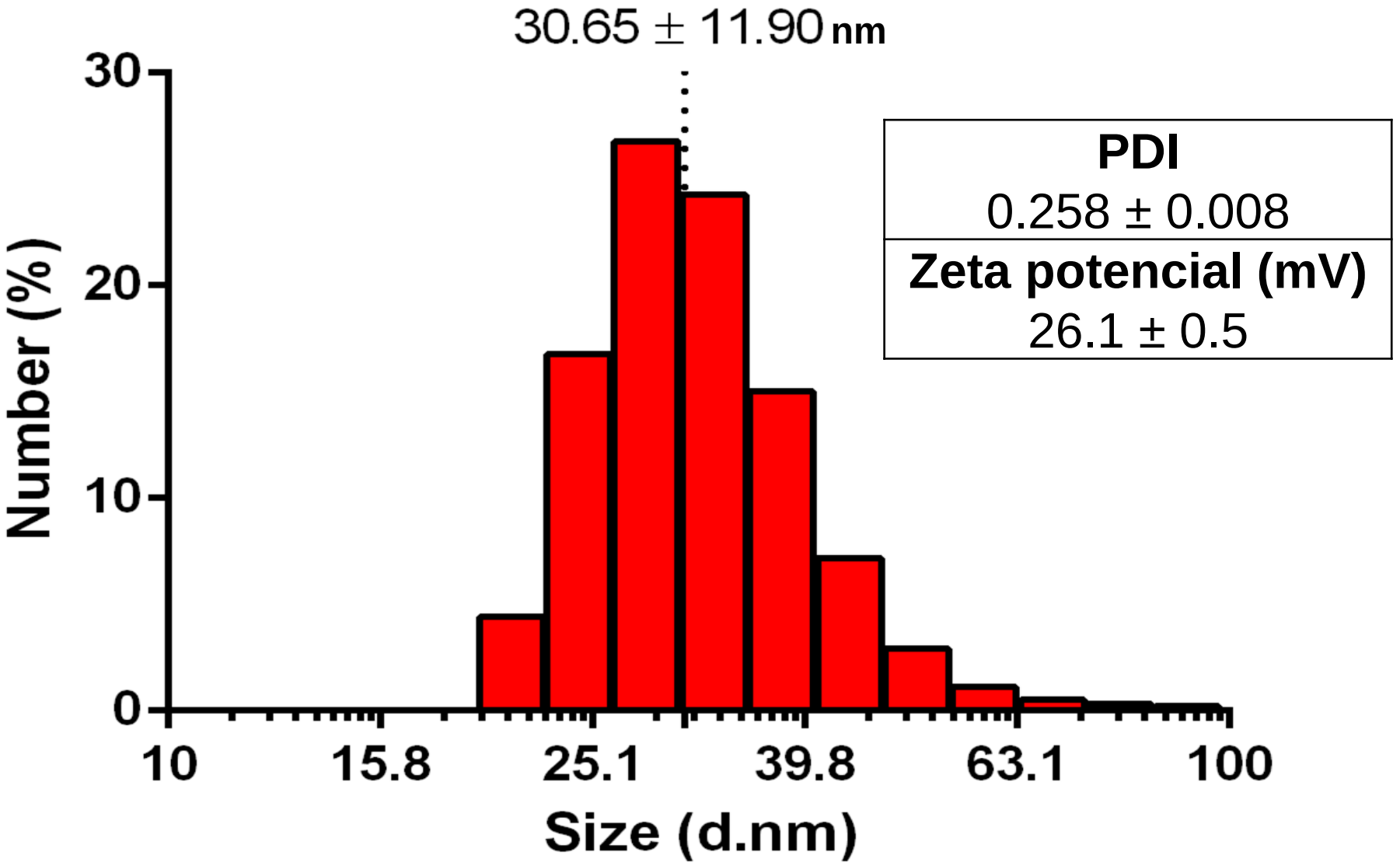
Synthesis of chitosan nanoparticles (CS NPs)



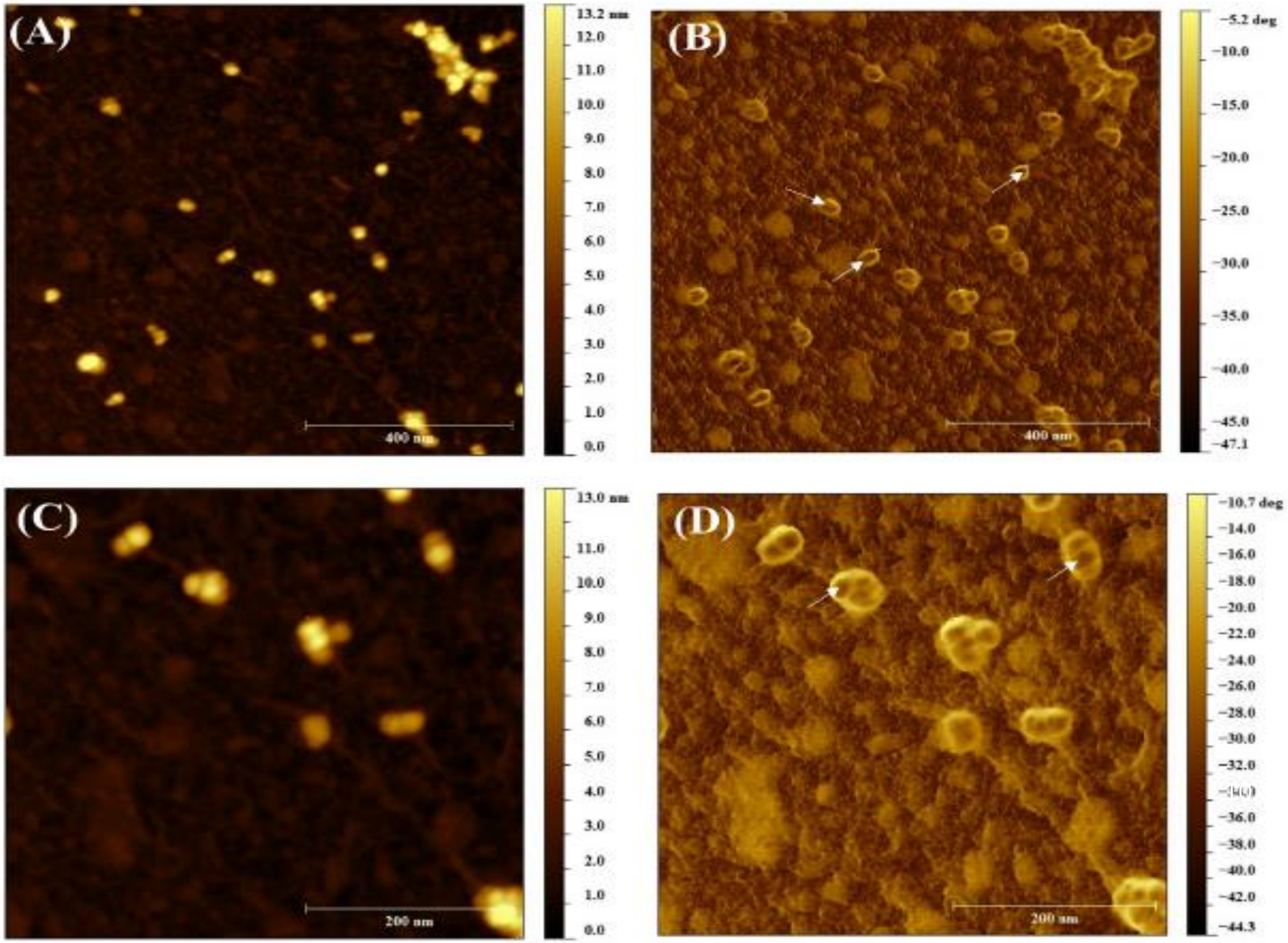
Encapsulation efficiency (EE%) 99.60 ± 0.01

(Tripathy et al., 2012)

Dynamic light scattering (DLS)

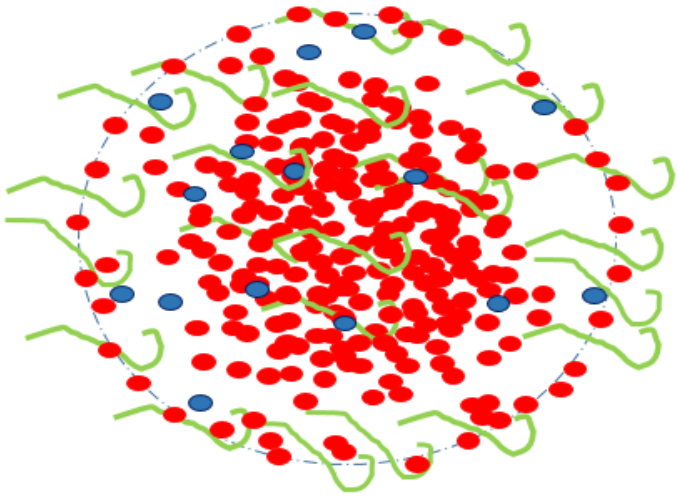


Atomic Force Microscopy (AFM)

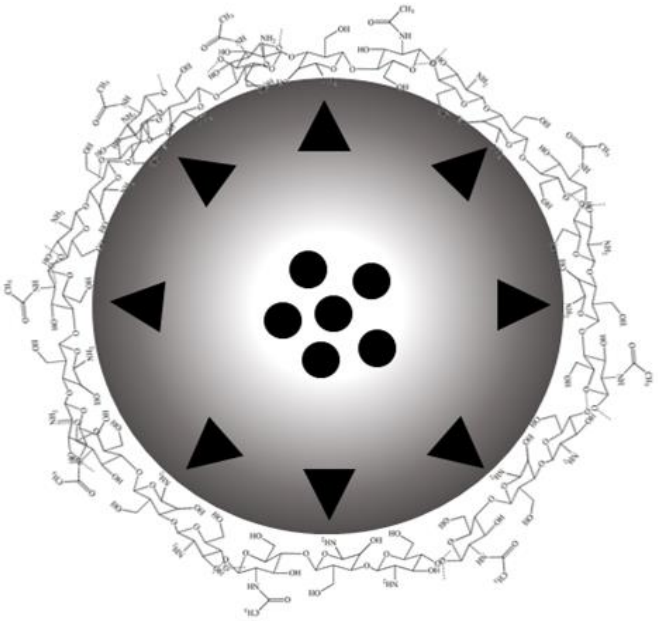


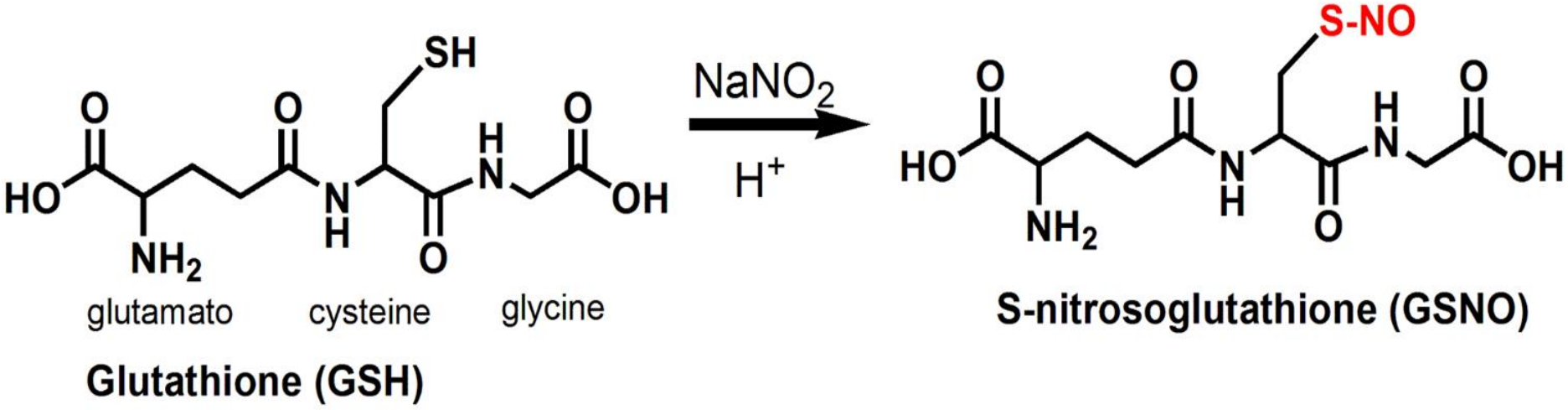
X-ray photoelectron spectroscopy (XPS)

- **Glutathione (GSH)**
- **Sodium tripolyphosphate (TPP)**
- **Chitosan**



Molar ratio	Total	Surface
Chitosan/TPP	3.5	7.8
GSH/Chitosan	17.5	3.29
GSH/TPP	60.5	25.93

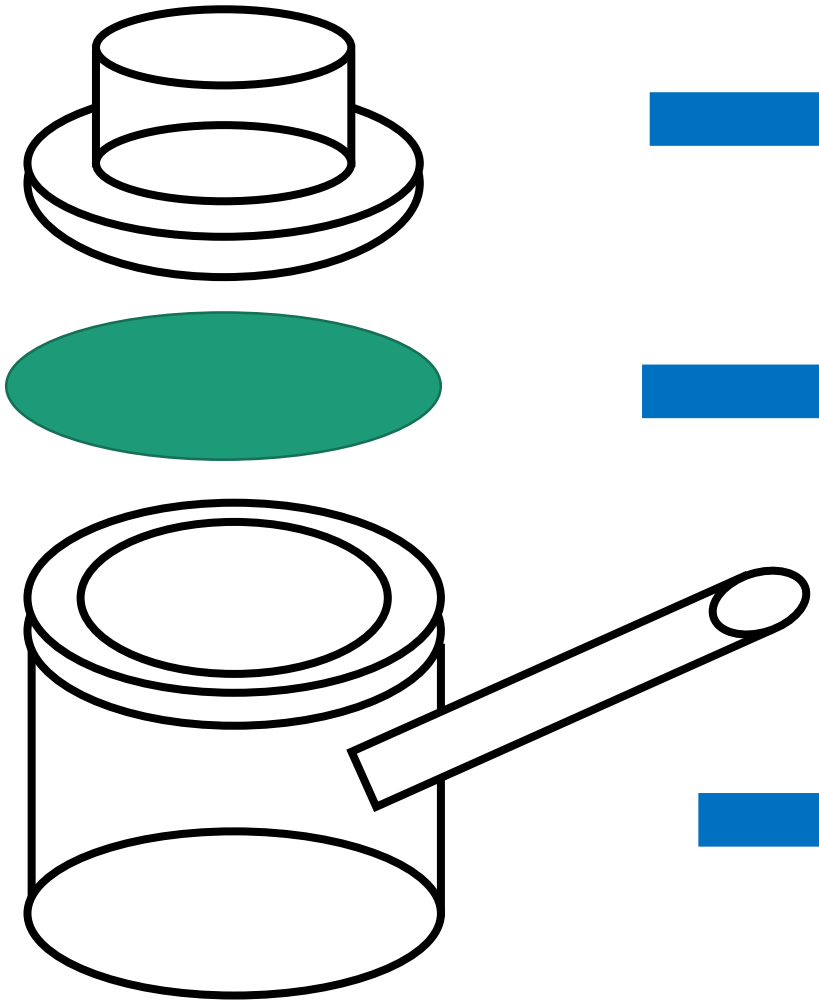




Glutathione (GSH)

S-nitrosoglutathione (GSNO)

In vitro GSNO release using a Franz cell

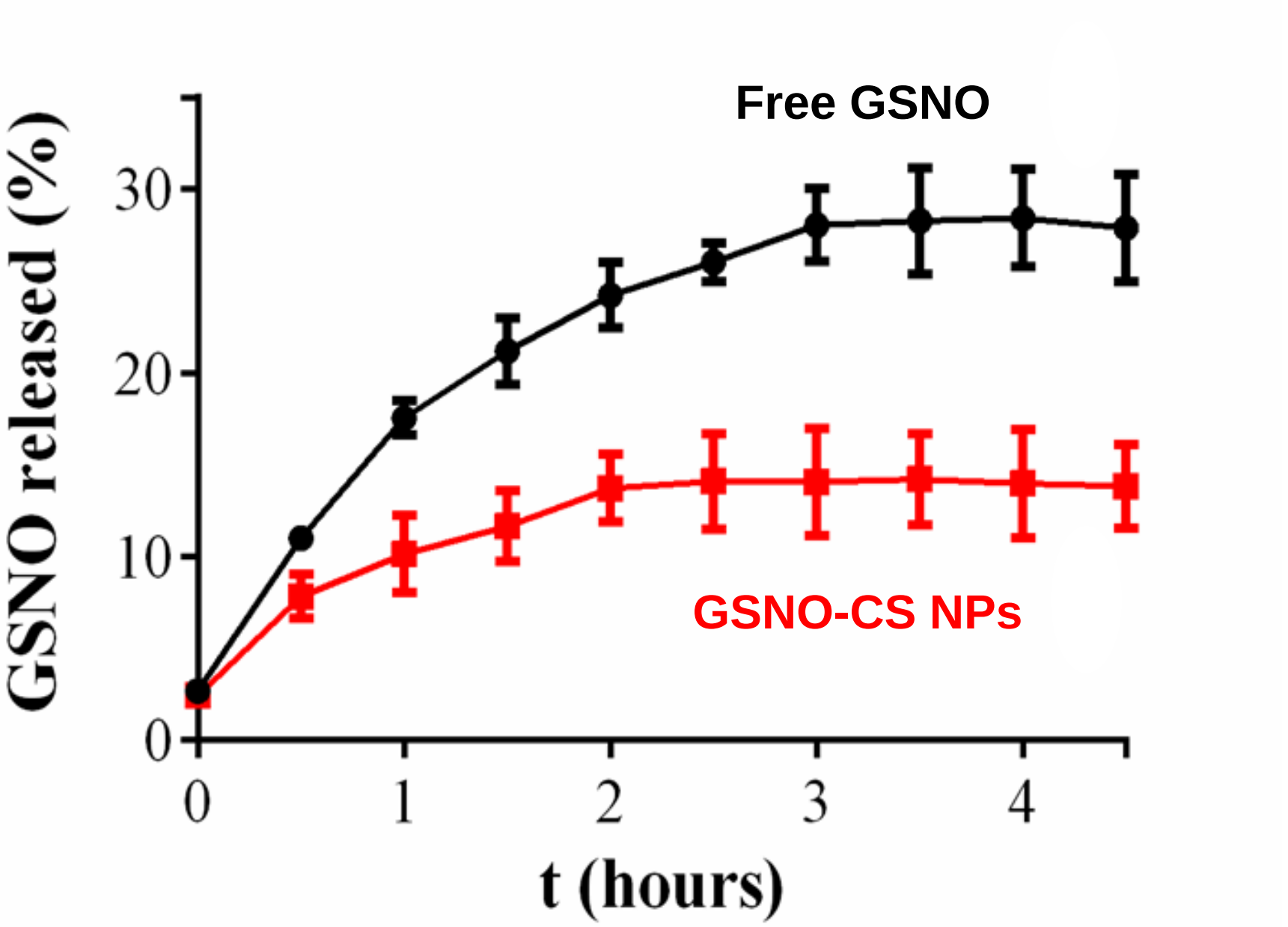


Donor chamber

Membrane

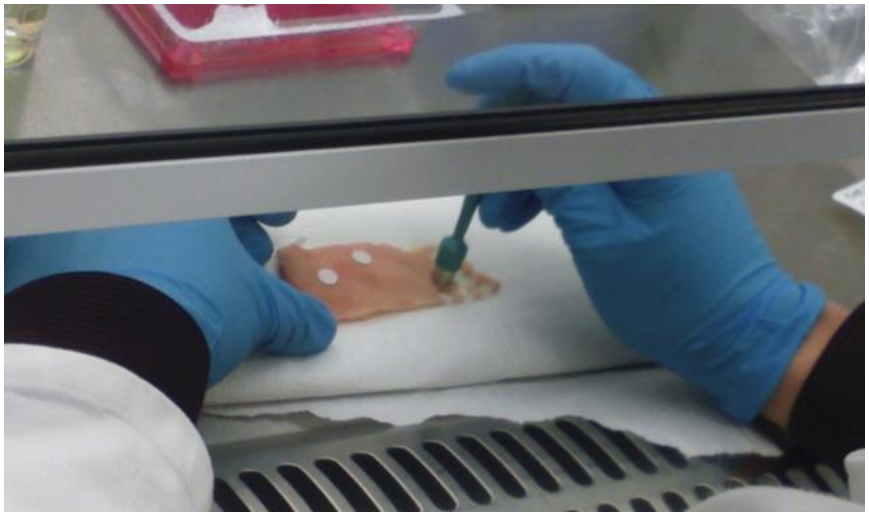
Samples each 30 min

Receptor chamber


Free GSNO
GSNO-CS NPs
GSNO released (%)
30
20
10
0
0
1
2
3
4
t (hours)

Ex vivo NO permeation

THE UNIVERSITY of EDINBURGH



Human skin section from the department of plastic surgery and incubated with DAF-2DA, an NO chromophore.

NPs (GSNO = 100 mmolL)

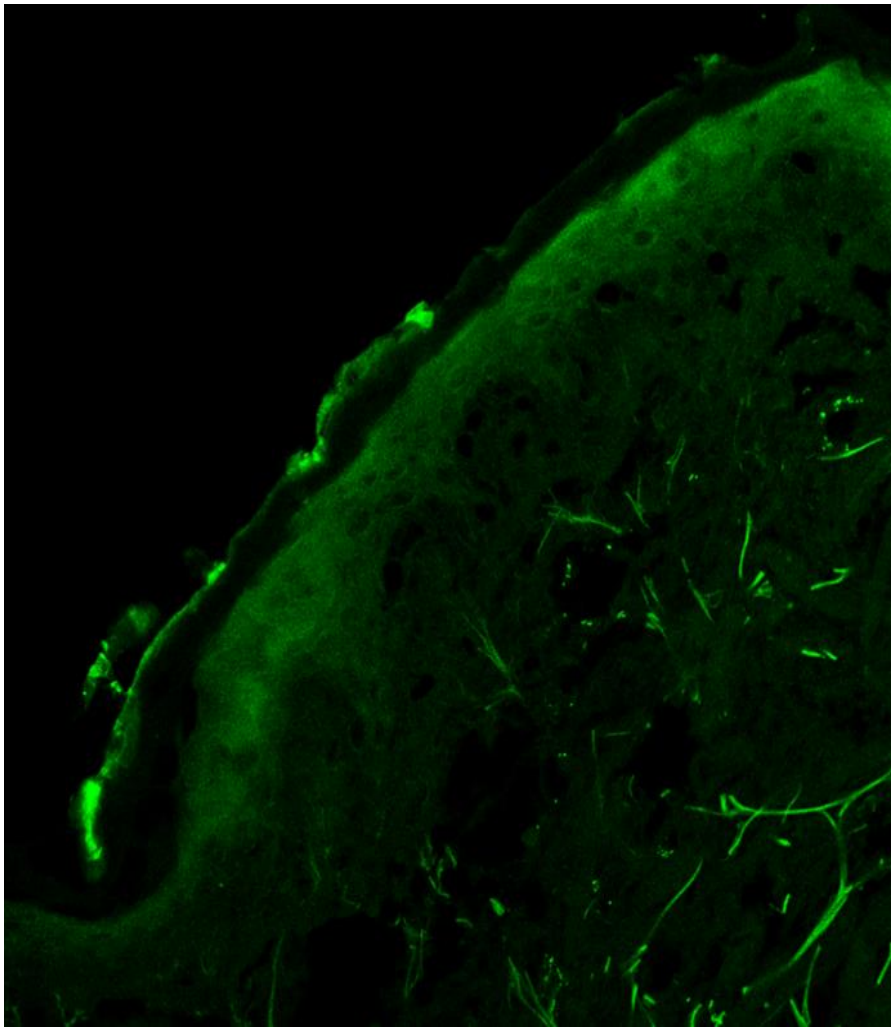
Skin

Dark condition

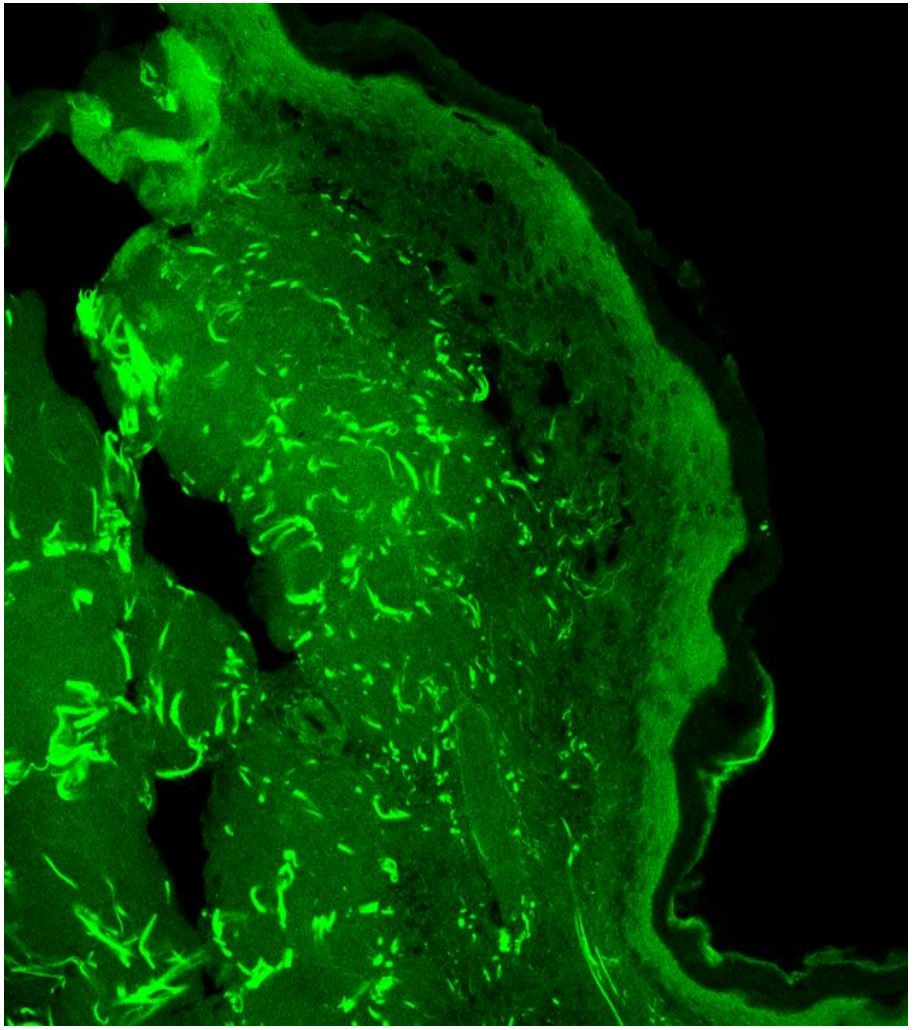
Irradiated with
UV light for 20 min
(λ = 290 nm)

NO permeated throught human skin: confocal microscopy

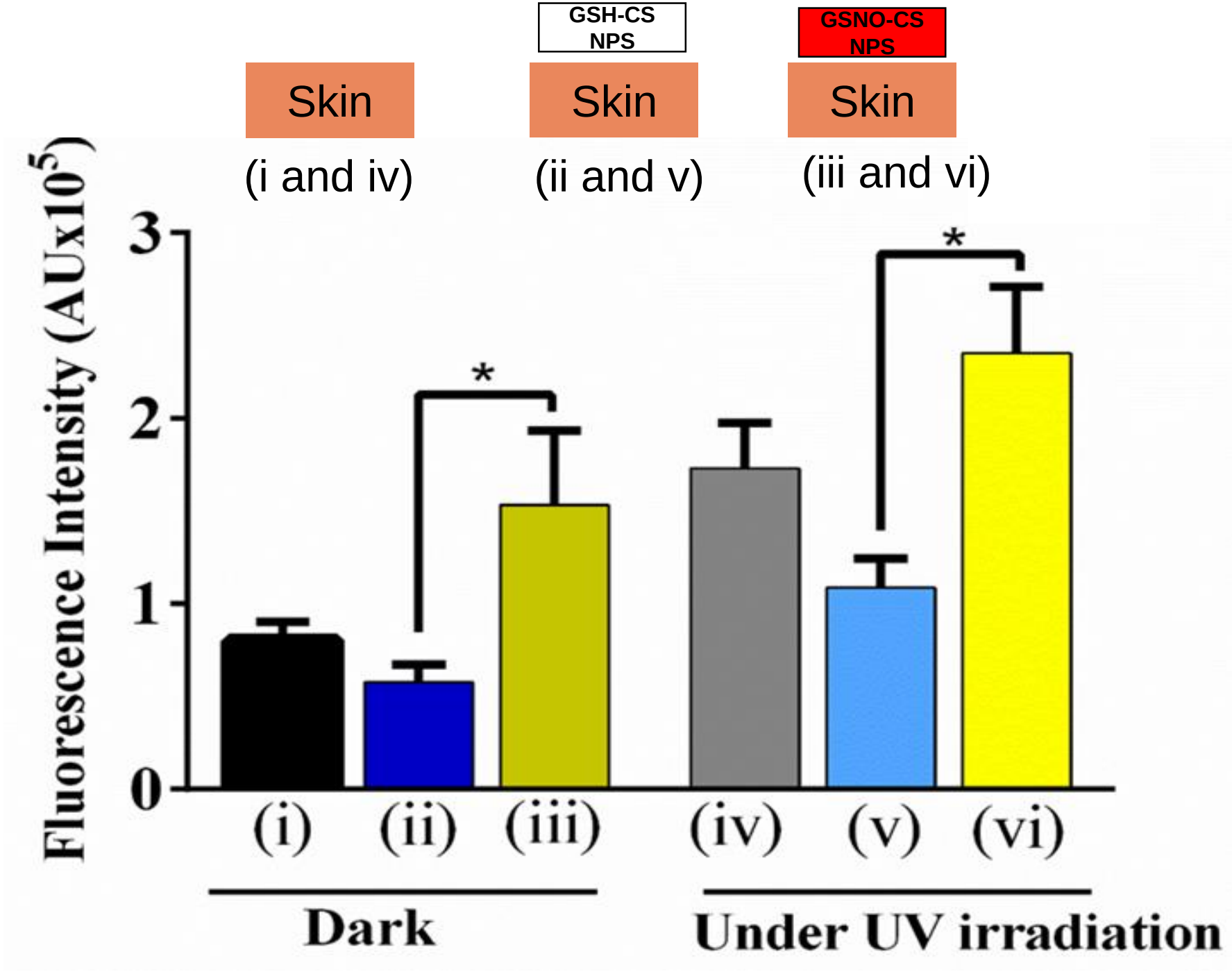
Green signal = NO derivatives



Control



NO-releasing CS NPs


GSH-CS NPS
GSNO-CS NPS
Skin
Skin
Skin
(i and iv)
(ii and v)
(iii and vi)
Fluorescence Intensity (AUx10^5)
3
2
1
0
*
*
(i)
(ii)
(iii)
(iv)
(v)
(vi)
Dark
Under UV irradiation

Conclusions

- GSH-CS NPs consist of a core-shell structure;
- The incorporation of GSNO into chitosan nanoparticles modulates the NO release;
- Topical application of GSNO-CS NPs significantly increase the levels of NO in the epidermis;
- In addition, the combination of cutaneous administration of GSNO-CS NPs followed by skin UV irradiation further enhanced the NO and its derivatives loaded in human skin.

Acknowledgments

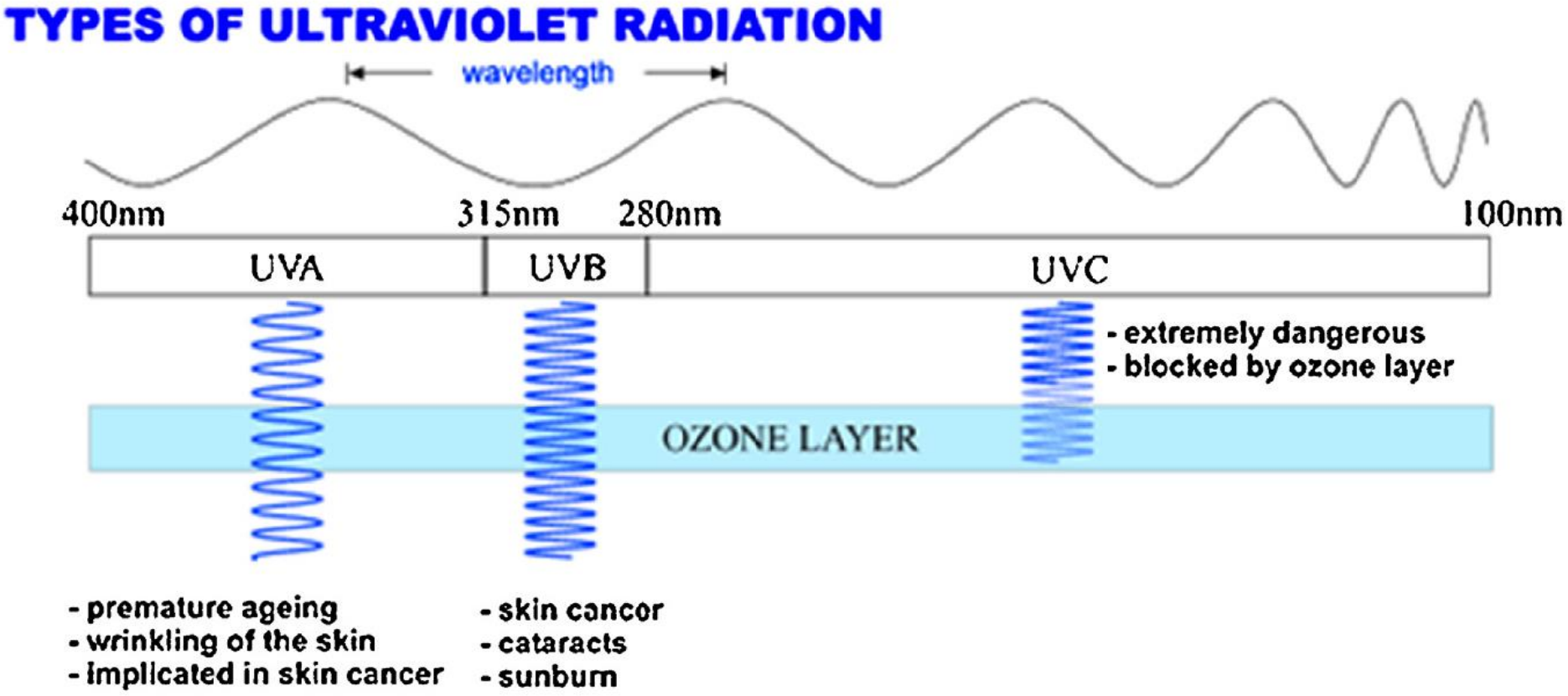
2015/00393-8

Brazilian Network on Nanotoxicology (552120/2011-1)

Newton Advanced Fellowship (The Royal Society NA140046)

Collaborators
Dr. Richard Weller
Drª. Juliana Bernardes

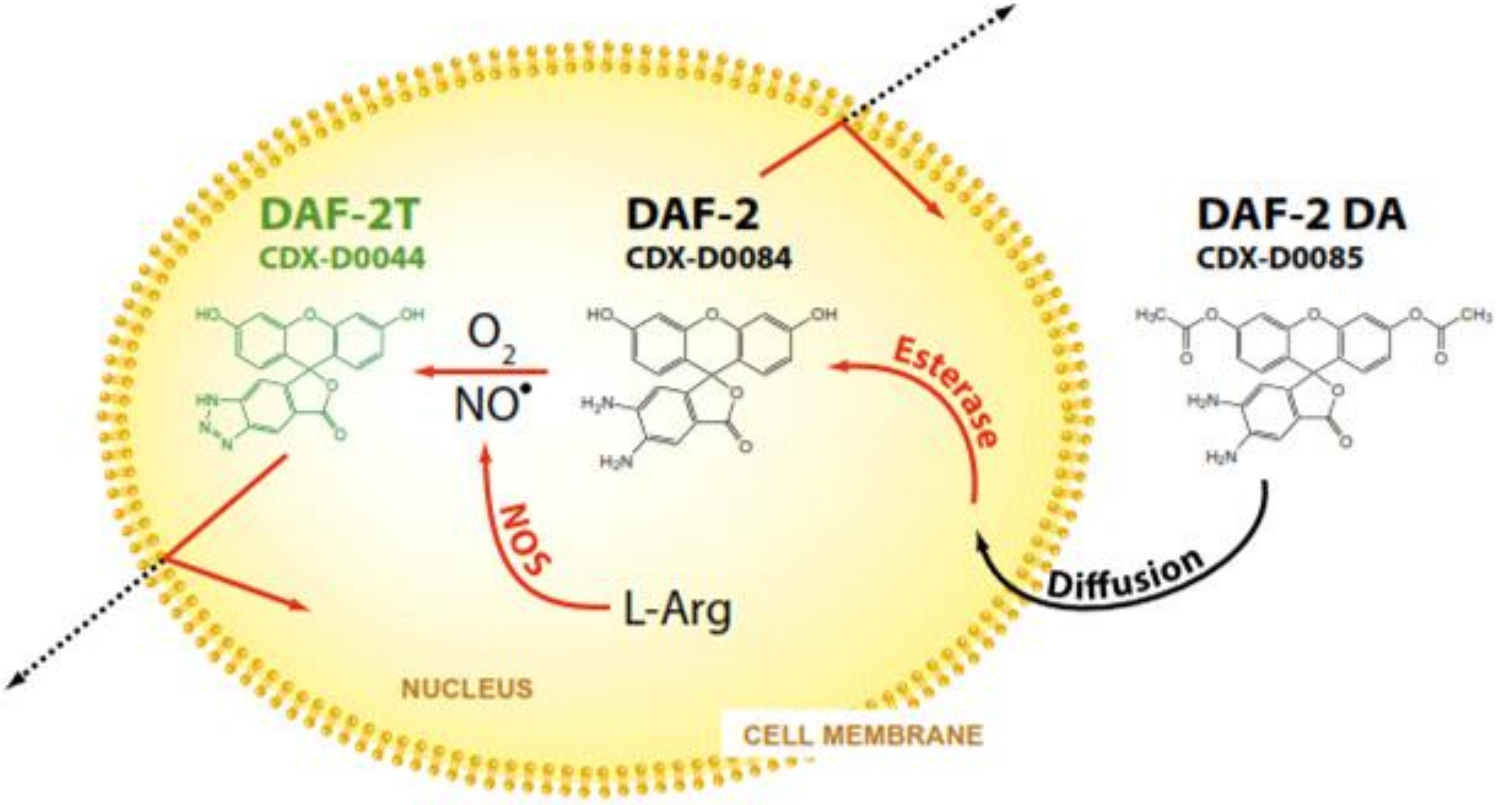
	Higuchi		Kosmeyer-Peppas			Hixson-Crowell	
	R^2	K_H (%·$h^{-1/2}$)	R^2	n	K_k (%·h^{-n})	R^2	K_s (%·$h^{-1/3}$)
GSNO	0.989	17.711	0.984	0.516	3.281	0.855	-4.144
GSNO-CS NPs	0.996	8.772	0.974	0.350	2.239	0.826	-2.564



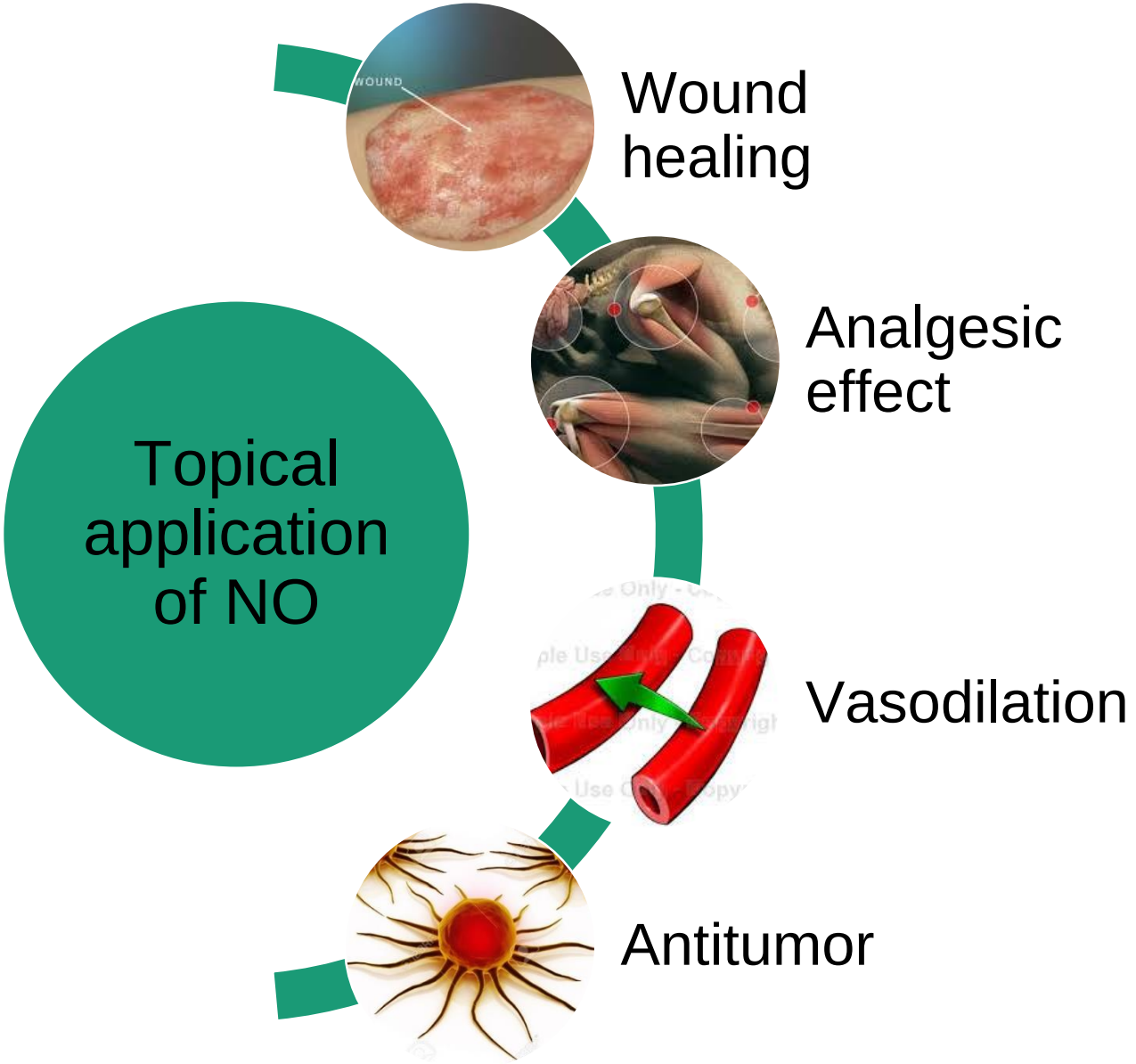
Vitamina D

(WRIGHT; WELLER, 2015)

DAF-2DA


DAF-2T
CDX-D0044
DAF-2
CDX-D0084
DAF-2 DA
CDX-D0085
O_2
$NO^{\bullet}$
Esterase
NOS
L-Arg
Diffusion
NUCLEUS
CELL MEMBRANE

While best known for its important signaling functions in human physiology, NO is also of considerable therapeutic interest



(VERCELINO, 2013; SEABRA, 2004,2007,2008 ;SCHANUEL, 2015 and CHAUDHARY et al. 2013)