MECCANISMI MOLECOLARI NELLA ATTIVAZIONE DEI MACROFAGI: RUOLO DEL FERRO E DELLA 5-LIPOSSIGENASI

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MOLECULAR AND CELLULAR MECHANISMS of IRON HOMEOSTASIS

UNITÀ DI RICERCA BIOCHIMICA
E BIOLOGIA MOLECOLARE

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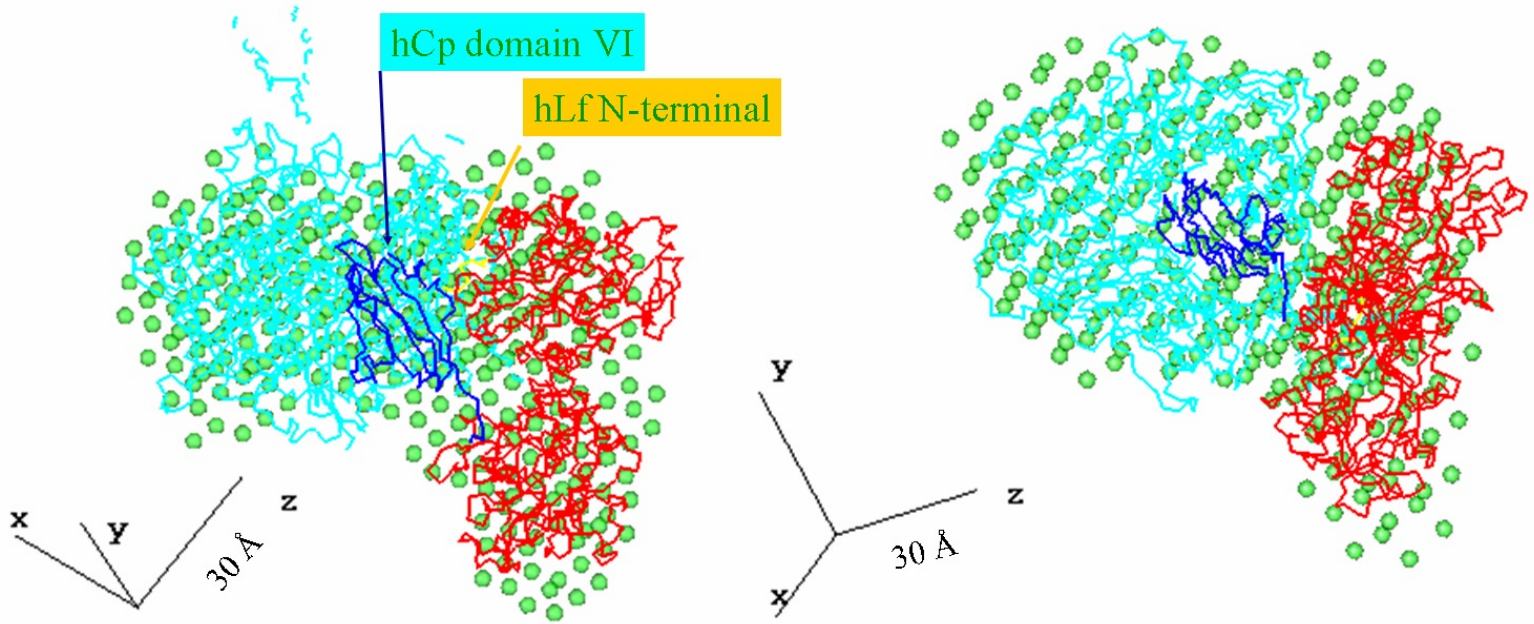
Ceruloplasmin has the correct orientation to facilitate the iron incorporation into transferrin



doi:10.1016/j.jmb.2007.05.089 J. Mol. Biol. (2007) 371, 1038–1046

JMB

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Structural Characterization of the Ceruloplasmin: Lactoferrin Complex in Solution

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Mariano Beltramini[2], Alexey Sokolov[5], Maria Pulina[5]
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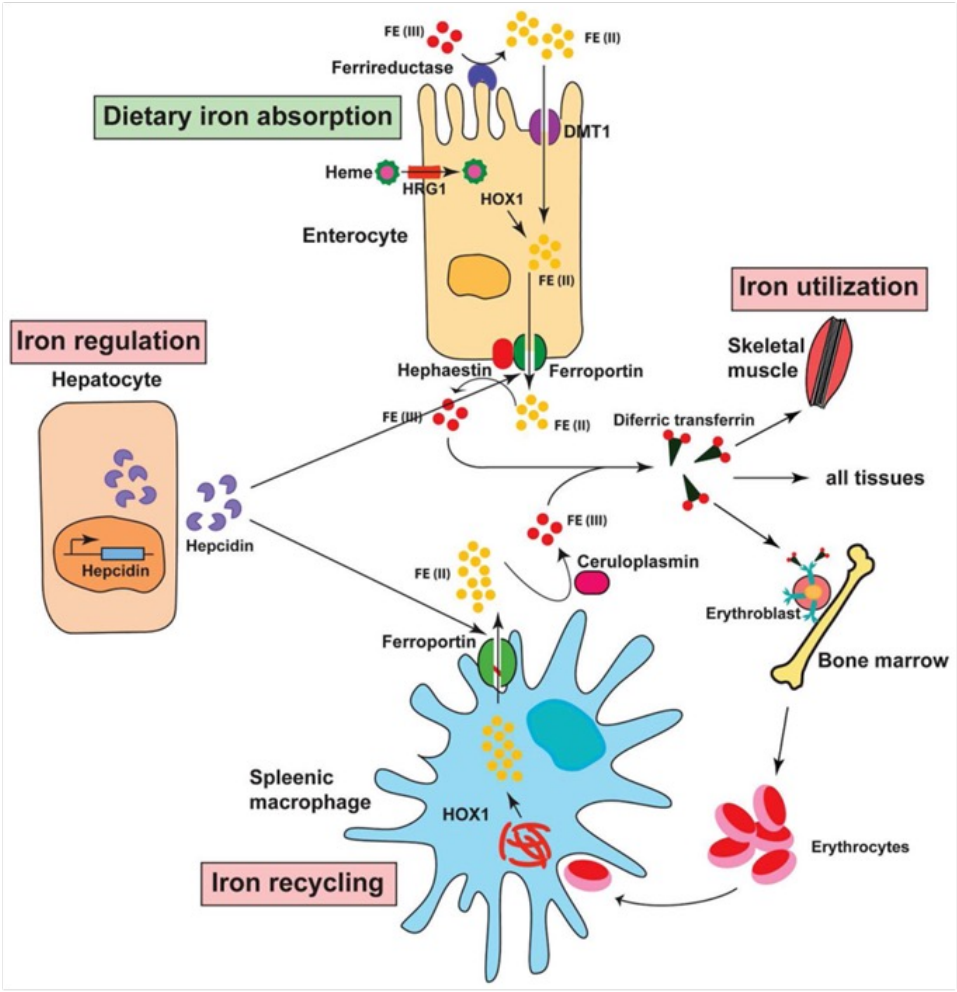
Ivo Cozzani[1] and Enrico Dainese[1]*

Ceruloplasmin in the Iron Homeostasis

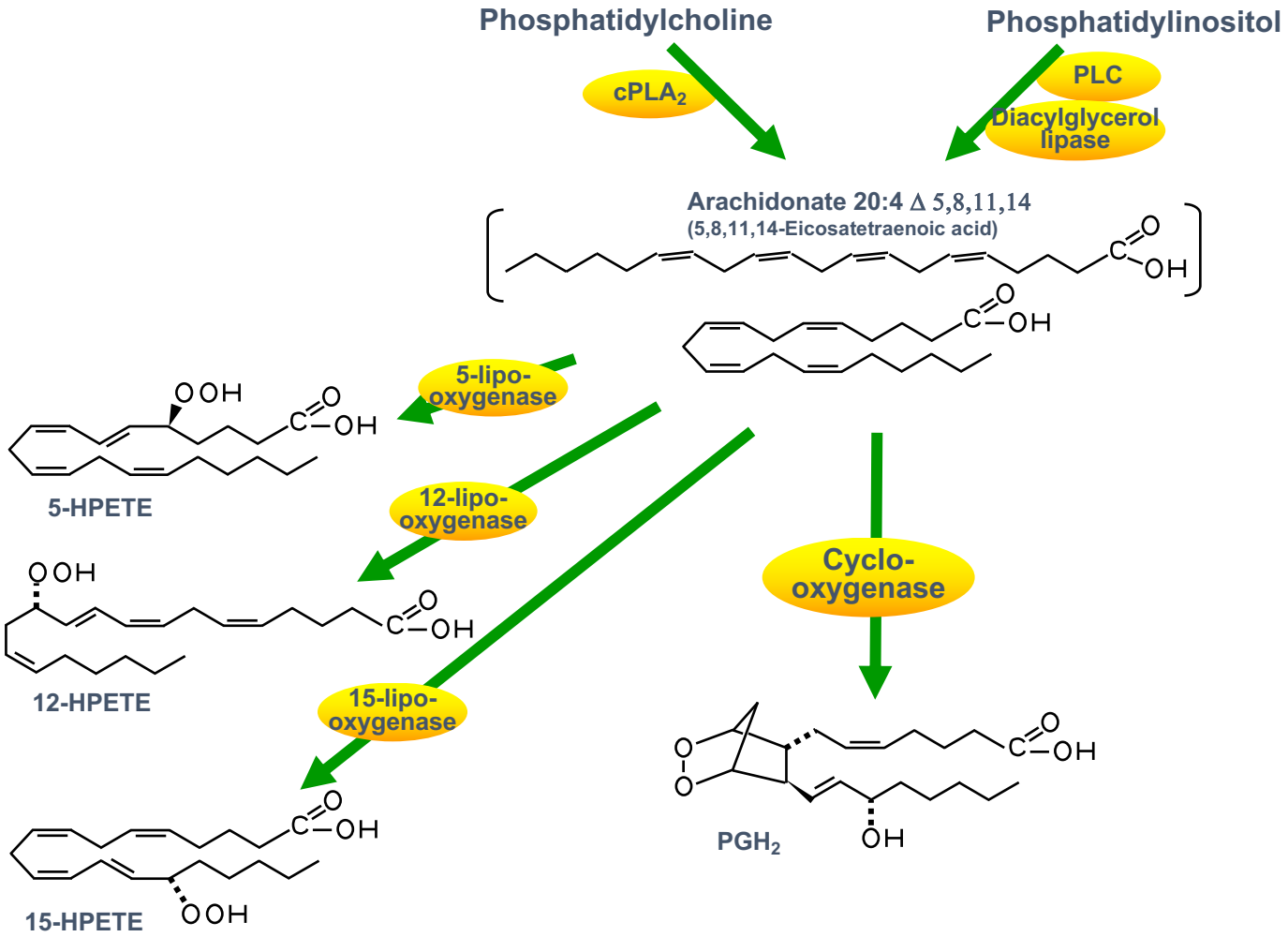


Lipoxygenases and Iron Homeostasis

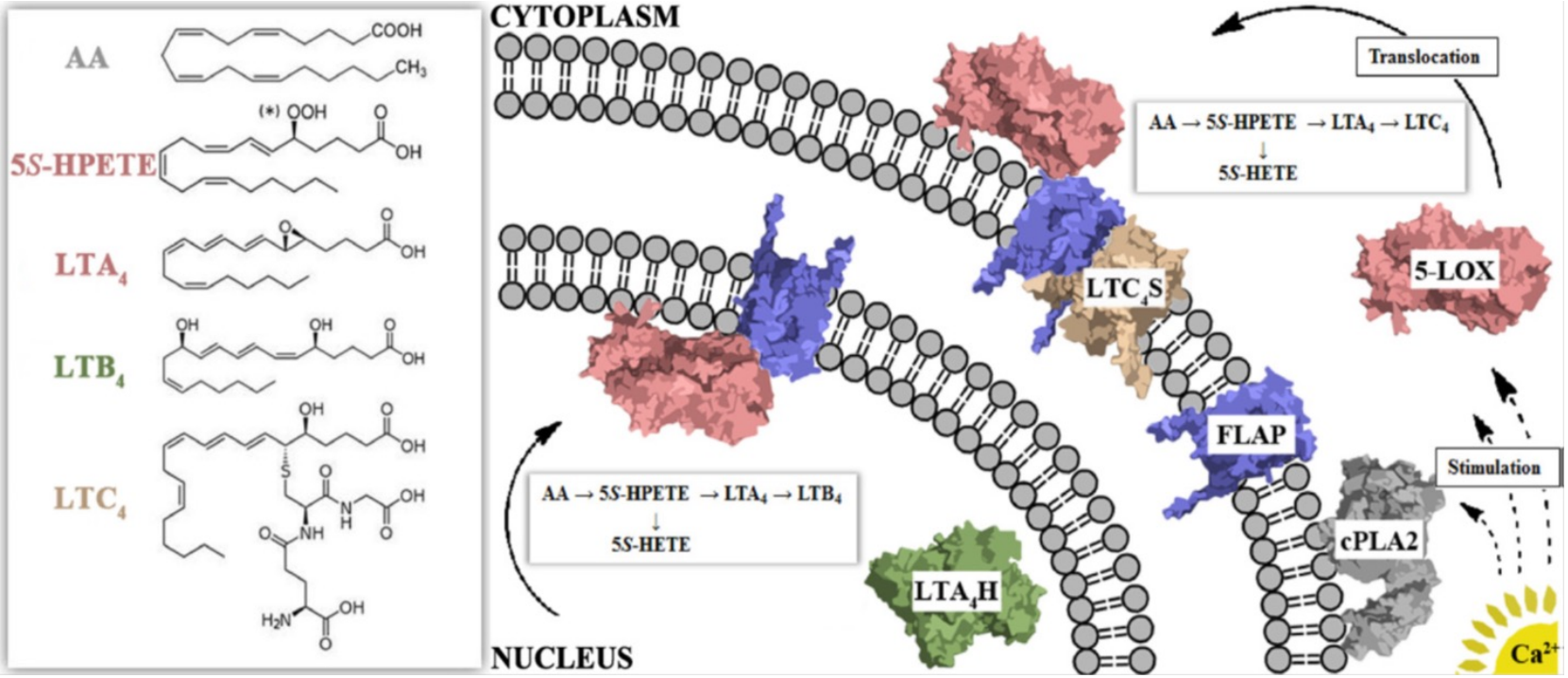
- The effect of inflammation on the regulation of iron metabolism is widely recognized, and a dysregulated iron homeostasis is a cornerstone of acute and chronic inflammatory processes involving cell-mediated immunity.

- Iron-associated oxidant damage is intimately involved with neurological disorders, from Alzheimer's disease to multiple sclerosis, where the accumulation of iron within microglia/macrophages has been documented.

The arachidonic acid cascade

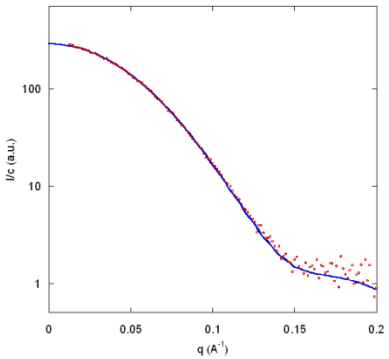


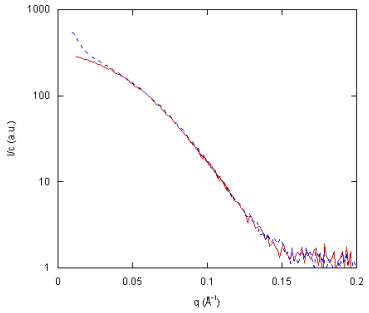
Nuclear translocation as key mechanism in 5-LOX activation

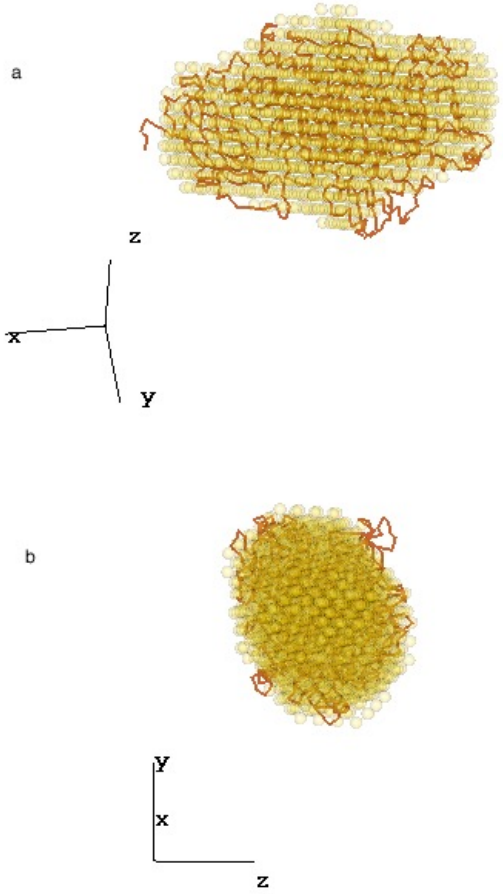


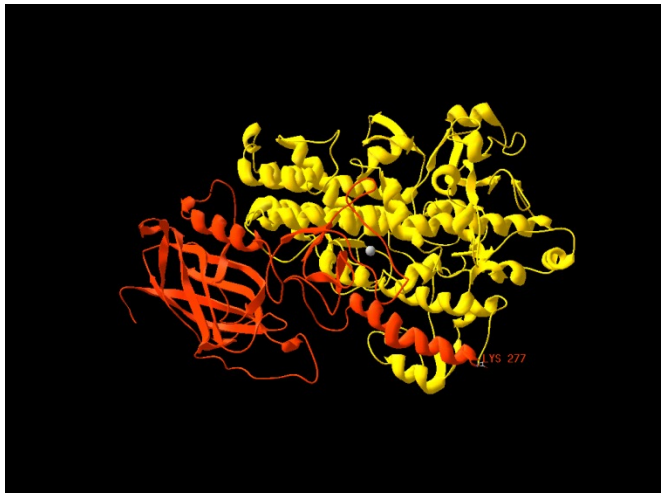
Newcomer & Gilbert, 2010 *JBC 28*, 25109–25114

Structural and functional properties of lipoxygenases: the interaction with membranes









LOX-1 does not require the N-terminal β-barrel domain movement proposed for the membrane binding of 15-LOX-1

Dainese et al., (2005) *JMB* 349,143-152.

- **High iron content has also been reported in in atherosclerotic lesions. However, the precise mechanism(s) underlying the relationship between iron and lipoxygenase are as yet unclear.**

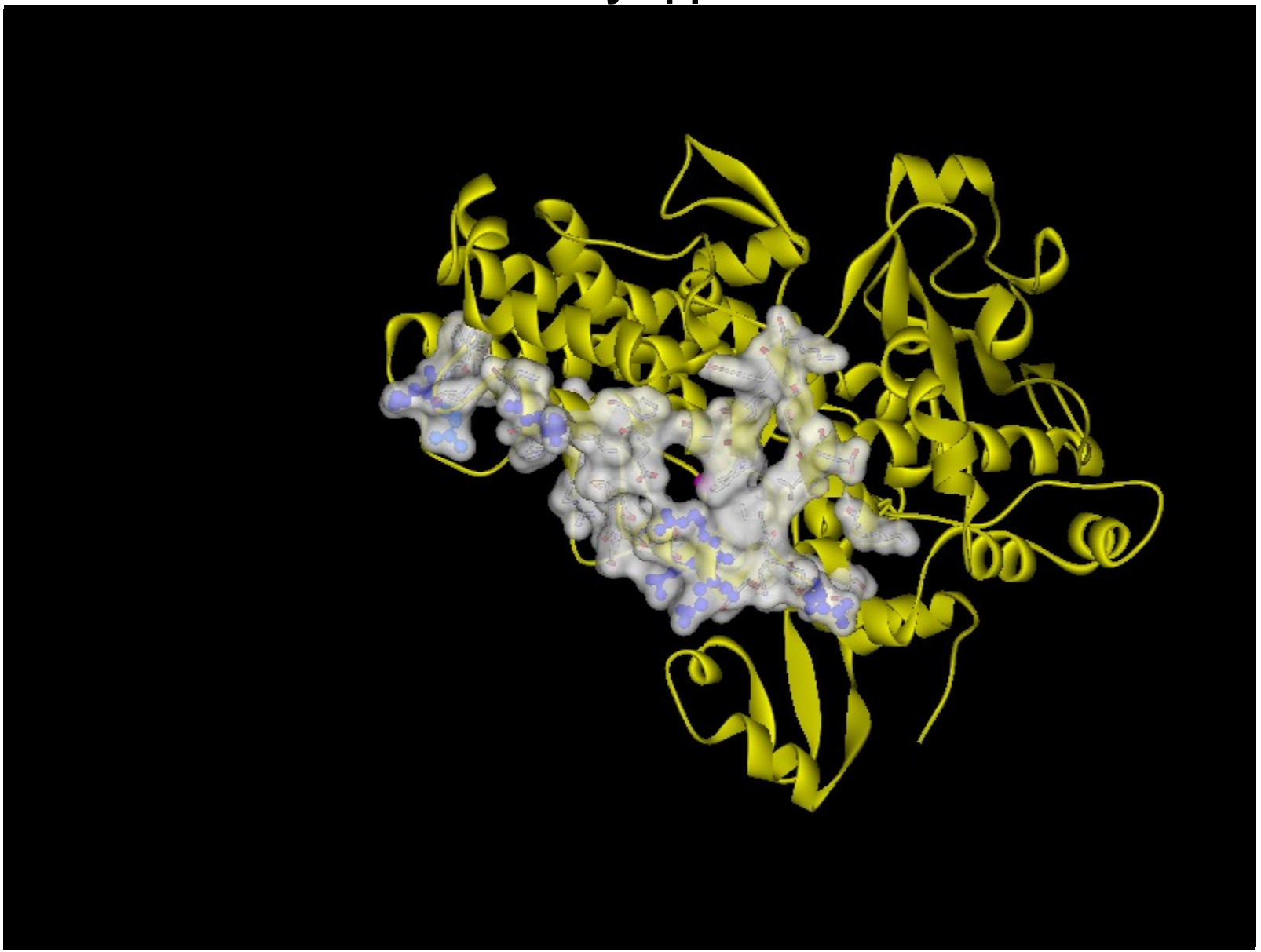
- **The effect of inflammation on the regulation of iron metabolism is widely recognized, and a dysregulated iron homeostasis is a cornerstone of acute and chronic inflammatory processes involving cell-mediated immunity.**

- **Iron-associated oxidant damage is intimately involved with neurological disorders, from Alzheimer's disease to multiple sclerosis, where the accumulation of iron within microglia/macrophages has been documented.**

Factor Regulating Hepcidin Level	Mechanism/Direction of Change	Effect on Hepcidin Level	Effect on Iron Cycling
Inflammation	Increased IL-6	Increased	Decreased
Iron			
Hepatic iron	Increased	Increased	Decreased
Tf saturation	Increased	Increased	Decreased
Erythropoietic activity	Increased	Decreased	Increased
O_2 tension	Low cellular pO_2	Decreased	Increased

IL-6, interleukin 6; Tf, transferrin.

Iron extraction, reconstitution and substitution have been successfully applied to miniLOX

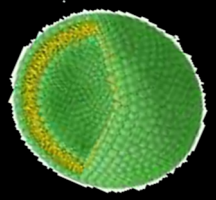


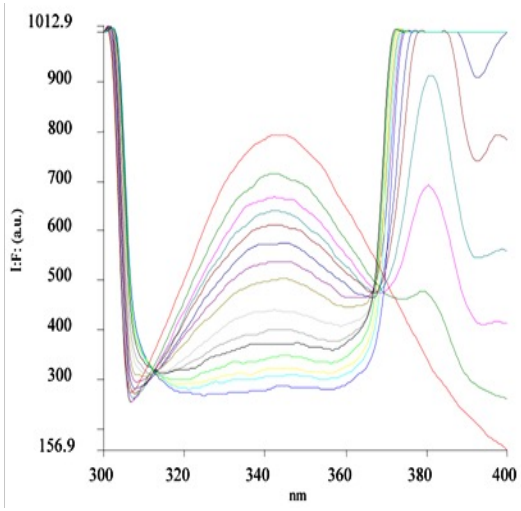
Iron modulates the membrane binding of lipoxygenase

F1 = fluorescence donors (Trp of the protein)
F2 = fluorescence acceptors (Py-PE)

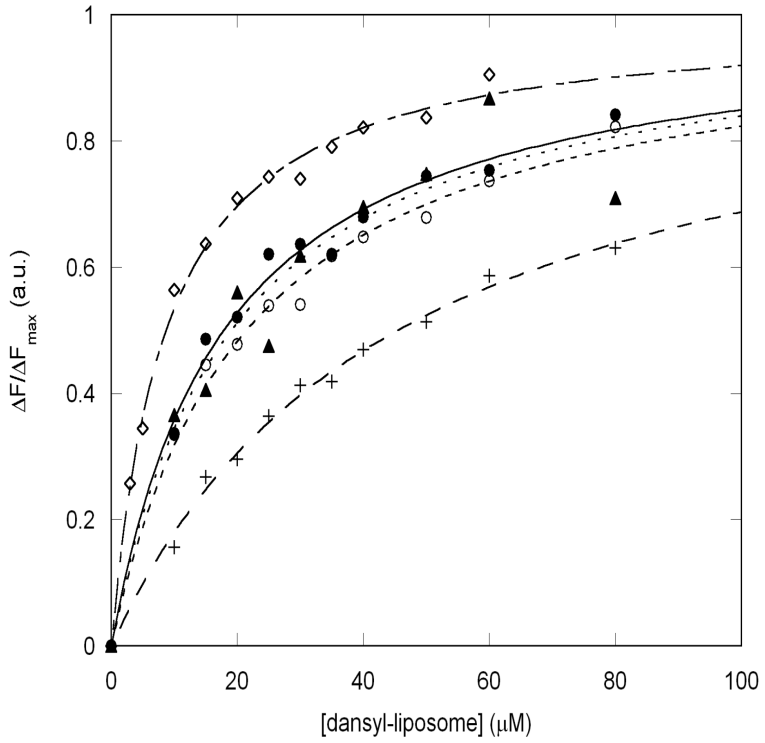
FRET

excitation → *F1* → *F2* → *F2 emission*
F1 quenching





Membrane binding affinity is modulated by the iron content



Protein form	K_d (μM)
(●) LOX-1	17.9 ± 2.0
(◊) miniLOX	9.2 ± 1.0
(+) apo-miniLOX	45.4 ± 4.3
(○) miniLOX-Fe(II)	21.4 ± 2.4
(▲) miniLOX-Fe(III)	18.9 ± 5.5

The FASEB Journal • Research Communication

A novel role for iron in modulating the activity and membrane-binding ability of a trimmed soybean lipoxygenase-1

Enrico Dainese,*,[1,2] Clotilde B. Angelucci,*,[1] Annalaura Sabatucci,* Vincenzo De Filippis,[†] Giampiero Mei,[‡] and Mauro Maccarrone*,[§,2]

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LOX Membrane binding affinity is modulated by the iron content

The FASEB Journal • Research Communication

A novel role for iron in modulating the activity and membrane-binding ability of a trimmed soybean lipoxygenase-1

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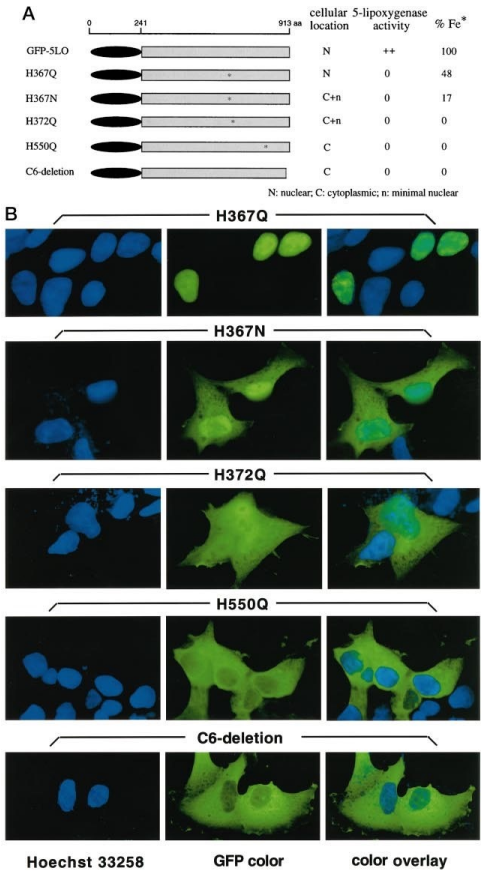
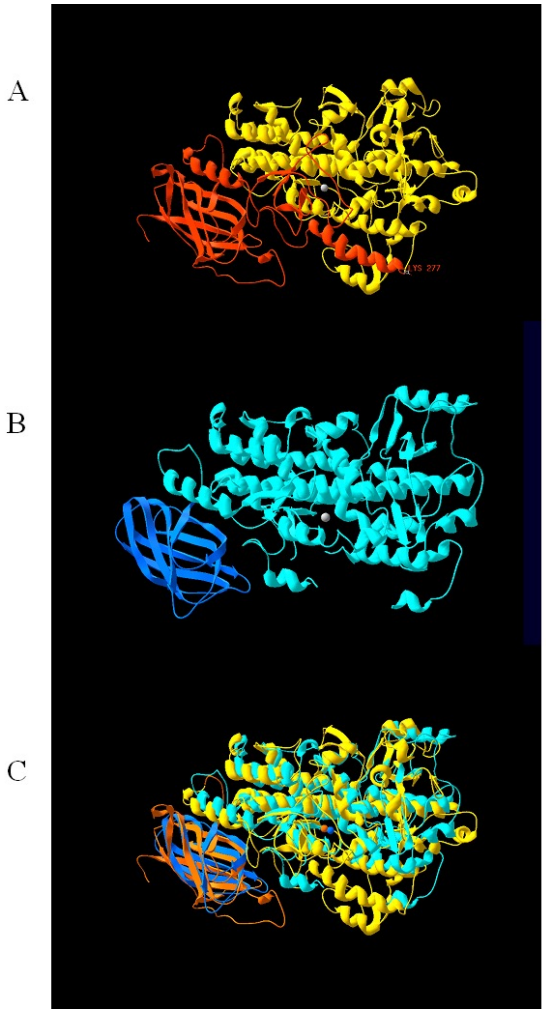
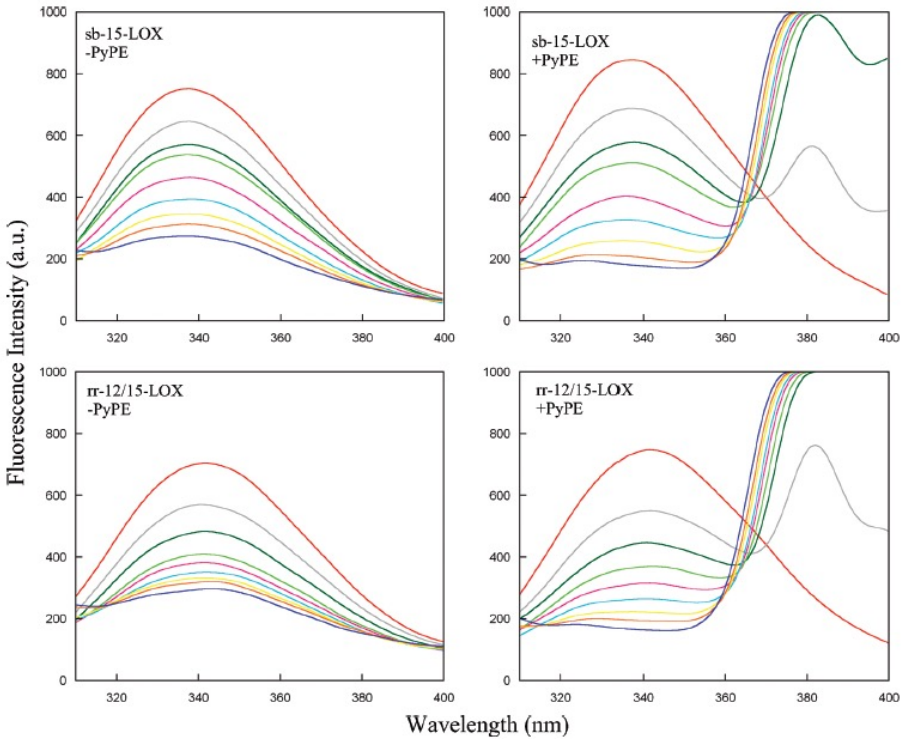


Plant and mammalian LOXs bind to membrane with different affinities





Mei et al., 2008, *Biochemistry* 47, 9234–9242
Di Venere et al., 2014, *BBA Cell Biology of Lipids* 841, 1–10

frontiers in Immunology

ORIGINAL RESEARCH
published: 28 June 2019
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Iron-Dependent Trafficking of 5-Lipoxygenase and Impact on Human Macrophage Activation

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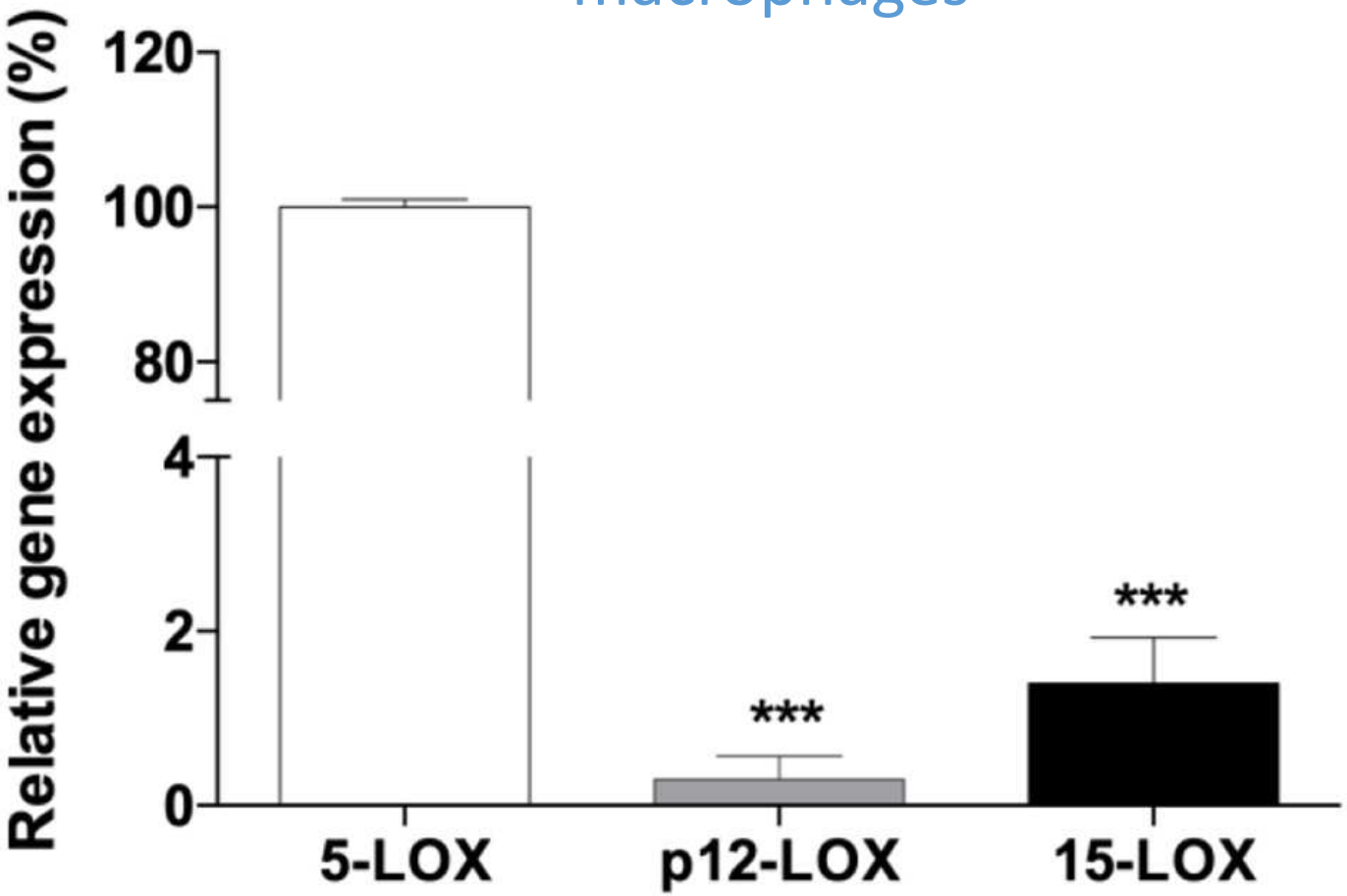
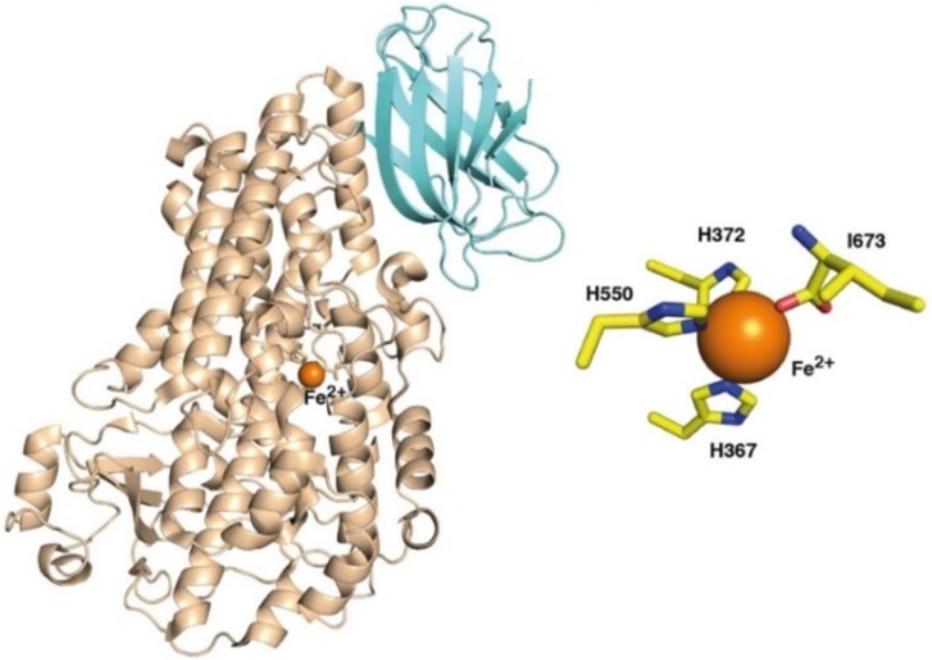
[1] *Faculty of Bioscience and Technology for Food Agriculture and Environment, University of Teramo, Teramo, Italy,* [2] *Faculty of*

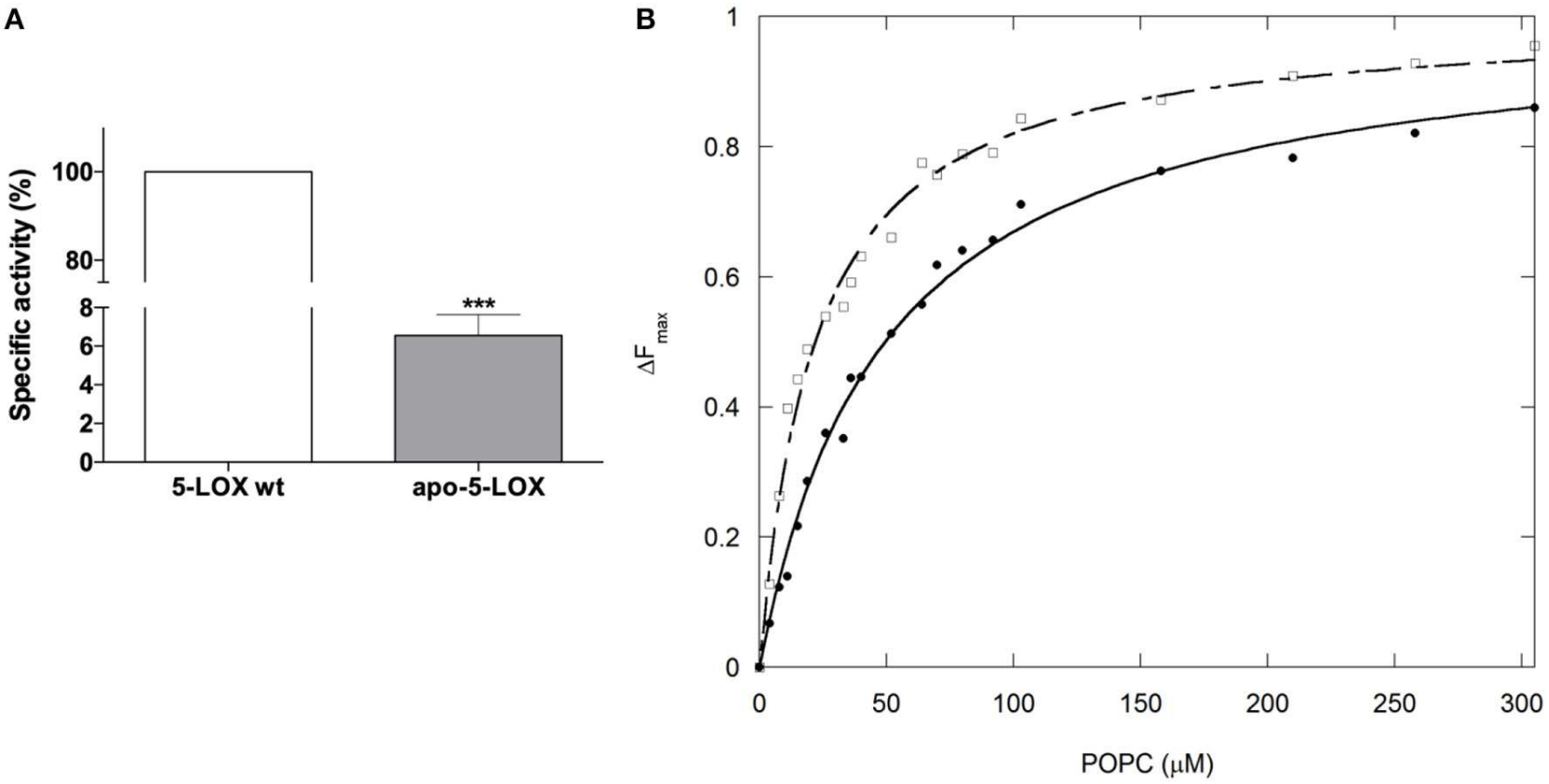
The gene of 5-LOX was the most expressed in macrophages



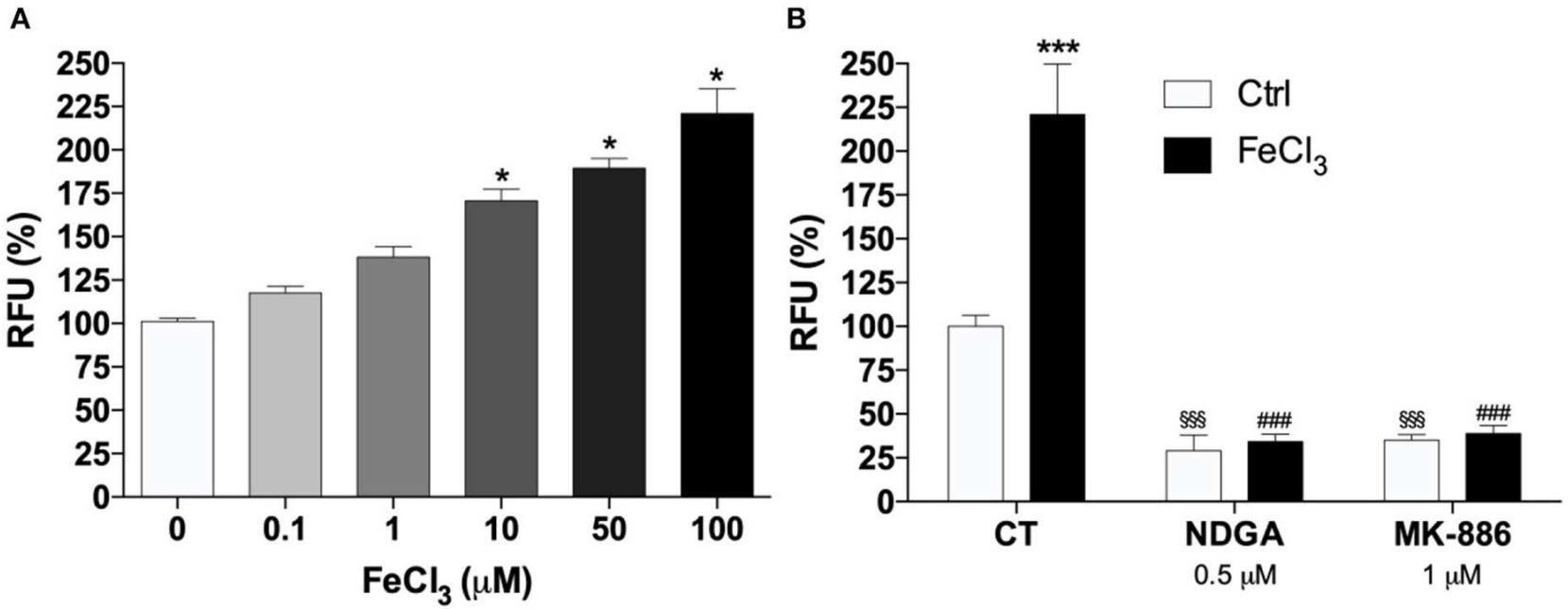
Human 5-Lipoxygenase



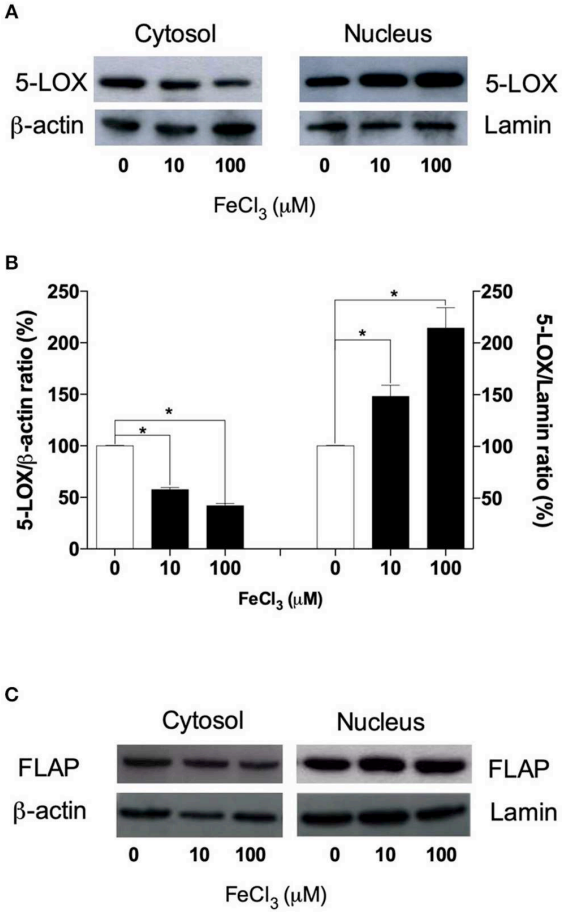
Human 5-LOX iron content modulates the interaction with membranes

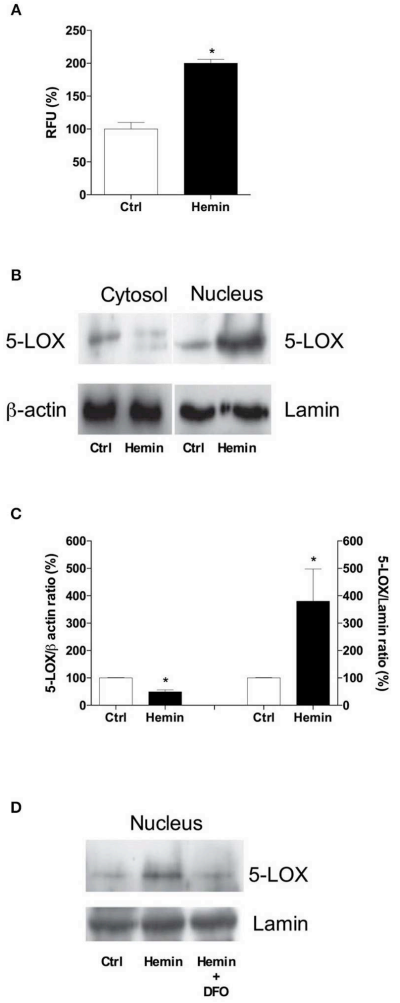


Effect of exogenous iron on 5-LOX activity in THP-1 macrophages

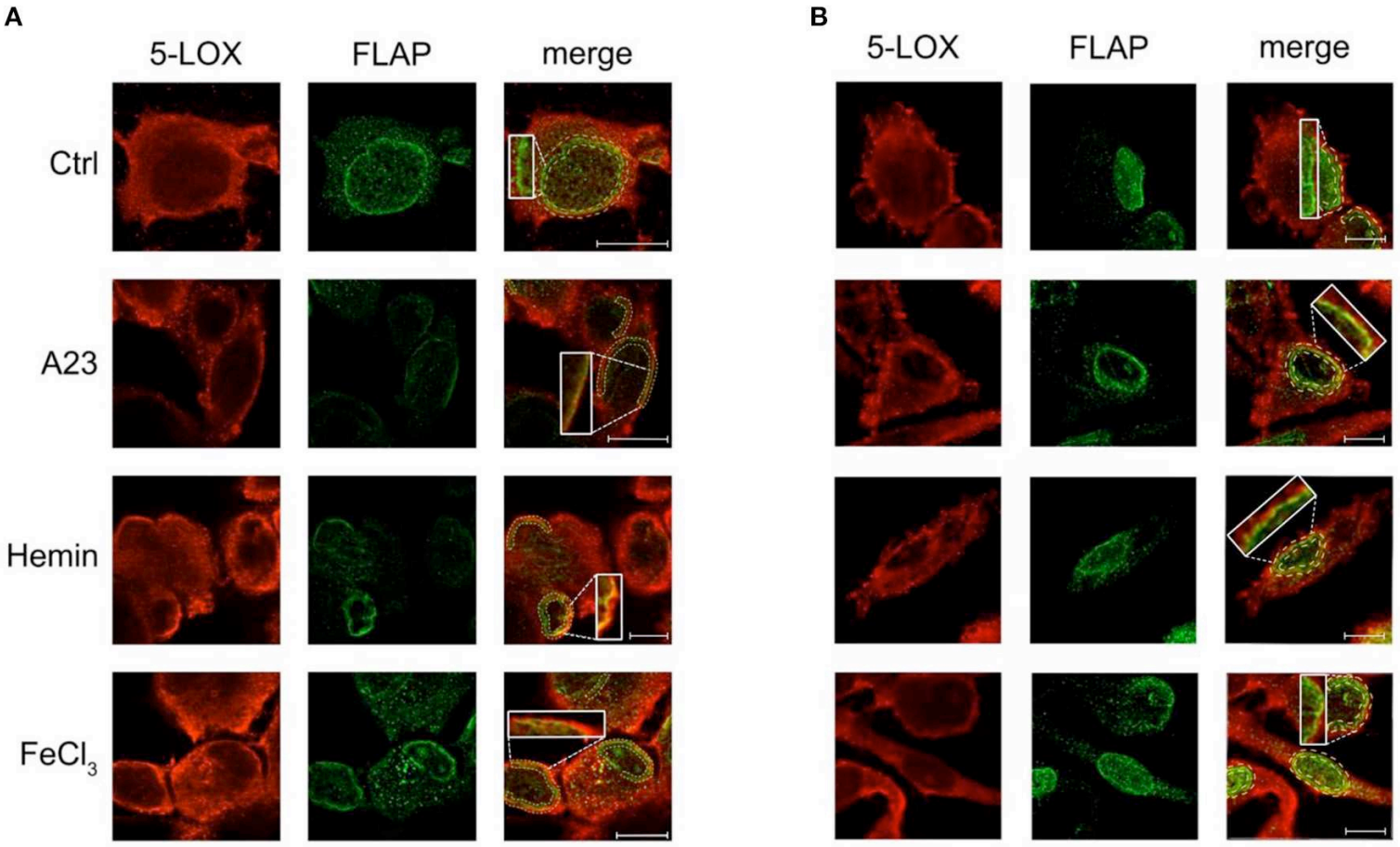


Iron and Hemin induce reversible 5-LOX nuclear translocation

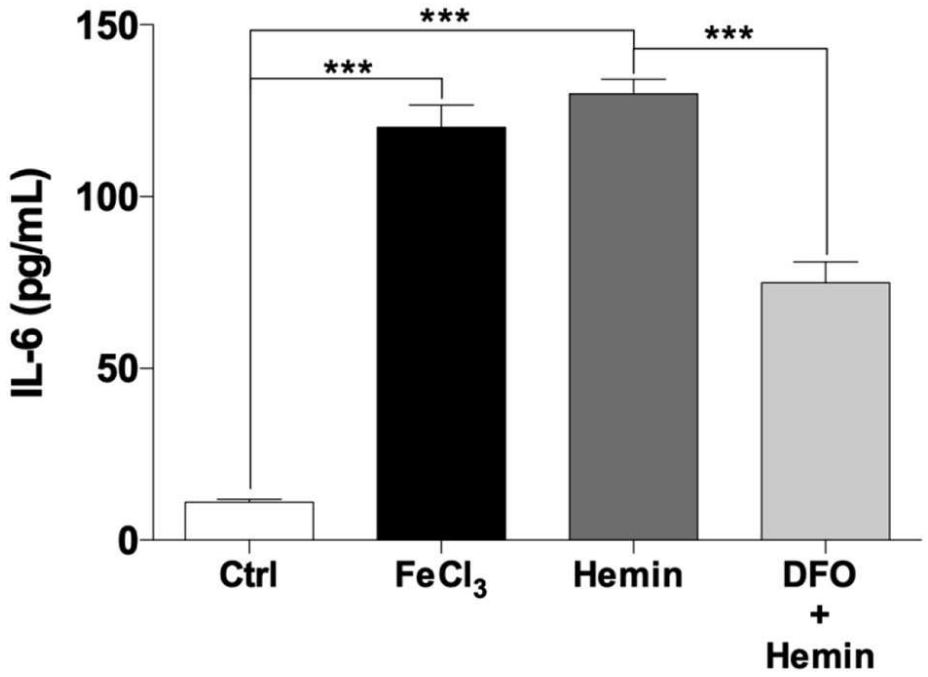




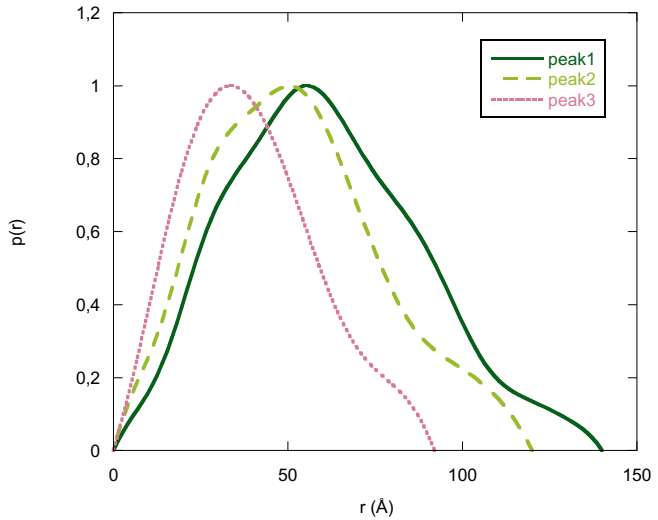
Iron and Hemin induce reversible 5-LOX nuclear translocation and FLAP interaction in human macrophages

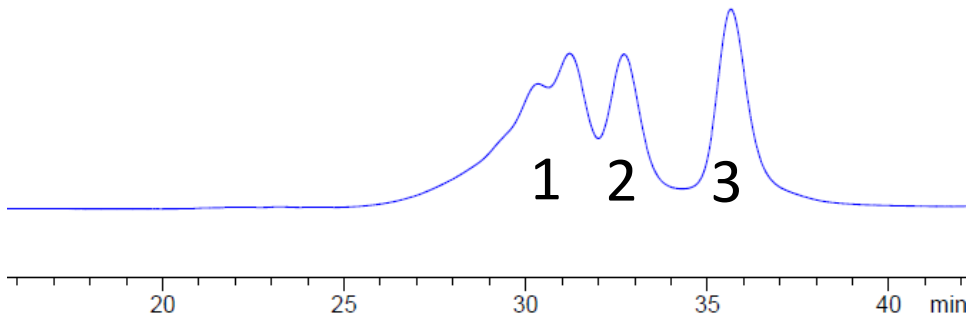


- The functional relevance of iron overloading was demonstrated by a marked induction of the expression of interleukin-6 in iron-treated macrophages.
- Pre-treatment of cells with the iron-chelating agent deferoxamine completely abolished the hemin-dependent translocation of 5-LOX to the nuclear fraction, and significantly reverted its effect on interleukin-6 overexpression.

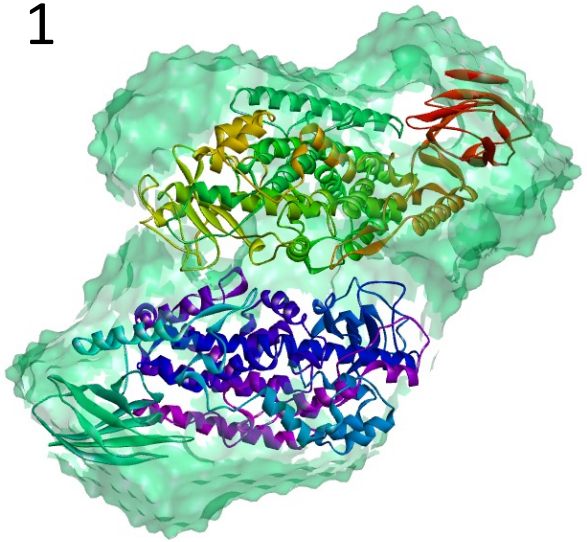


SAXS and SE-HPLC analyses revealed that human 5-LOX in solution is present as homodimer and monomer





1



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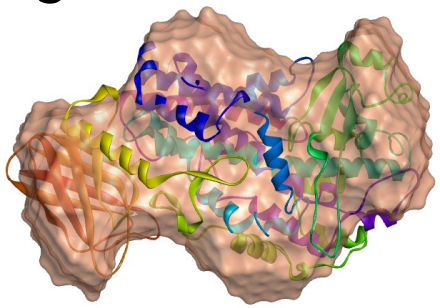
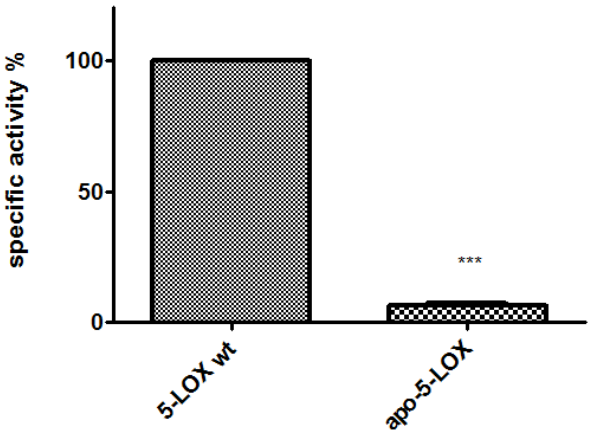
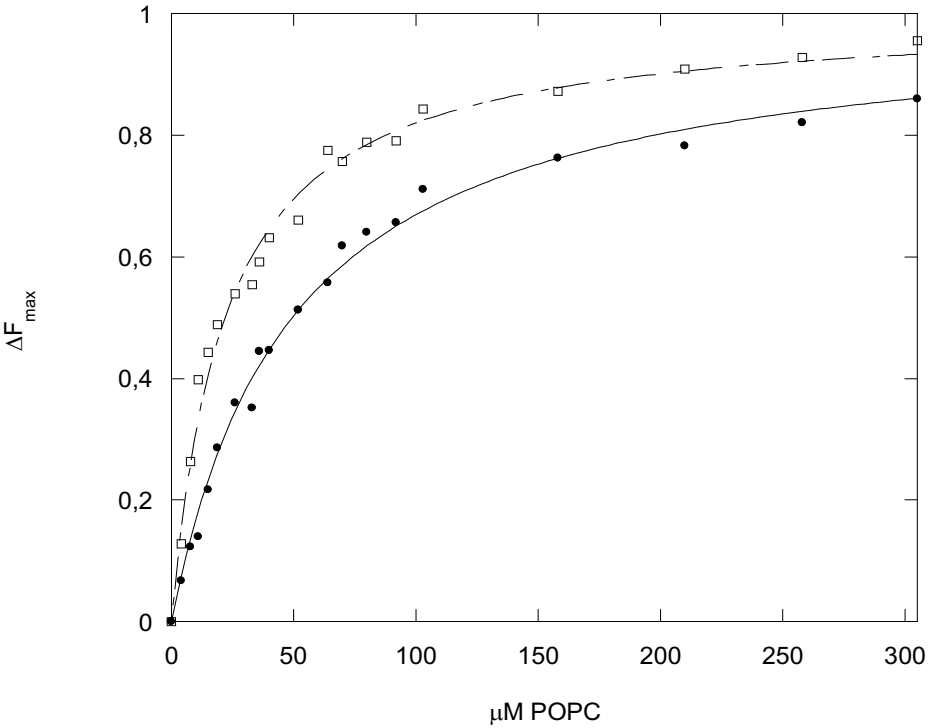
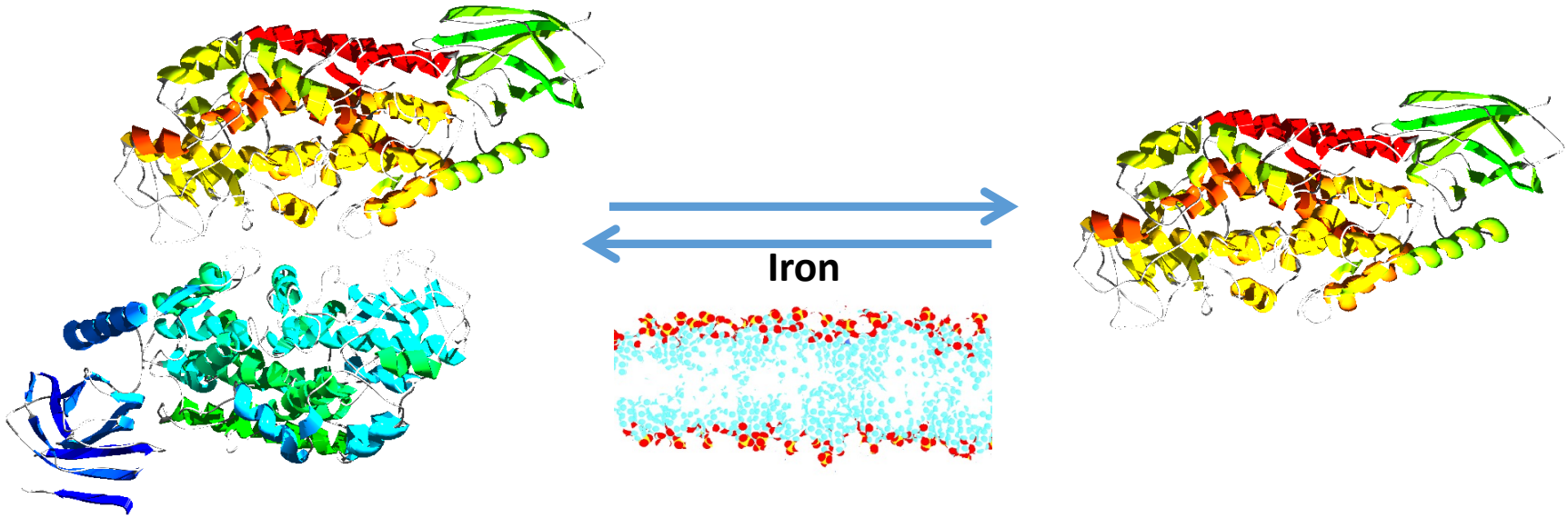


Human 5-LOX iron content modulates the interaction with membranes





Iron could be the main effector in modulating inter- subunit interactions and membrane binding

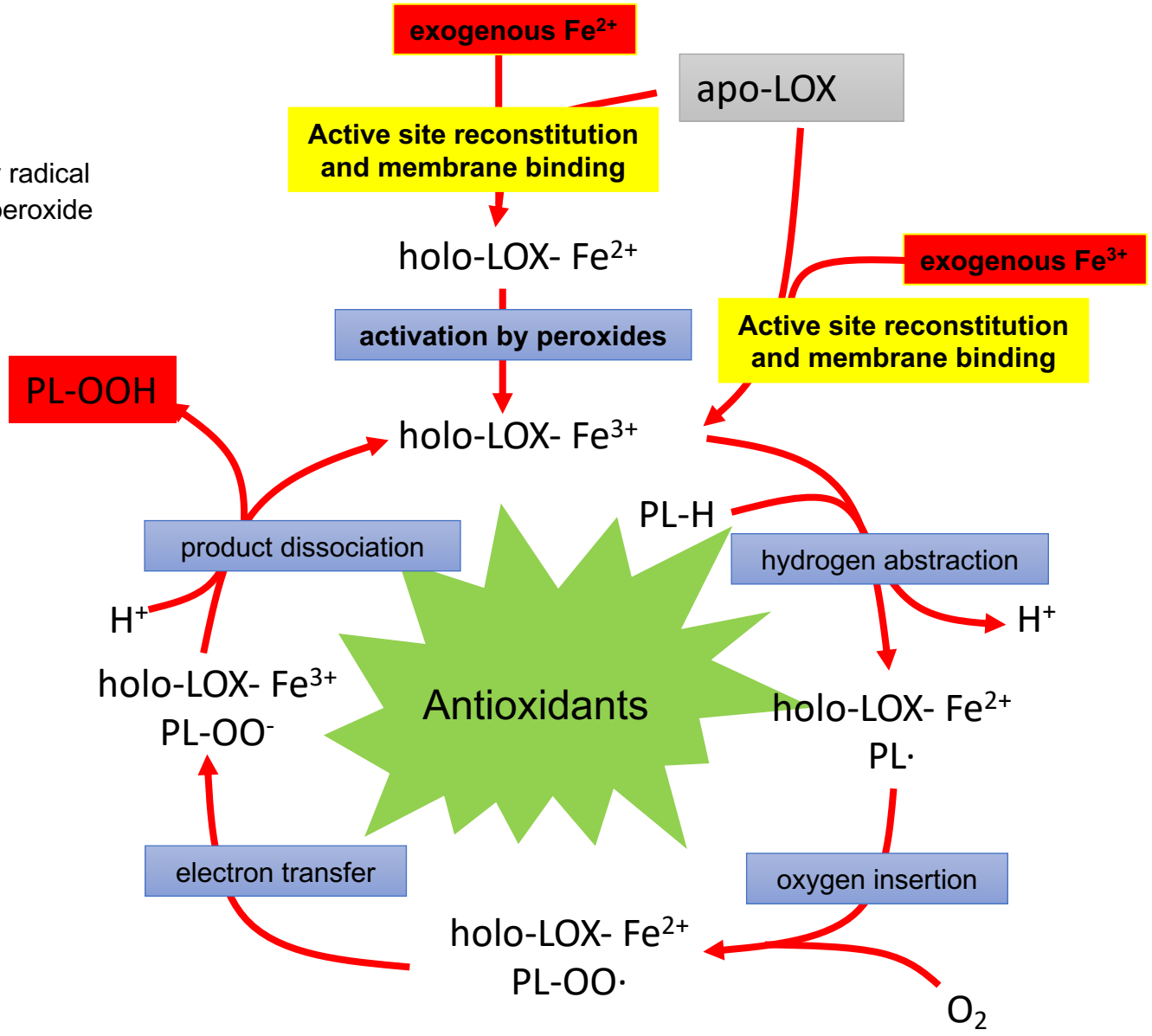


A scheme of new molecular mechanisms leading to a modulation of the LOX-dependent lipid peroxidation

LOX = lipoxygenase
PL-H = phospholipid
PL · = phospholipid radical
PL-OO · = phospholipid peroxy radical
PL-OOH = phospholipid hydroperoxide

= Anti-AD

= Pro-AD



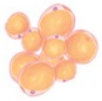
Macrophages are the most plastic cells of the hematopoietic system and are present in all tissues

Macrophages have roles in almost every aspect of an organism's biology ranging from development, homeostasis, to immune responses

Physiological roles of resident macrophages

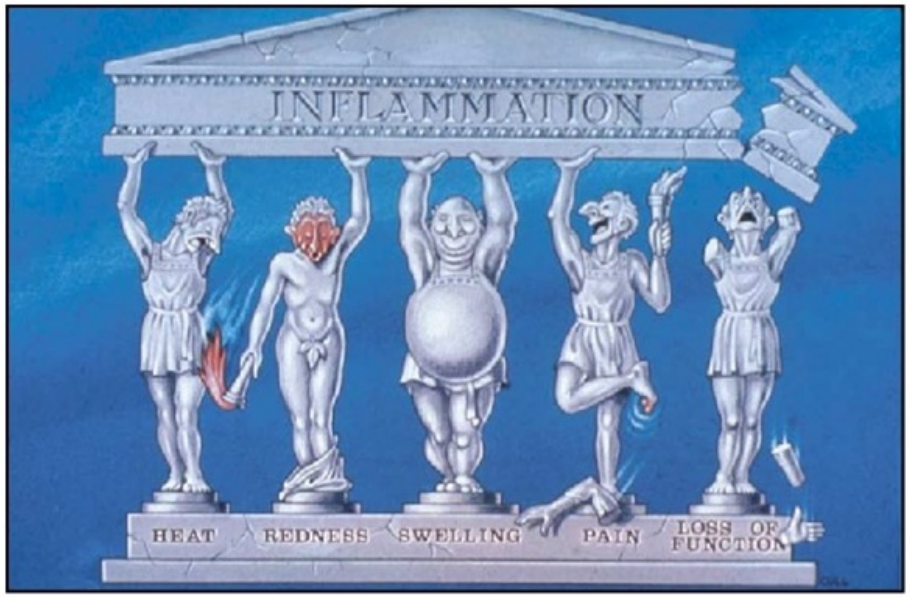
Cell	Tissues	Physiological role
Microglia	Brain	Brain development by sustaining neuron viability and neuronal activity
Kupffer cells	Liver	Clearance of microorganisms and cell debris from the blood, and clearance of aged erythrocytes
Osteoclasts	Bone	Support of erythropoiesis and maintain hematopoietic stem cells in stem cell niches
Alveolar macrophages	Lung	Homeostatic regulation of tissue function
Metallophilic macrophages Red/white pulp macrophage	Spleen	Erythrocyte clearance and iron metabolism

Macrophages are also involved in a variety of disease conditions such as autoimmune disorders, atherosclerosis and tumorigenesis

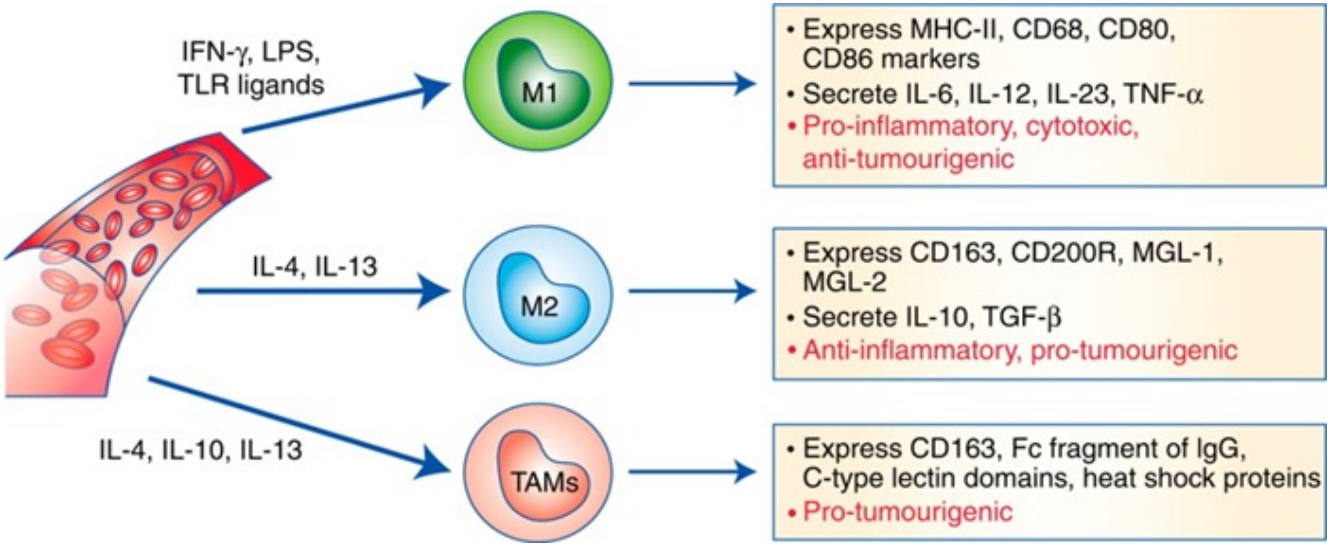
Normal physiology		Pathology
Microglia, (neuronal patterning, fluid balance)		Neurodegeneration
Osteoclasts and macrophages (bone remodelling; haematopoiesis)		Osteoporosis and osteopetrosis Leukemia
Heart and vasculature		Atherosclerosis
Kupffer cells (lipid metabolism, toxin removal)		Fibrosis
Branching morphogenesis		Cancer and metastasis
Metabolism; adipogenesis		Obesity and diabetes
Immunity		Arthritis, EAE, IBD

Wynn et al. Nature 496, 445-455 (2013)

The balance between physiological and phatological function of macrophages is linked to a correct inflammation response



During inflammatory response macrophages undergo to a ***'polarization'*** *process* wherein they express different surface markers and functional programs in response to microenvironmental stimuli, such as cytokines and others signaling mediators.

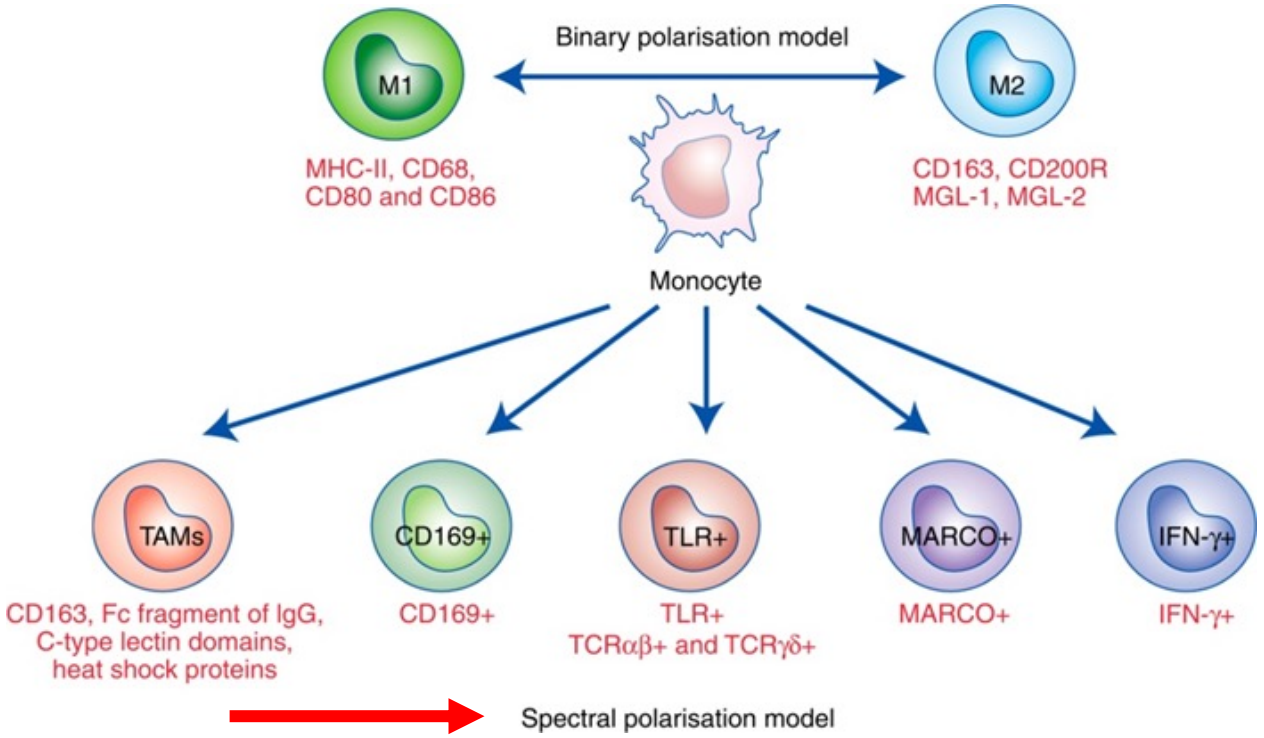


Binary Polarization Model

M1, pro-inflammatory	M2, anti-inflammatory
pro-inflammatory cytokines reactive oxygen/nitrogen species phagocytosis pathogens tumour suppressive effects	anti-inflammatory cytokines tissue homeostasis and repair tumour progression effects

the historical **binary classification** of macrophages is oversimplified for the presence of a network of transcriptional regulators, which determine a **spectrum** of differentiated tissue-macrophages

Spectral Polarization model



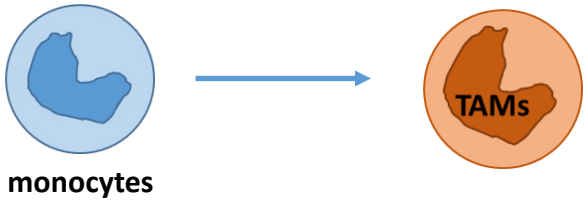
This **spectral polarization model** suggests that various subtypes of differentiated macrophages are contemporary present in tissue.

5-LIPOXYGENASE AND CANCER

Tumour-Associated Macrophages (TAMs)

chemokines:
CCL2, M-CSF
CSF-1, CCL2, IL6

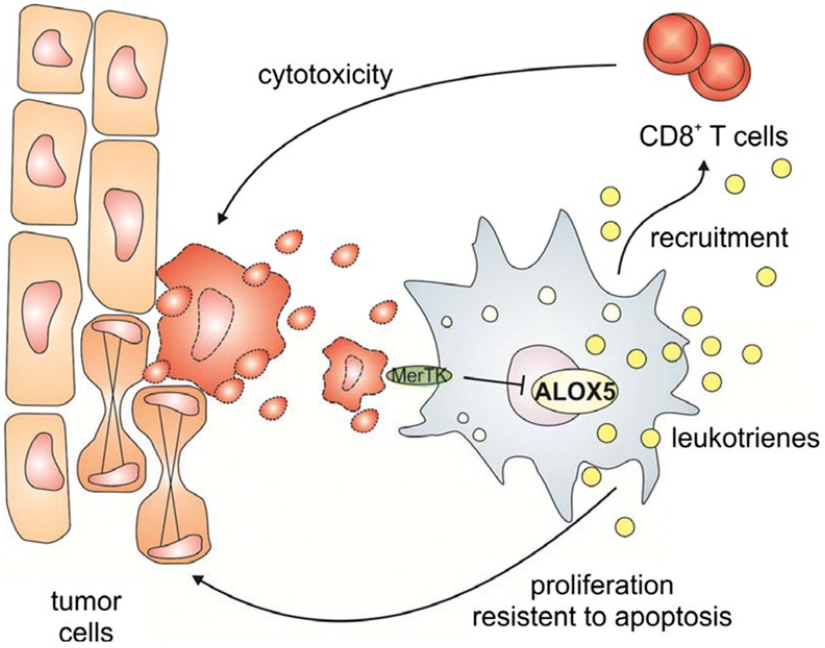
hypoxia-inducible chemotactic factors:
CXCR4 ligand
CXCL12,
Angiopoietin-2



Angiogenesis
Immunosuppression
Migration and Ivasion
Epithelial Mesenchymal Transition
Interaction with Cancer Stem Cells

the role of 5-Lipoxygenase in TAMs

- increased expression of 5-LOX and its products are found in several tumors including breast, colon, and prostate cancer;
- the expression of 5-LOX correlated with the density of TAMs;
- the metabolites of 5-LOX promote TAM infiltration.



Weigert et al., cancer and metastasis reviews 37, 317–334 (2018)

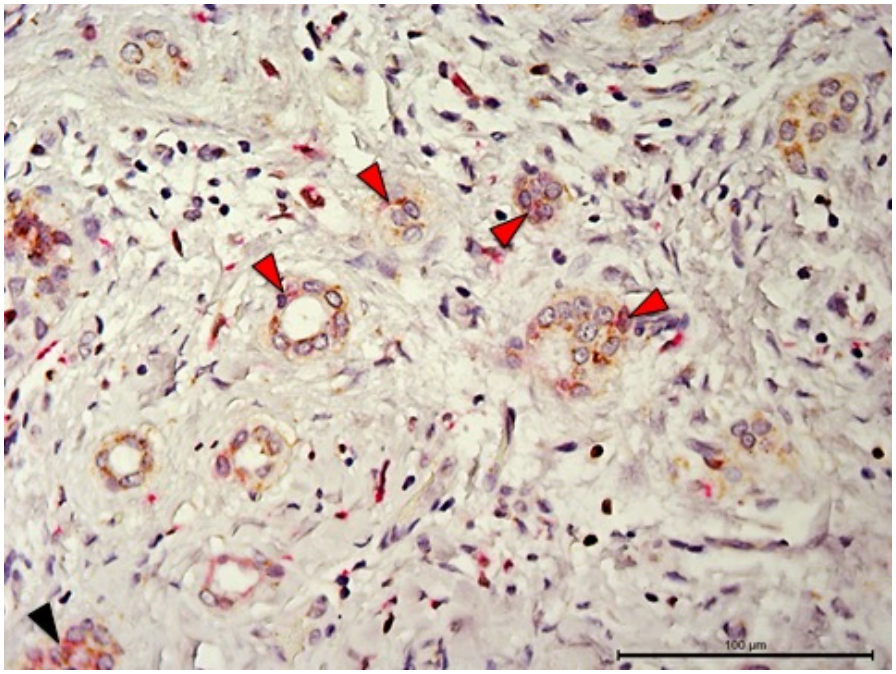
5-LIPOXYGENASE AND CANCER

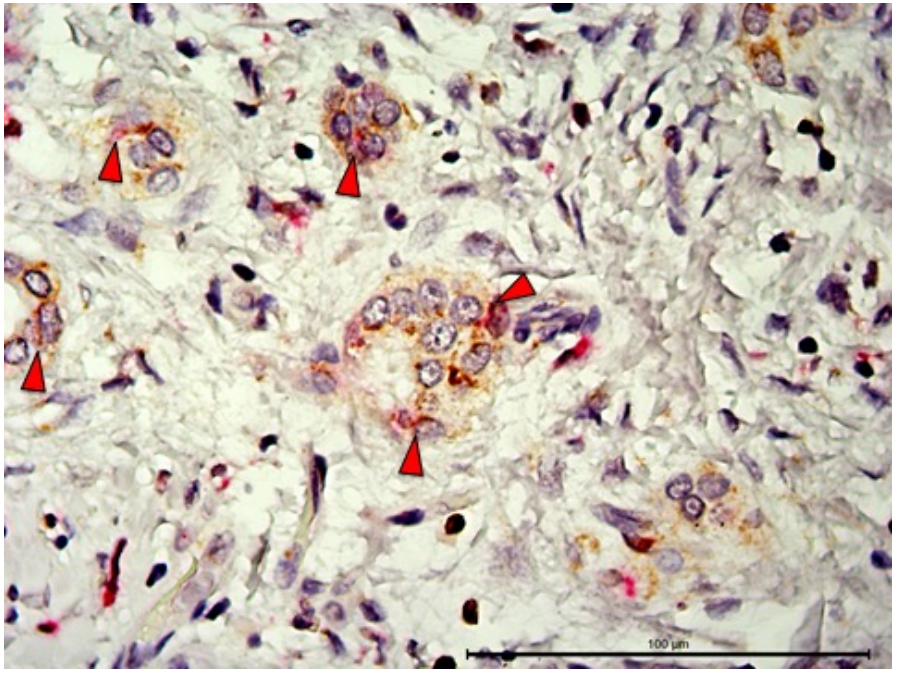
Aberrant expression of 5-LOX and its products is found in several types of tumors such as breast, prostate, pancreatic, and colon cancer.

Enhanced expression and activity of 5-LOX control cancer cell viability, cell migration, invasion through extracellular matrix destruction and metastasis.

Tumor Associated Macrophages (TAMs) are the major leukocyte component of the tumor microenvironment that contributes to all stages of tumor progression.

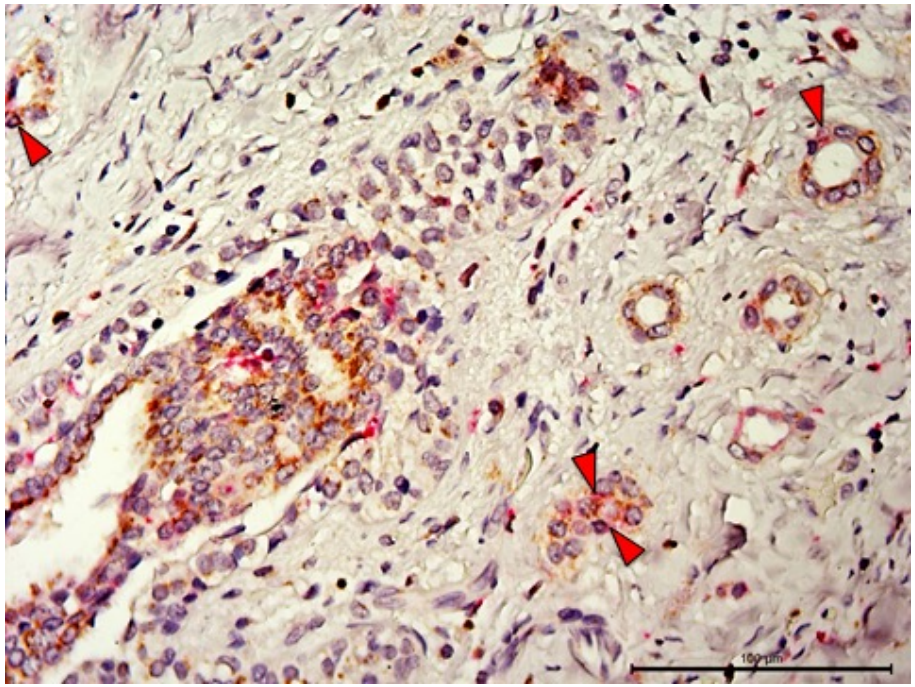
5-LOX / TAMs breast cancer

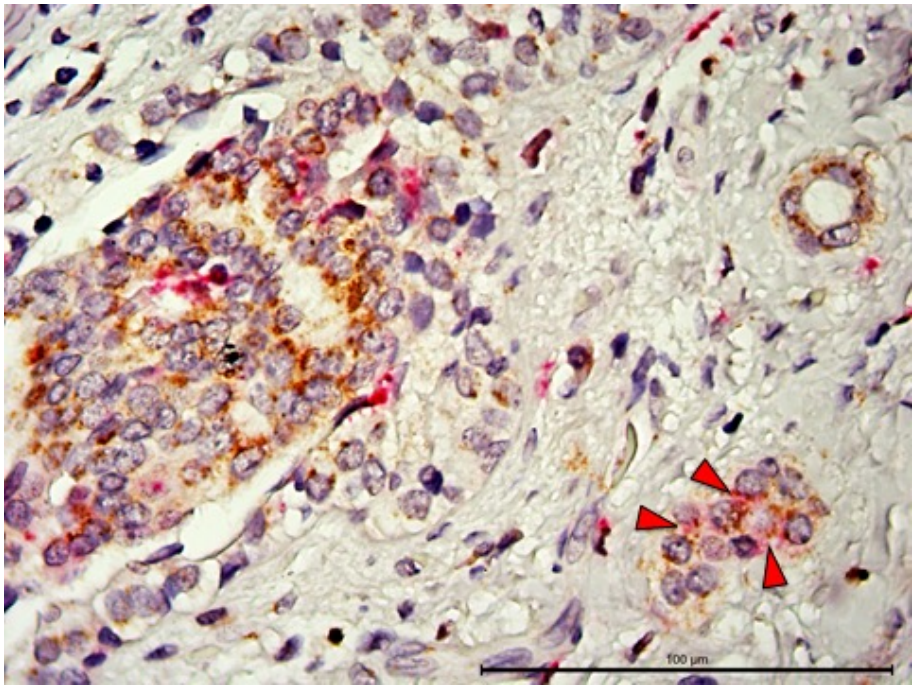




Invasive breast cancer cells showed higher 5-LOX expression
scattered CD68 positive cells were found between tumor cells

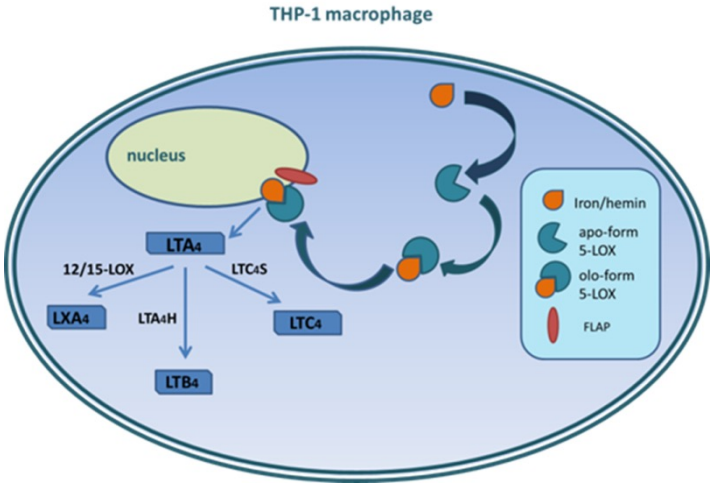
5-LOX / TAMs breast cancer





Invasive breast cancer cells showed higher 5-LOX expression
Diffusely CD68 positive cells were present in peritumoral stroma

- These results showed a crucial role for iron in preserving the structural stability and membrane binding ability of the enzyme;
- Being the presence of an apo-form of the enzyme previously documented in several mammalian cells, iron controls *in vivo* the 5-LOX trafficking among different compartments of the cell, and hence modulating their signalling;
- In keeping with the *in vitro* studies, the localization of the enzyme has a also a clinical value. The 5-LOX localization depending by iron can be translated to the clinical practice as a prognostic factor; c-LOX-5High expression was significantly associated with higher **local relapse free survival (LRFS).**



CONCLUSIONS

- Aberrant formation of 5-LOX metabolites is tightly associated with several neurodegenerative diseases (e.g. AD) and different tumors such as breast, prostate, pancreatic, and colon cancers.
- Iron dependent 5-LOX subcellular localization and trafficking are fundamental for macrophage activation;
- The amount of apo-form found *in vivo* in mammalians is very high (up to 70%), but a structural and functional characterization of this form is still lacking;
- Iron modulates 5-LOX intracellular localization by increasing the ability of the enzyme to bind to nuclear membranes thus activating the 5-LOX-mediated inflammatory processes.
- New potentially important mechanism regarding the role of 5-LOX in the functional activation of macrophages, and may advance our understanding of the risks associated to iron overloading.