

# 1<sup>st</sup> Workshop - Novel Compounds from Renewable Sources

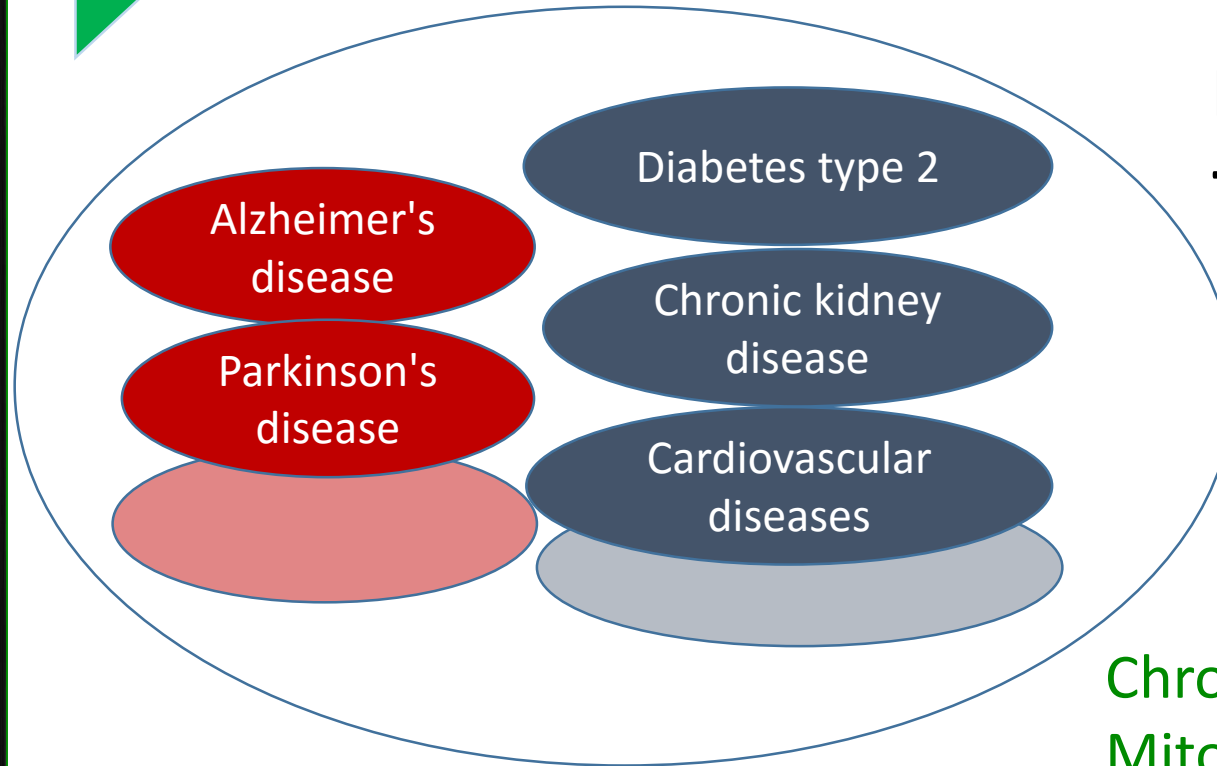


## Drug Discovery

Romeu A. Videira, Fátima Fernandes, Patrícia Valentão, Andreia P. Oliveira, M. Figueiredo González,  
João Bernardo, Daniela Correia, Rui Gonçalves, David M. Pereira, Paula B. Andrade



## AGE-RELATED DEGENERATIVE DISEASES - THE GREAT CHALLENGE OF OUR TIME



**How we can address this challenge?**

**What is common at all age-related degenerative diseases?**

Chronic low-grade inflammation  
Mitochondrial dysfunctions  
Oxidative stress

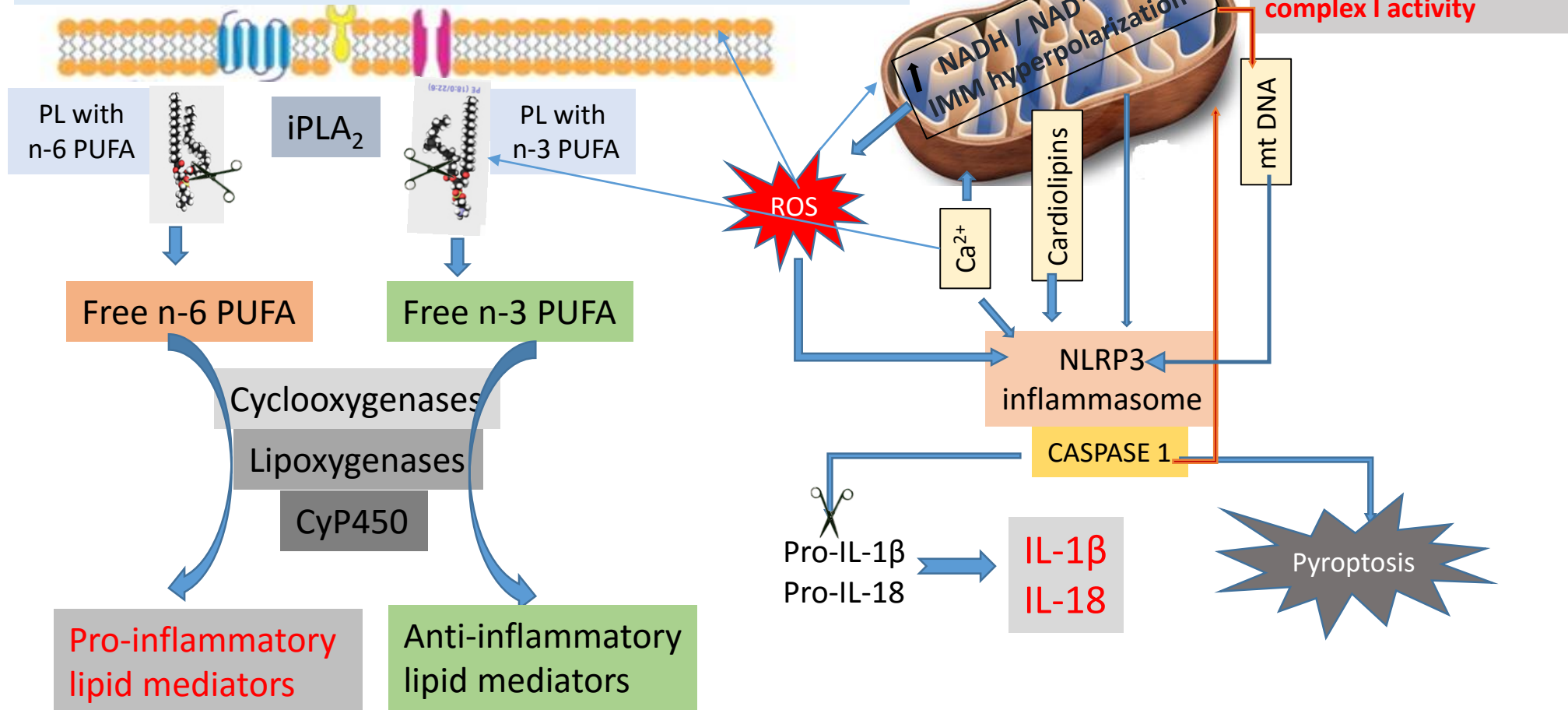
**How these pathological markers are connected in biological systems and how they can trigger “apparently” different diseases?**

# Cross talk among mitochondrial function, oxidative stress and Chronic low grade inflammation

Organs/ tissues / cells affected by age-related degenerative process

Membrane LysoPL content increasing – changes in the functional membrane organization and dynamics

iPLA<sub>2</sub> affinity: PL (PUFA<sub>oxidized</sub>) > PL(PUFA) >> PL(MFU) >> PL (SFA)





**Project Proposal:** Natural bioactive compounds to control age-related chronic low-grade inflammation using an integrative multi-target strategy

**Strategy:** Natural bioactive compounds:

- i) To increase cell capacity to quenching ROS and other reactive species
- ii) To increase n-3/n-6 PUFA in membrane phospholipids, decreasing the production pro-inflammatory lipid mediators;
- iii) To improve the fitness of IMM to allow the functional assembling of protein complexes involved in the oxidative phosphorylation, decreasing ROS release and avoiding the NLRP3 inflammasome activation

**Our long-term vision** is to develop a multi-target therapy and/or specific diets to reduce chronic low-grade inflammation process, associated with several age-related diseases in order to promote healthy aging and increasing Human longevity.





## Skills and Expertise

**Know how** to obtain, from natural sources, extracts with a well-defined chemical composition and to use them to prepare mixtures enriched in (pre)selected physiologically-active compounds, using methodologies with scalable potential;

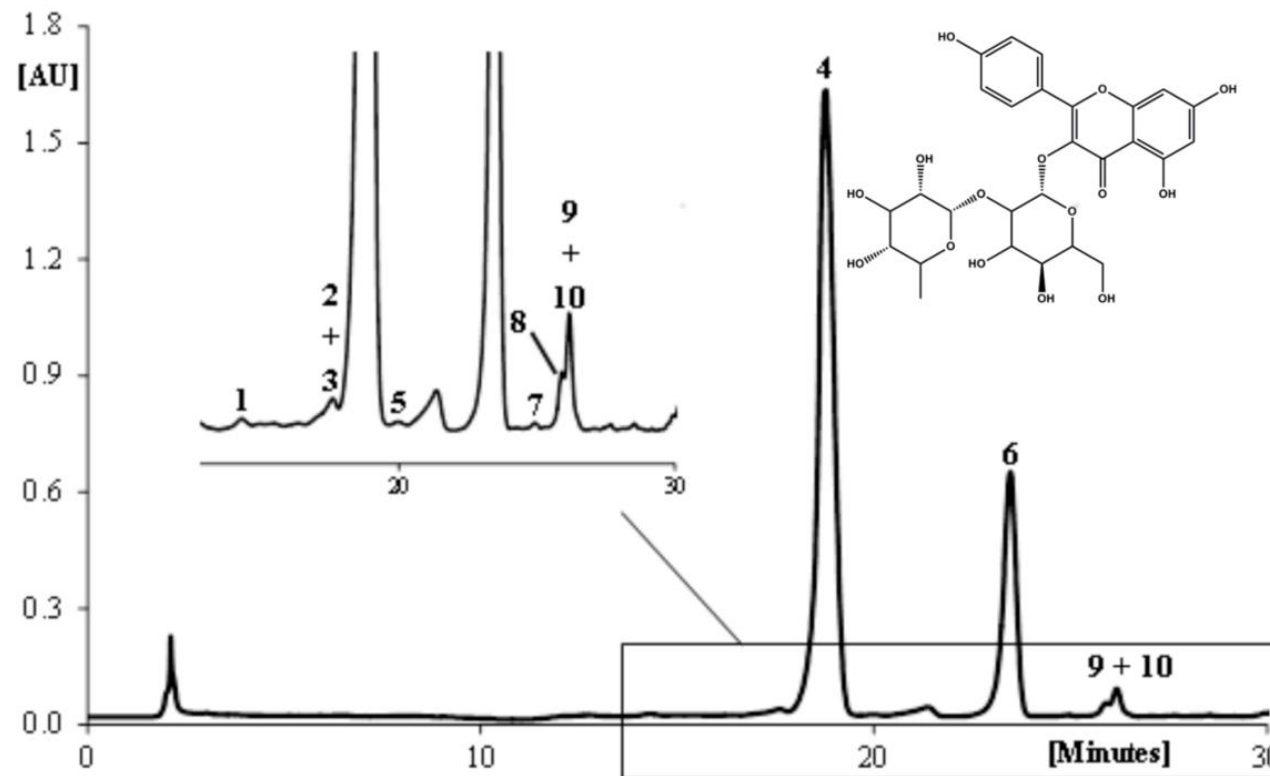
**Know how** to characterize the antioxidant and antiradical activities of the extracts as well as to evaluate their effects on the activity of key enzymes involved in the inflammation cascade, by using cell-free assays;

**Know how** to evaluate the extracts ability to modulate the biochemical pathways connected with the age-related chronic low-grade inflammation process and with the redox state of cells (oxidative stress), using *in vitro* (different types of cells) and *in vivo/ex vivo* (animal models for different diseases such as Alzheimer's disease) assays;

**Know how** to evaluate the extracts ability to modulate the bioenergetic and lipidomic profiles of different types of cells, tissues and organs and its relationship with both chronic low-grade inflammation process and redox state of cells



## *Echium plantagineum* L. bee pollen **flavonoids**-rich extract

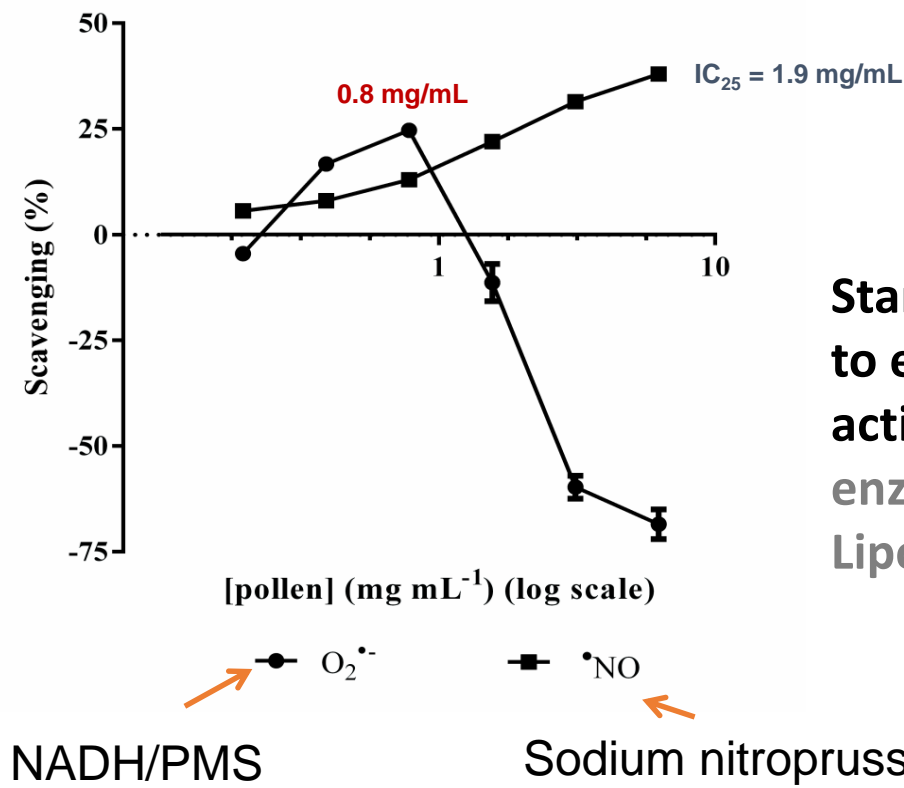


HPLC-DAD chromatogram

# Skills and Expertise



*Echium plantagineum* L. bee pollen **flavonoids**-rich extract exhibit antioxidant/antiradical activity

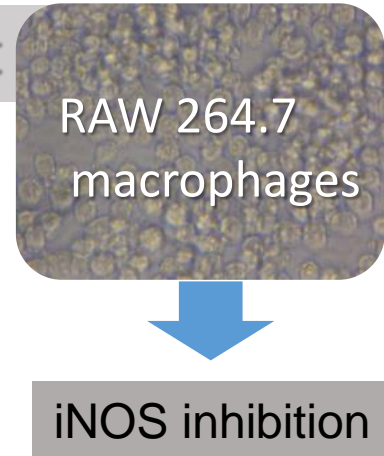
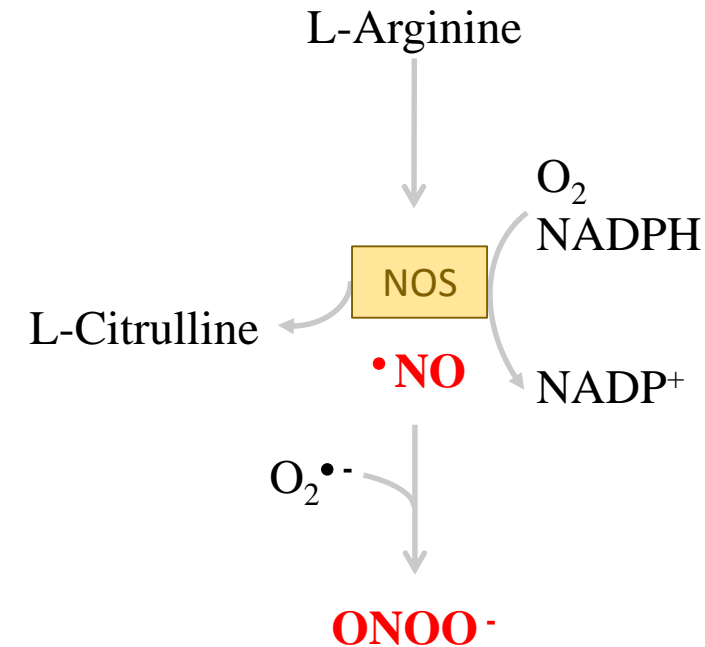
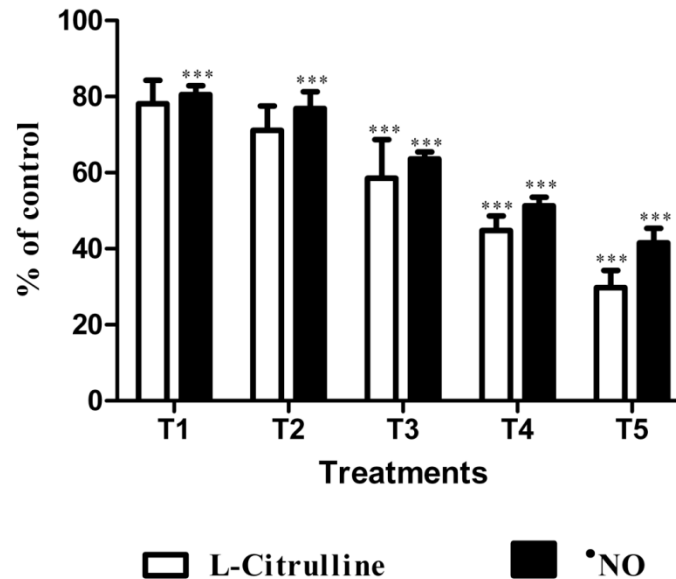


**Standardized Cell-Free Assays** to evaluate the antioxidant/antiradical activity as well as the effects on key enzymes (e.g. phospholipases A2, Lipoxygenases, Cyclooxygenases)

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*Echium plantagineum* L. bee pollen **flavonoids**-rich extract

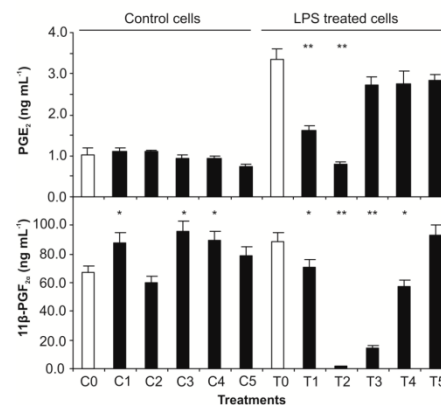




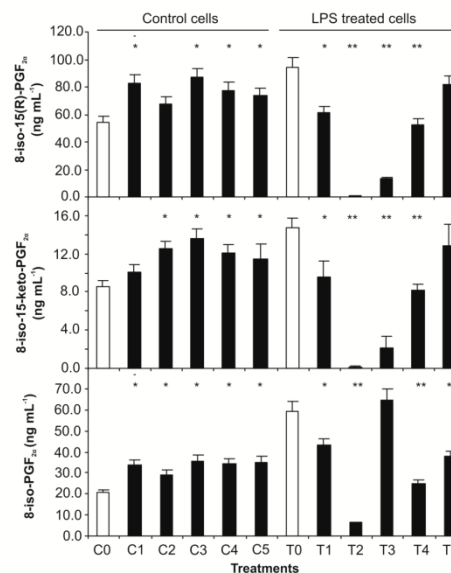
*Echium plantagineum* L. bee pollen **flavonoids**-rich extract has ability to modulate the prostaglandins release by RAW macrophages stimulated by LPS



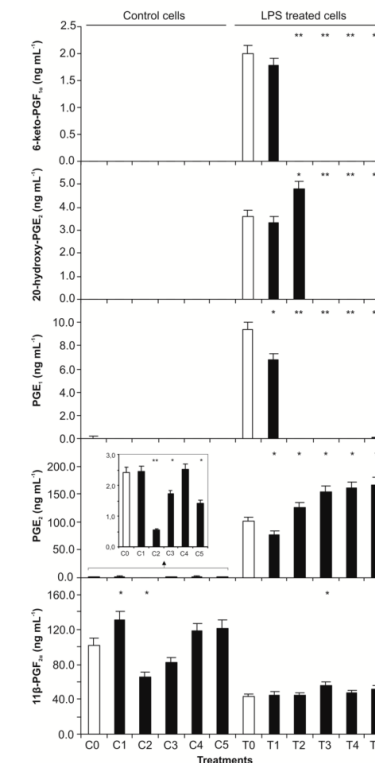
A PROSTAGLANDINE METABOLITES



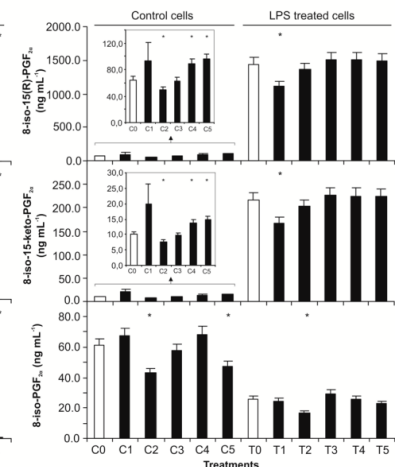
B ISOPROSTANES



A PROSTAGLANDINE METABOLITES



B ISOPROSTANES



PGs metabolites



UPLC-MS

# Skills and Expertise



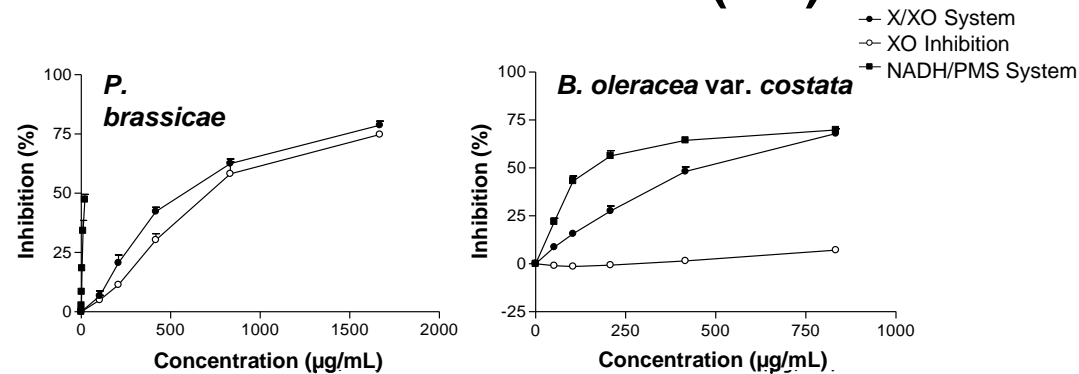
*Brassica oleracea* L. vs *Pieris brassicae* L.

Ecological duo as source of bioactive compounds

Standardized aqueous extracts of *Pieris brassicae* larvae exhibit antioxidant and antigenotoxic activities

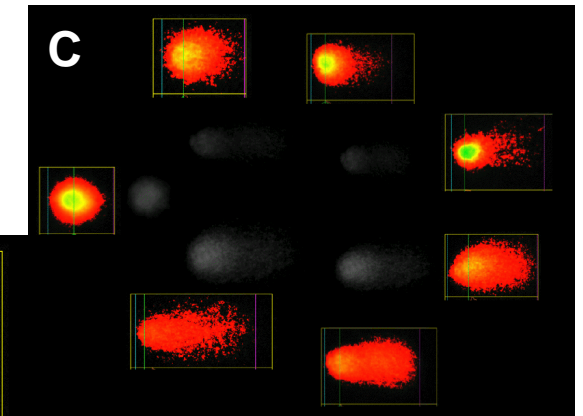
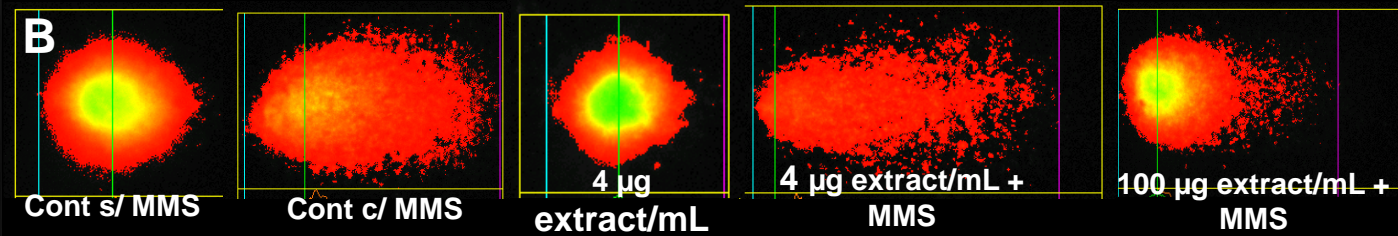


## Xanthine oxidase inhibition (XO)



**Patent:** Andrade, P. B.; Valentão, P.; Sousa, C.; Pereira, D. M.; Ferreres, F. Aqueous extract of *Pieris brassicae* larvae and its use as antioxidant. Portuguese Patent nr. 103931.

## Antigenotoxic activity detected by comet assay

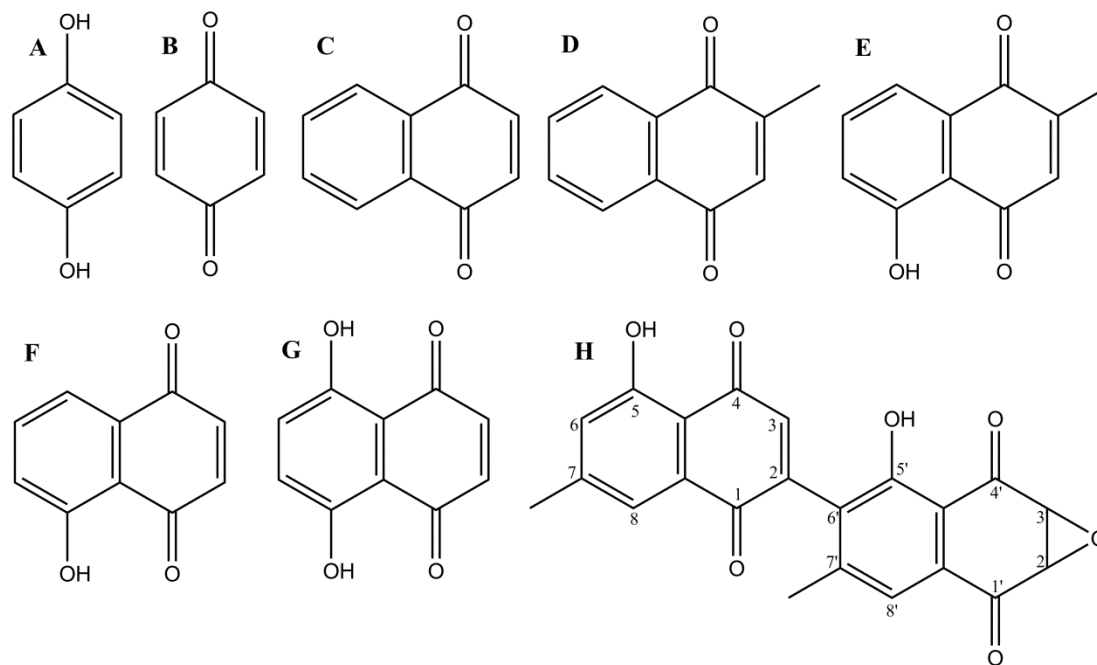


**1<sup>st</sup> PRIZE – CIENTIFIC PHOTOGRAPHY CONTEST**  
 (Fátima Fernandes)

Sousa C, et al. (2009). *J Agric Food Chem*, 57:2288-2294



## Naphthoquinones from *Diospyros chamaethamnus* Dinter ex Mildbr.

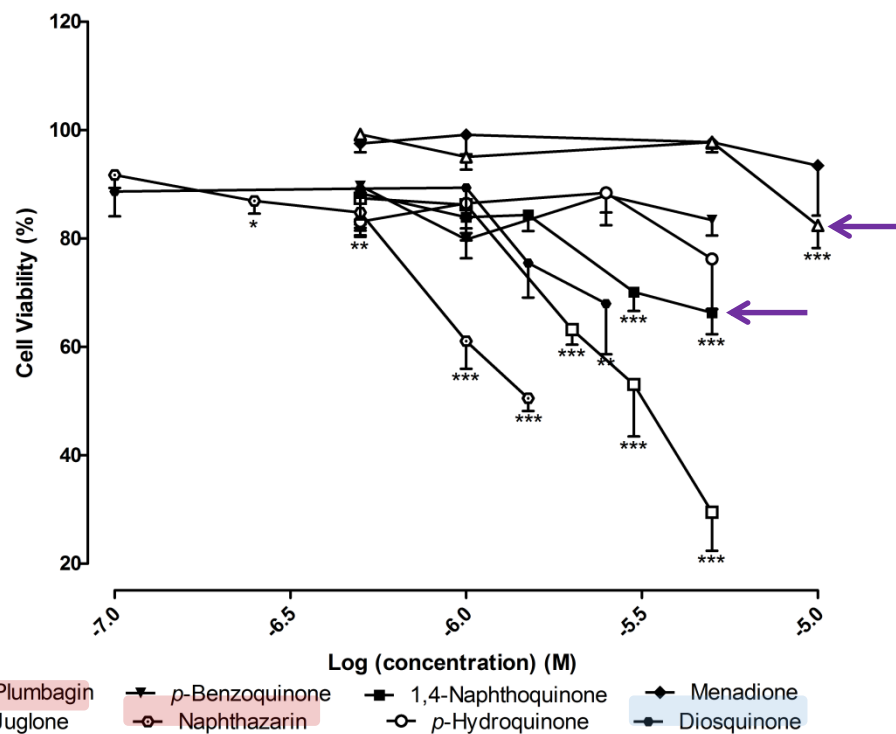


- (A) *p*-Hydroquinone
- (B) *p*-Benzoquinone
- (C) 1,4-Naphthoquinone
- (D) Menadione
- (E) Plumbagin
- (F) Juglone
- (G) Naphthazarin
- (H) Diosquinone

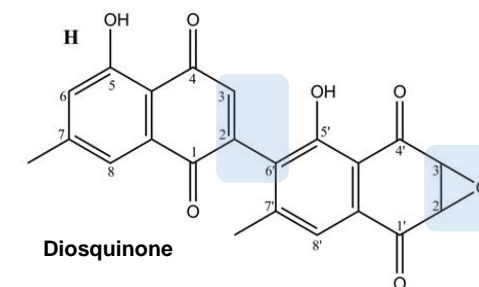




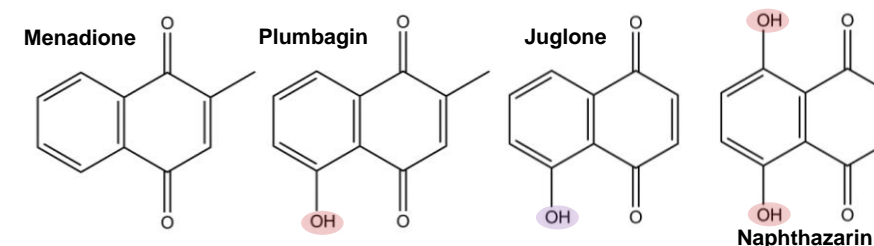
## Biological behaviour of Naphthoquinones from *Diospyros chamaethamnus* Dinter ex Mildbr. - molecular mechanism of action



Nucleophilic attack to thiol groups involves C2 and C3



Introduction of electron-donating hydroxyl groups increases the pro-oxidant potential



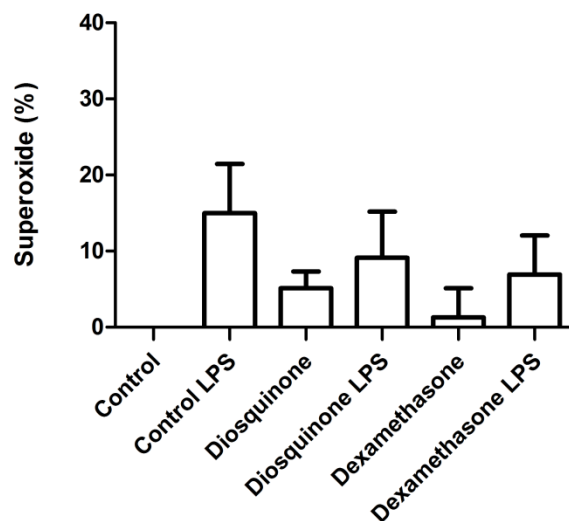
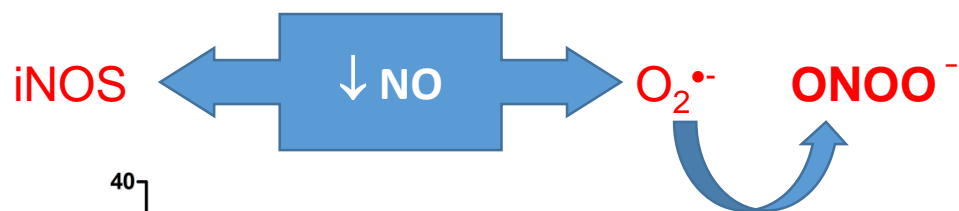
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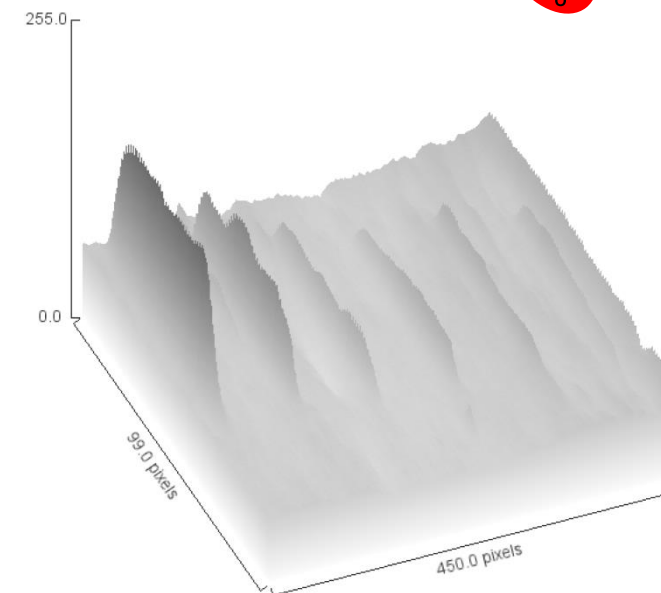
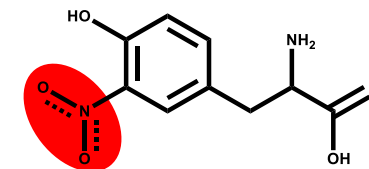
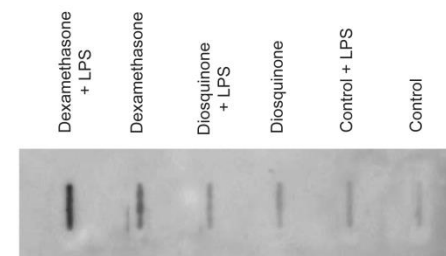
## Biological behaviour of Naphthoquinones from *Diospyros chamaethamnus* Dinter ex Mildbr. - molecular mechanism of action

**Diosquinone dampens the inflammation cascade induced by LPS**, as detected by the reduction of proinflammatory cytokines: tumour necrosis factor alpha (TNF-alpha) and interleukin 6 (IL-6).



Superoxide radical quantification.

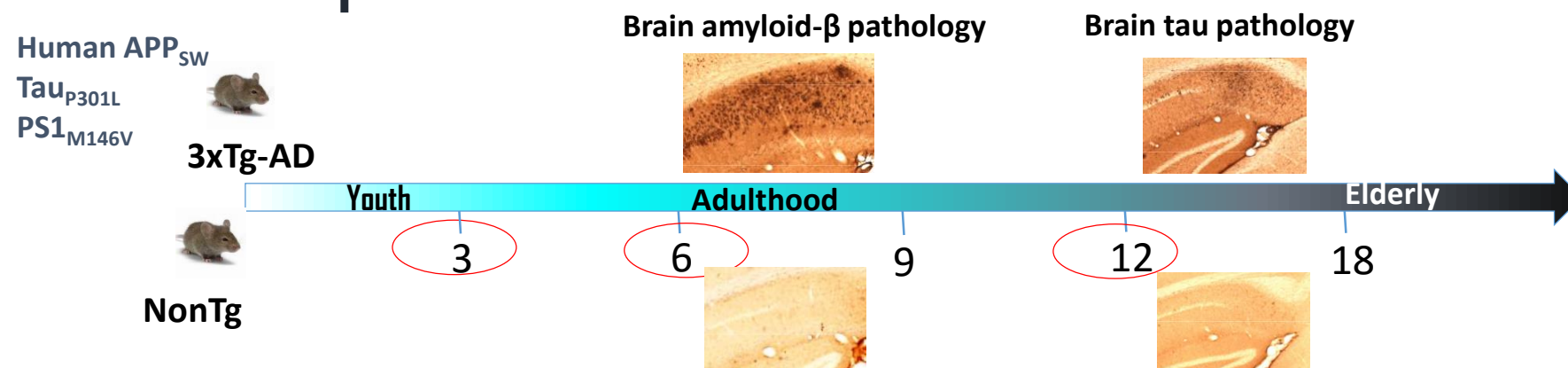
$\downarrow$  TNF- $\alpha$ : 51%  
 $\downarrow$  IL-6: 39%



**Semi-quantitative analysis of 3-nitrotyrosine.** Image of slot-blot film, where 3-nitrotyrosine was detected by immunoblotting and respective surface plot analysis (Y axis represents intensity of bands).



# Skills and Expertise

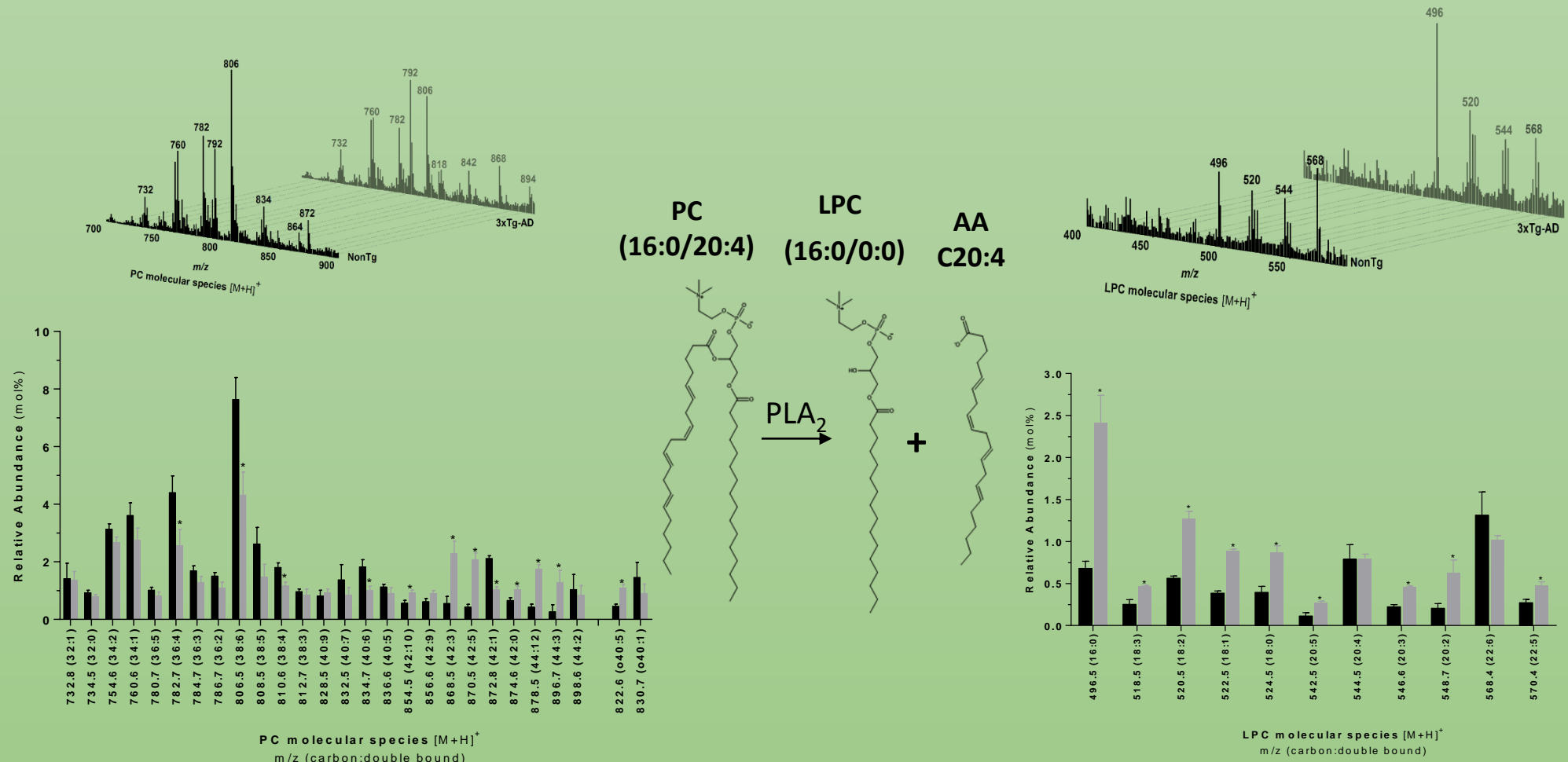


- 3xTg-AD mitochondrial dysfunction connected with **a decay of complex I activity and with an aberrant mitochondrial in CL profile** occurs mainly in synaptic mitochondria preceding the traditional pathological hallmarks of disease (senile plaques and neurofibrillary tangles)
- Age-dependent low catalase activity and high levels of ROS detected in 3xTg-AD mice suggests that oxidative stress should be connected with mitochondrial dysfunction
- The increased LPC levels simultaneously with a decreased levels of PC containing arachidonic acid in both brain and skeletal muscle of 3xTg-AD mice indicate an enhancement of cPLA<sub>2</sub> activity and is an evidence of systemic low grade inflammation.

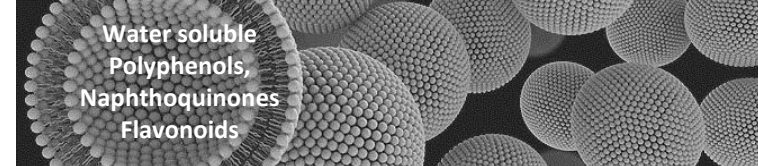
## Membrane Lipid Changes and Chronic Low Grade Inflammation



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# Project Design



## Work package 1 – Preparation and analysis of standardized bioactive formulations for clinical applications and food industry

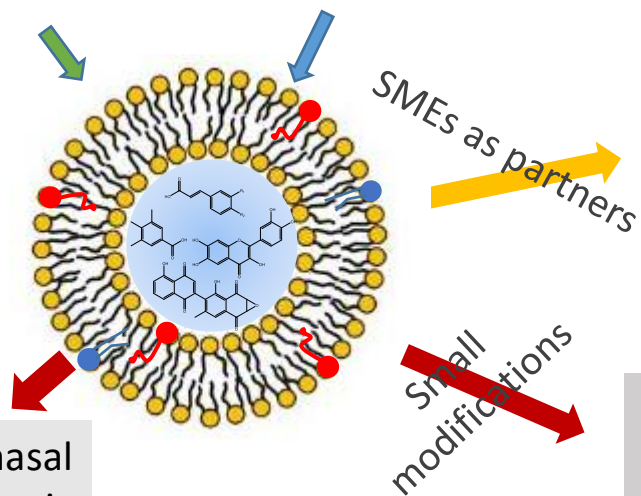
Extraction and purification of Polyphenols, Flavonoids, Naphthoquinones (mainly of water-soluble ) from dietary sources

Extraction and purification of a selected pool of phospholipids (PC, PE, PG) with high-content of n-3 PUFA (n-6 /n-3 ≤ 1) from Red algae (*Palmaria palmate*) and brawn algae of genus *Sargassum*

SMEs as partners



Unilamellar liposomes with size close to 100 nm and Zeta potential positive or close to zero



SMEs as partners

Small modifications

Incorporation in food matrices (e.g. dairy products) for the production of specialized foods for the elderly and to be used in pilot and interventions studies

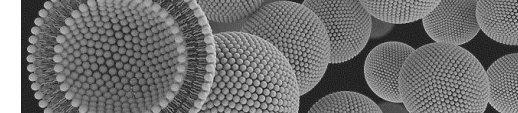
Nanomedical platform for nasal administration to control the chronic low grade inflammation in brain

Nanomedical platform for oral and intravenous administration to control the chronic low grade inflammation in peripheral organs

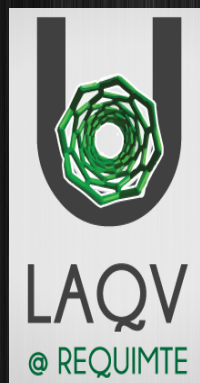


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## Work package 2 – In vitro studies: molecular mechanisms of action and toxicological screening

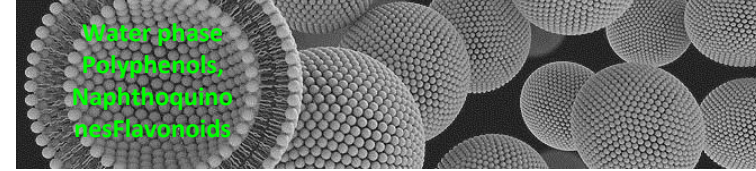


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The therapeutic success of nanomedical platform is dependent of five main steps to which the experimental approaches will be addressed using immortalized cell lines and primary cell cultures:

- i) cell internalization pathway in order to escape from the endosome and subsequent fusion with mitochondrial membranes;
- ii) ability to modulate the lipid composition of cell membranes, increasing the phospholipid content with n-3 PUFA (increasing n-3/n-6 PUFA rate);
- iii) ability to restore both mitochondrial functionality and redox state of cells induced by different types of mitochondria-active drugs that also promote oxidative stress;
- iv) ability to modulate the inflammation cascade induced by different types of pro-inflammatory agents
- v) Toxicological screening to find the dose range with potential therapeutic effects without cytotoxicity

# Project Design



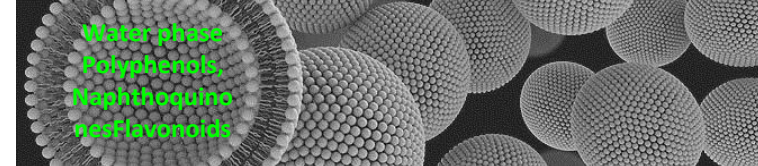
**Work package 3 – Validation the novel therapeutic concept (liposomal formulations) to control the chronic low-grade inflammation associated with age-related diseases by using animal models: *e.g.* Alzheimer’s disease, diabetes type 2, chronic kidney disease**

The therapeutic approach should be tested considering, at least, three different diseases: one associated with brain degeneration (e.g. AD) and two with chronic low grade inflammation in peripheral organs, such as diabetes type 2 and chronic kidney disease. However, the choice of diseases and corresponding animal models for the investigation are dependent on the expertise of the research partners that we join to form the project consortium

**Require the use of the integrative “omics” approaches to investigate molecular mechanisms underlying the biological effects of the liposomal formulations in different organs, tissues and cell types and may have also the goal to find new disease biomarkers.**

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# Project Design



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**Work package 4** – Evaluation the effectiveness of a bifunctional diet enriched in omega-3 fatty acids and specific pool of polyphenols/flavonoids/naphthoquinones to control the chronic low-grade inflammation and prevent/delay the degeneration associated to several age-related diseases, by using animal models and, eventually, performing pilot studies with humans.

## **Work package 5** – Management, Coordination, Dissemination and Exploitation

### **Objectives**

To ensure the project meets its objectives within the time and the budget limits

To manage the evolution of the project and coordinate activities

To facilitate communication between the partners, and with the EU commission

To disseminate the project's findings and promote the results on a national and European level to stakeholders, media and society.

To establish an exploitation plan for the milestones achieved in the project

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## Drug Discovery

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Thank you for your attention



