

PHD PROJECT PRESENTATION



**STUDY OF THE MOLECULAR
MECHANISMS UNDERLYING
IRON DEPLETION-INDUCED PEXOPHAGY**

SUPERVISOR

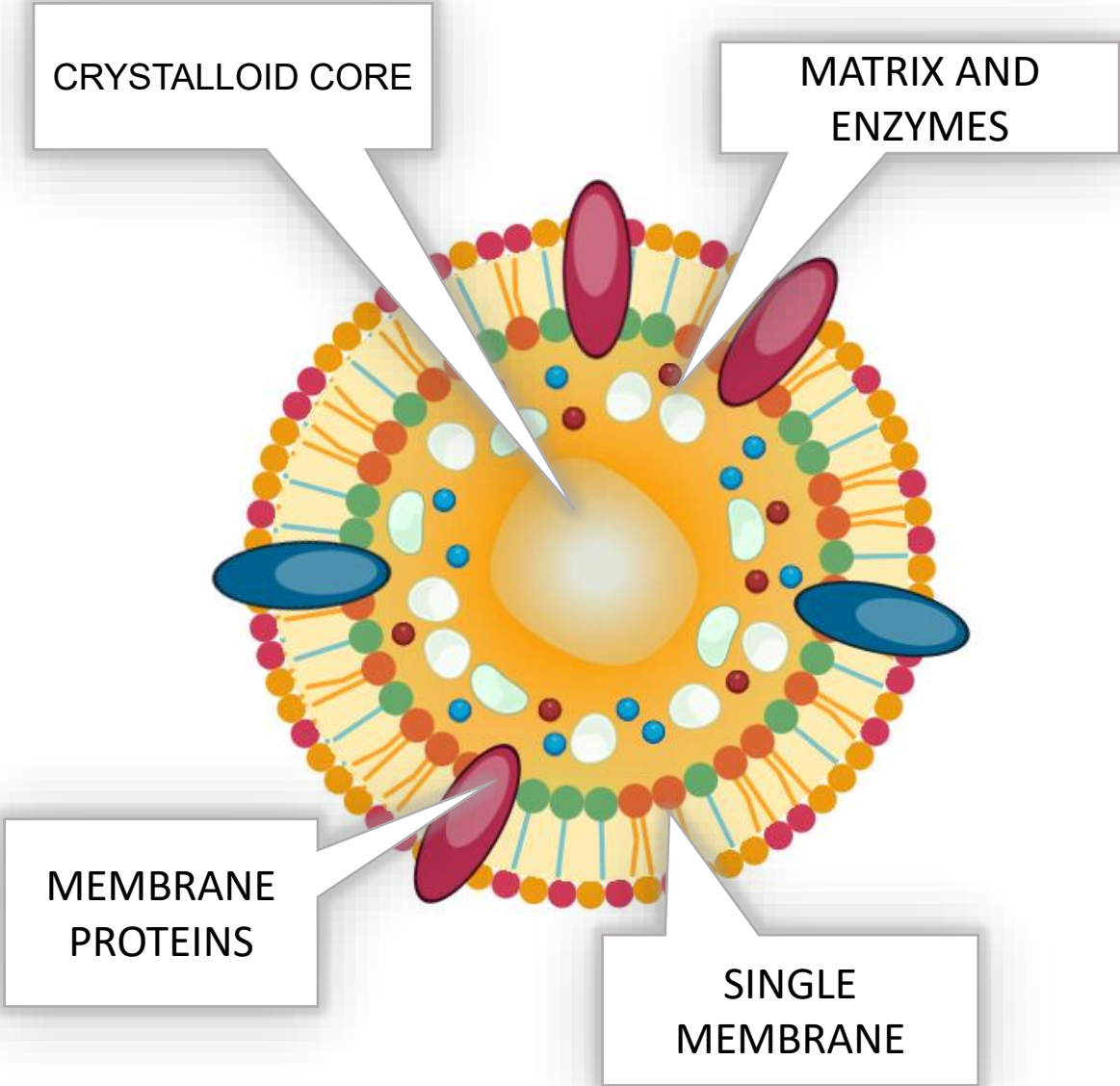
Assist. Prof. Valentina Cianfanelli

Rome, March 14th 2025

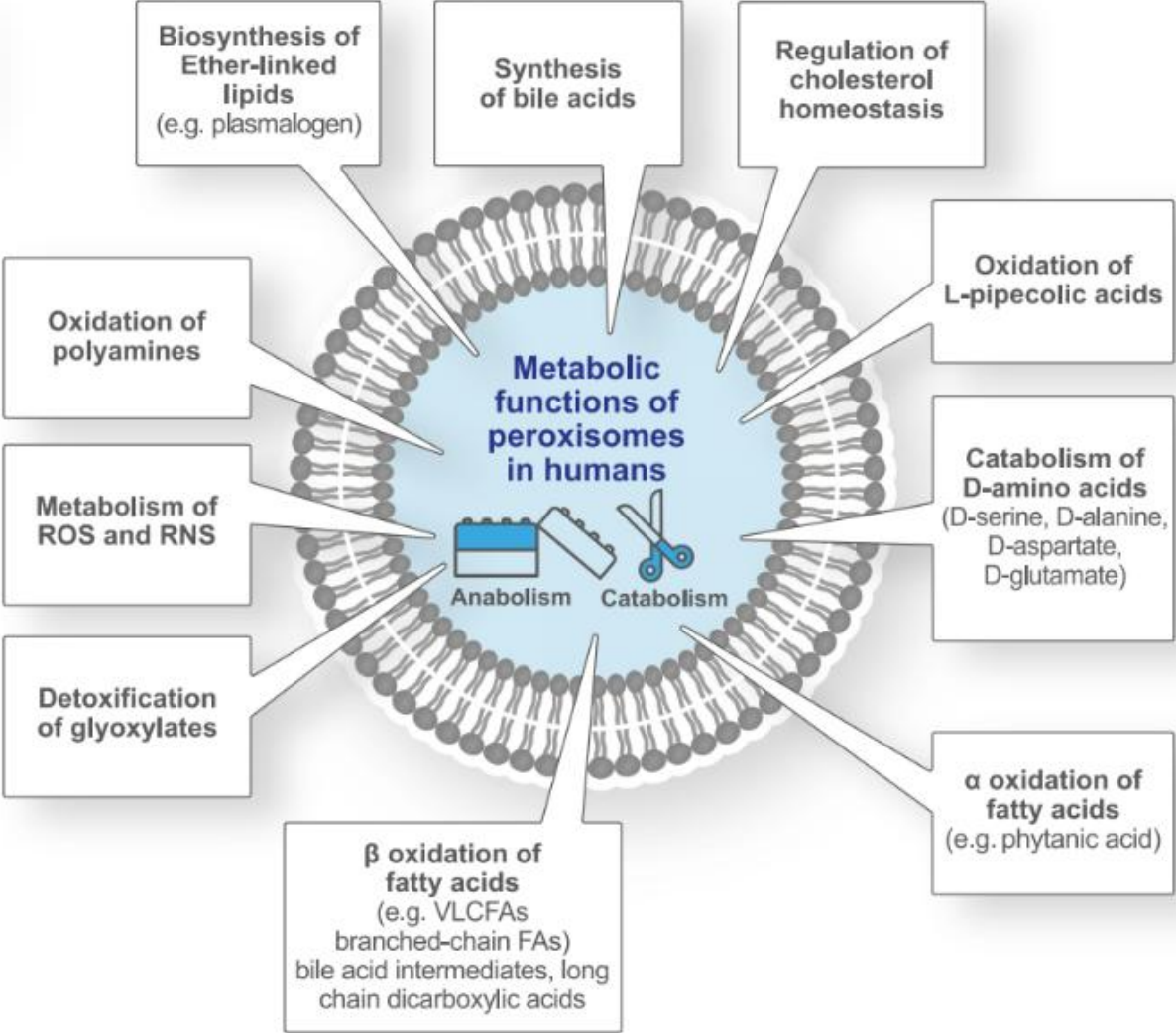
PhD STUDENT

Michele Petraroia

BACKGROUND: PEROXISOME STRUCTURE AND FUNCTIONS

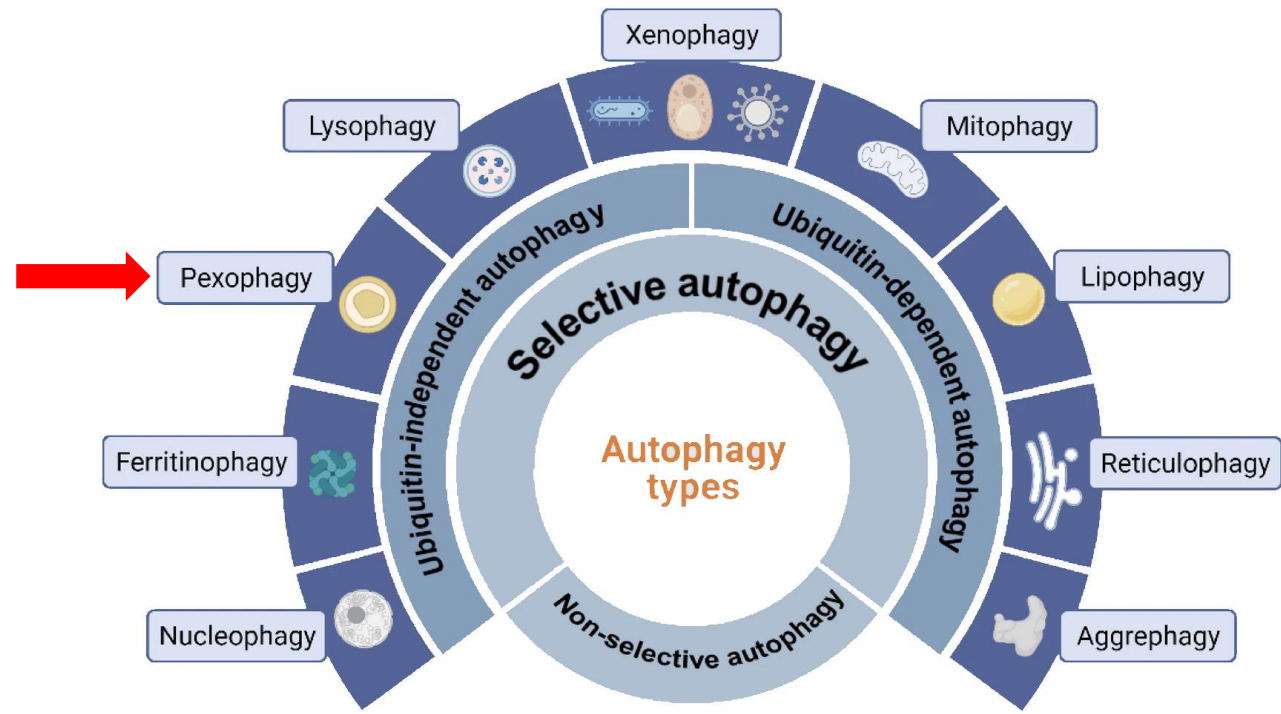
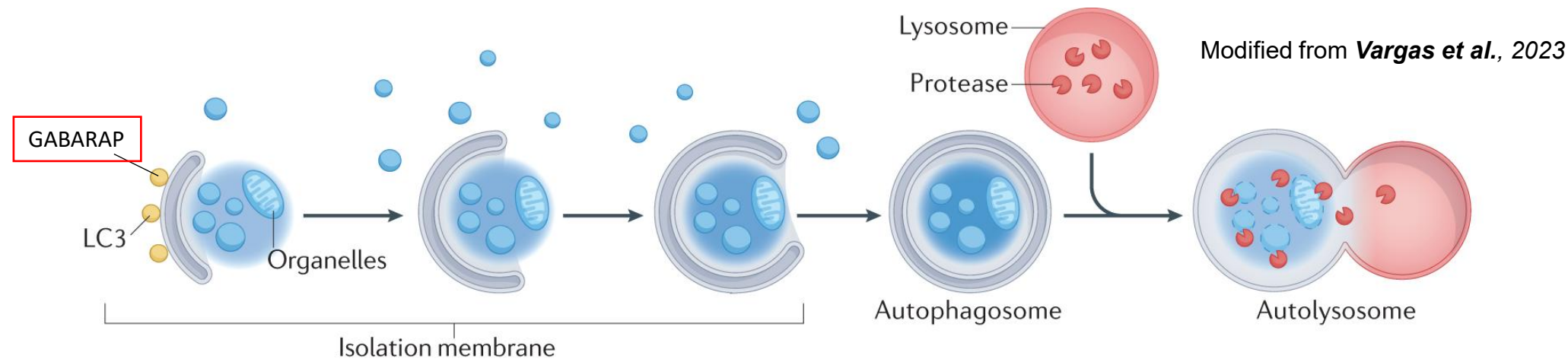


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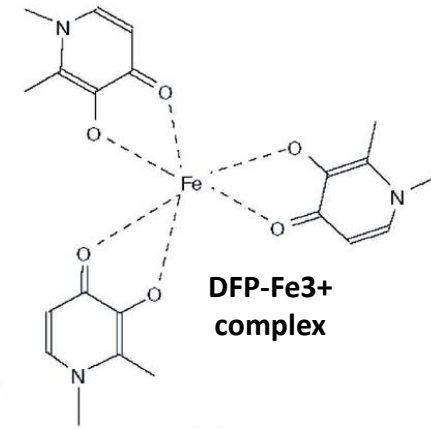
Zalckvar & Schuldiner, Molecular Cell, 2022

BACKGROUND: AUTOPHAGY – GENERAL FEATURES

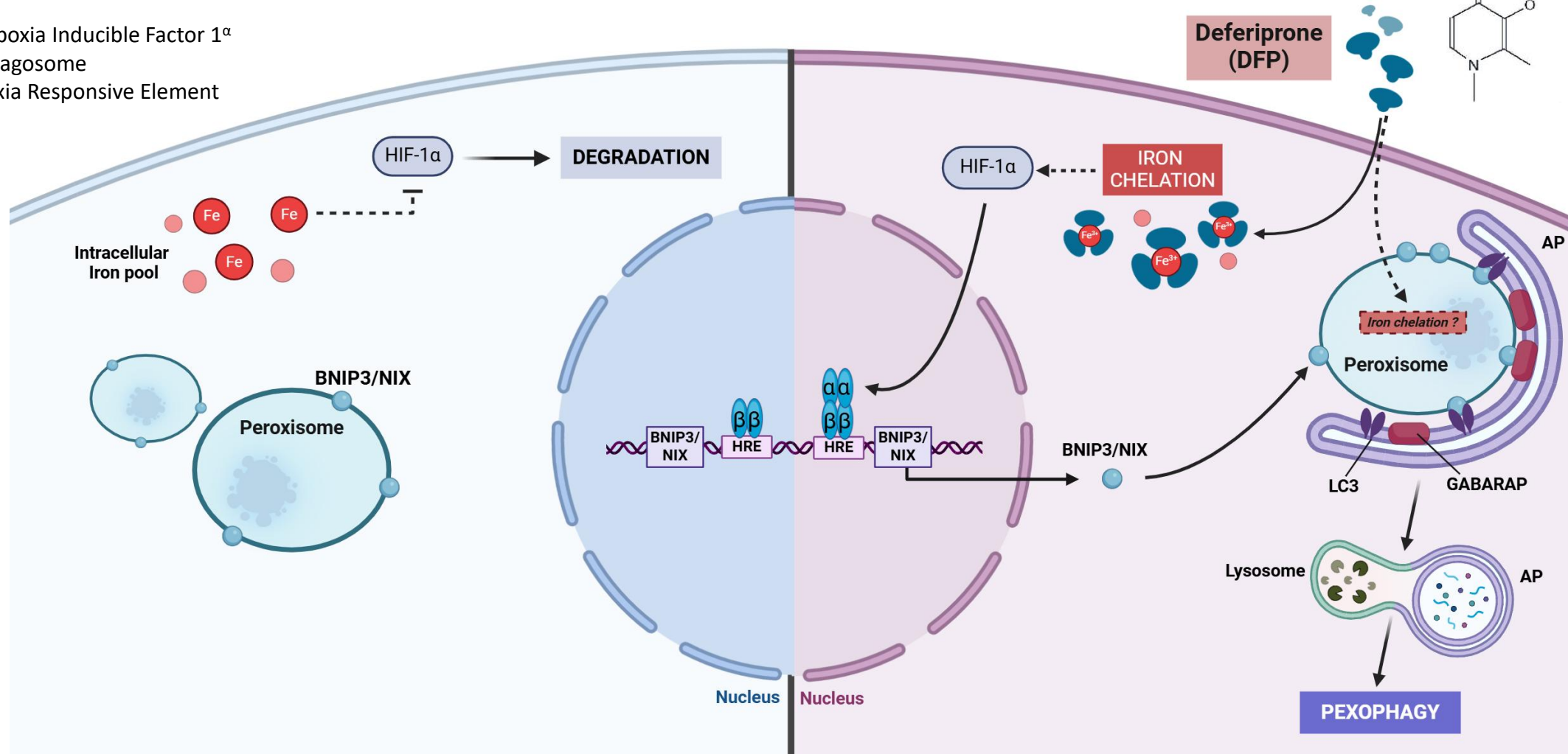


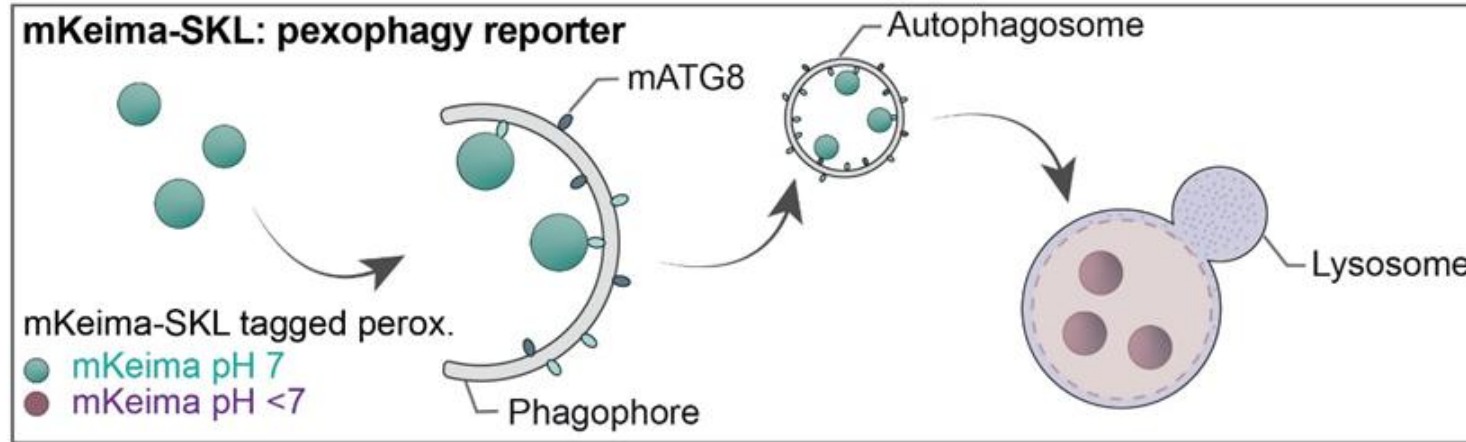
Iron depletion has been identified as a pexophagy inducer

(Wilhelm *et al.*, 2022)



HIF-1 α : Hypoxia Inducible Factor 1 α
 AP: Autophagosome
 HRE: Hypoxia Responsive Element



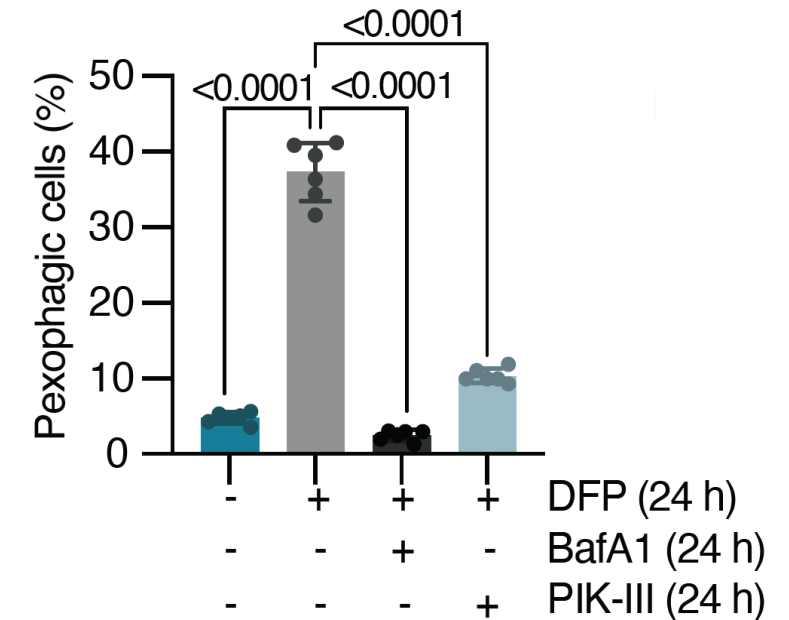


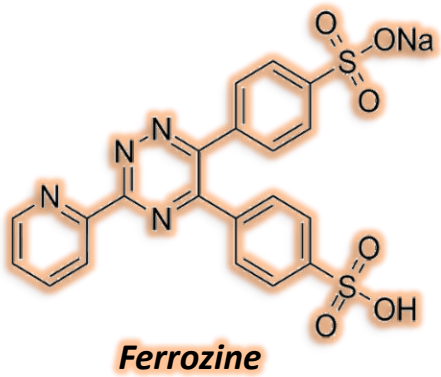
Exogenous reporters of pexophagy

- SKL-Keima: fluorescent reporter to monitor pexophagy through cytofluorimetry and fluorescence microscopy

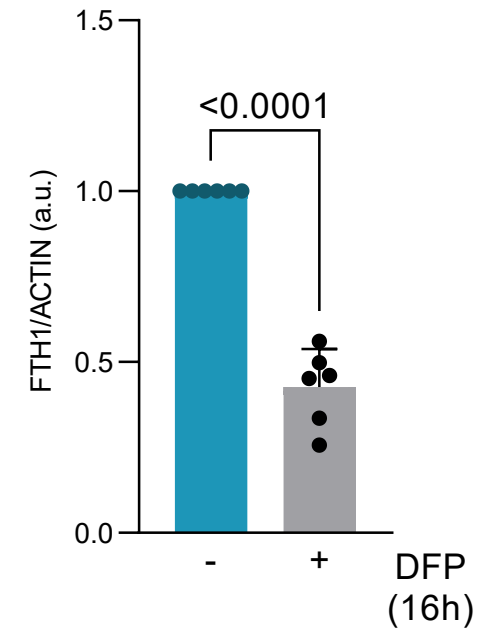
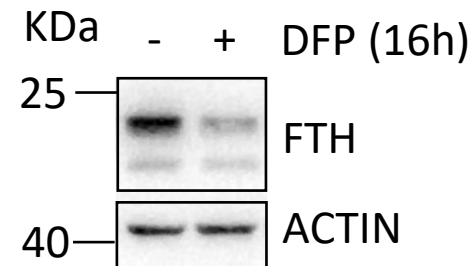
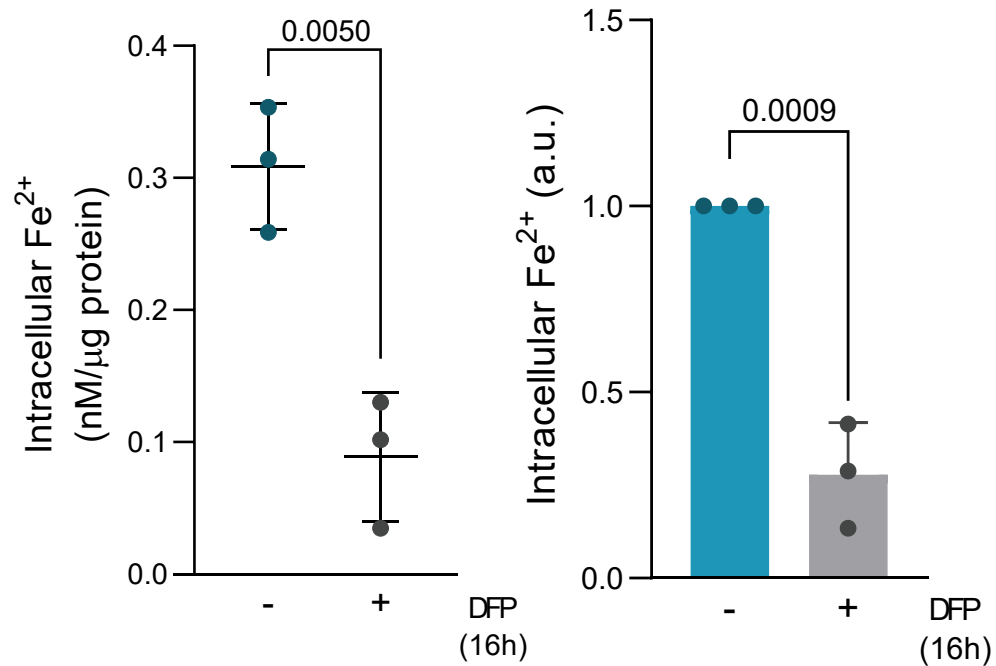
Endogenous reporters of pexophagy

- Peroxisomal markers (e.g. PMP70);

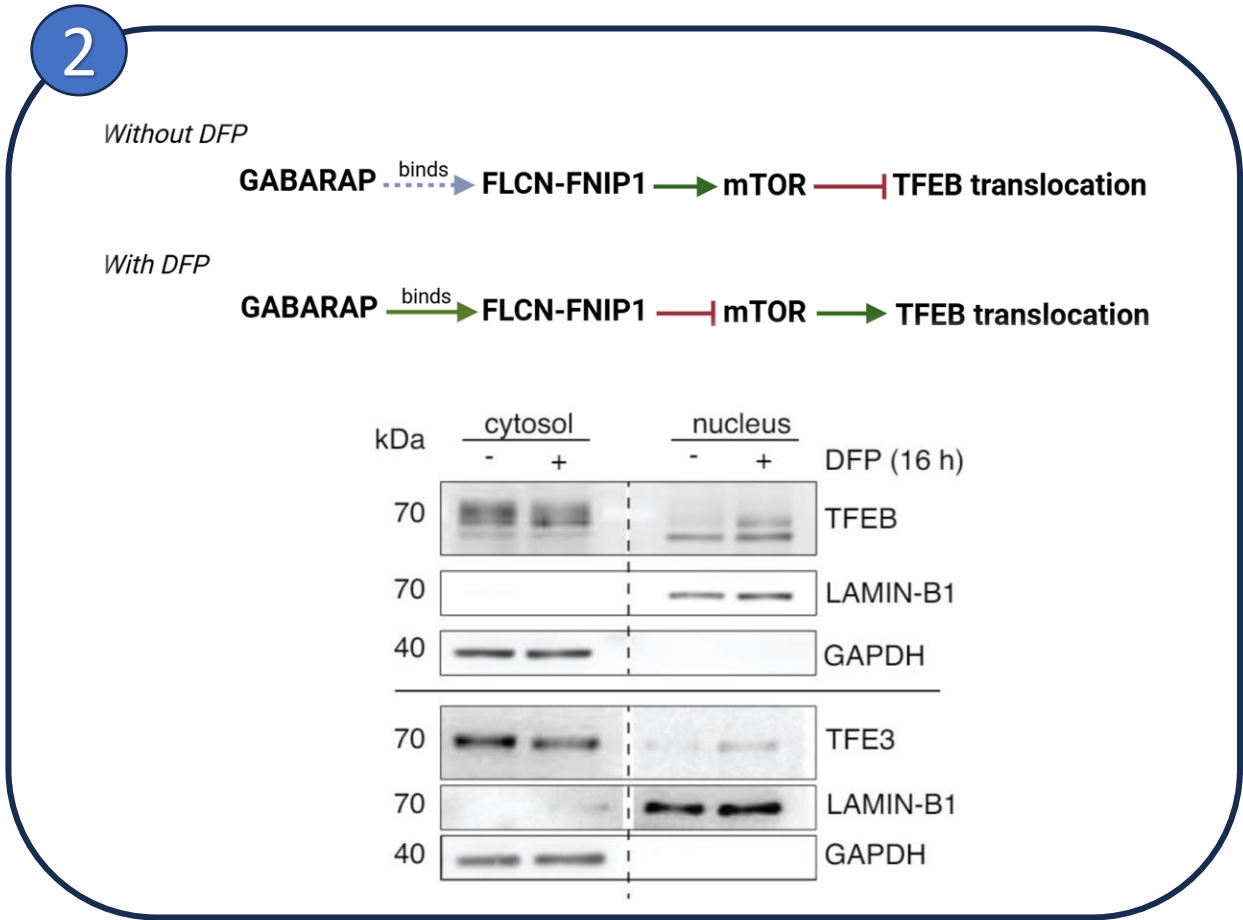
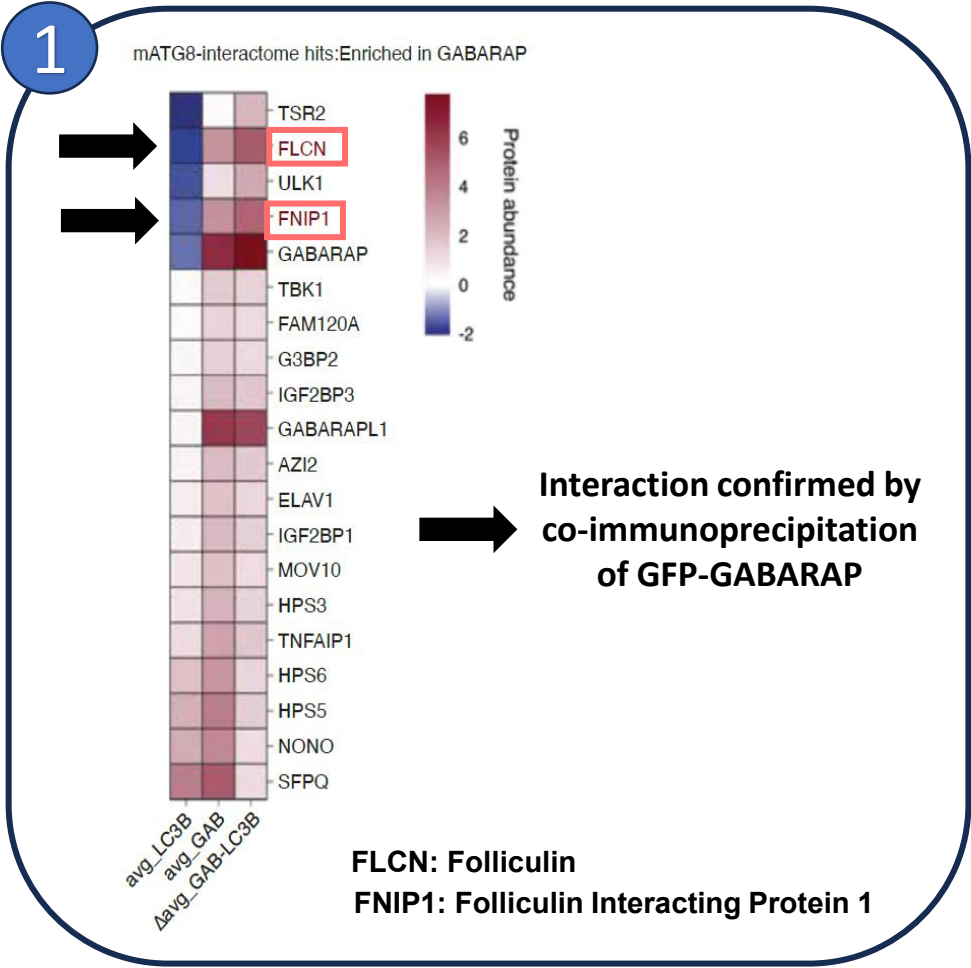




DFP treatment reduces total intracellular iron



Iron-deficiency activates the GABARAP-FLCN/FNIP1-TFEB axis to promote pexophagy



OVERALL GOAL OF THE PROJECT

Characterization of novel molecular mechanisms underlying **iron chelation-induced pexophagy**, and involving **protein phosphorylation**, **peroxisomal iron homeostasis**, and **ROS production at peroxisomes**

AIM1

Study of protein phosphorylation in the activation of FLCN-FNIP1-GABARAP axis in DFP-induced pexophagy

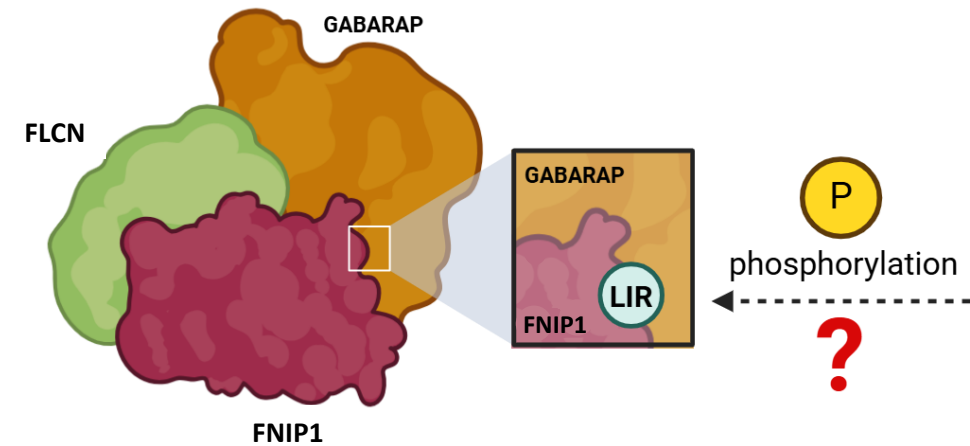
AIM2

Role of the ubiquitin ligase enzyme MARCH5 and ROS as triggers for iron depletion-induced pexophagy

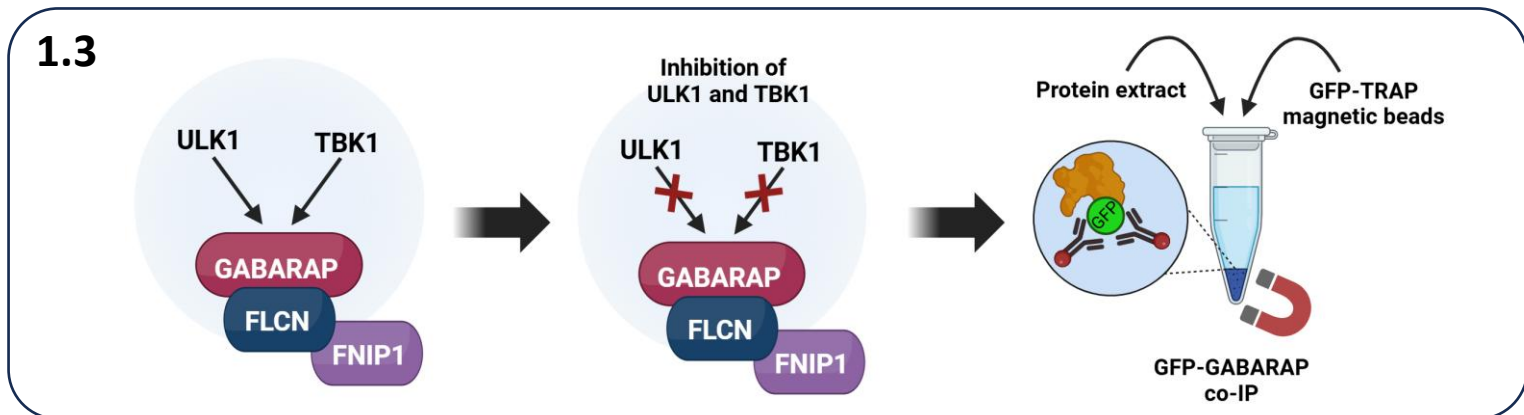
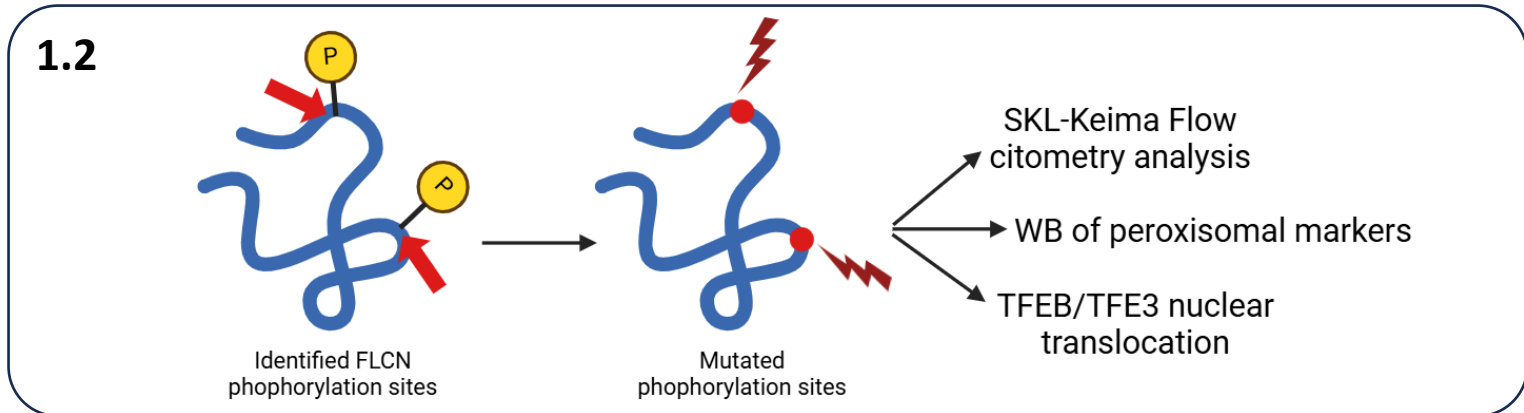
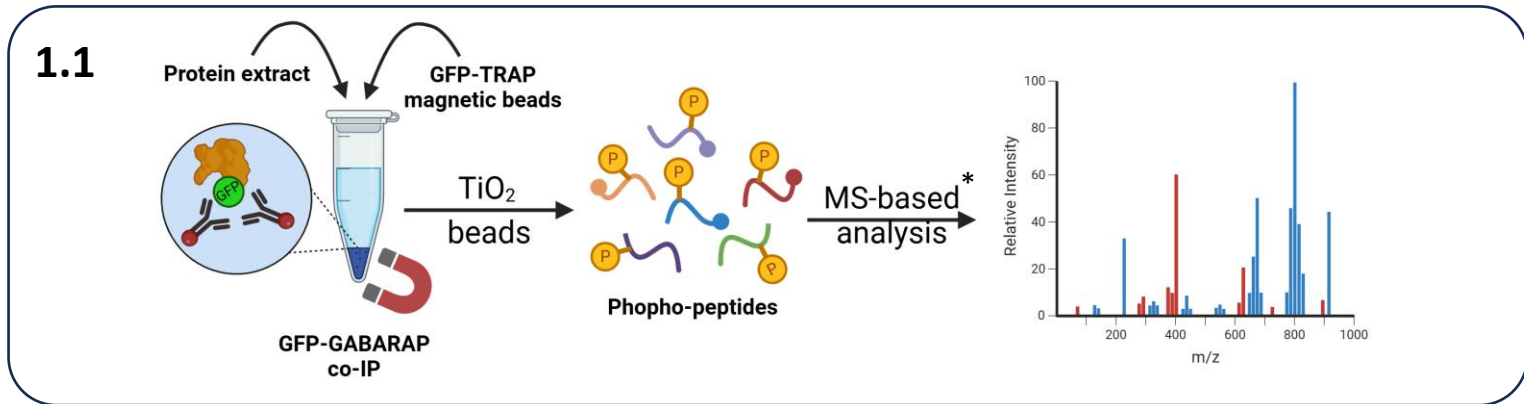
AIM3

Characterization of cytosolic and peroxisomal iron depletion as a mechanism of pexophagy induction

AIM 1. REGULATION OF FLCN/FNIP1-GABARAP AXIS IN DFP-INDUCED PEXOPHAGY



LIR: LC3-Interacting Region

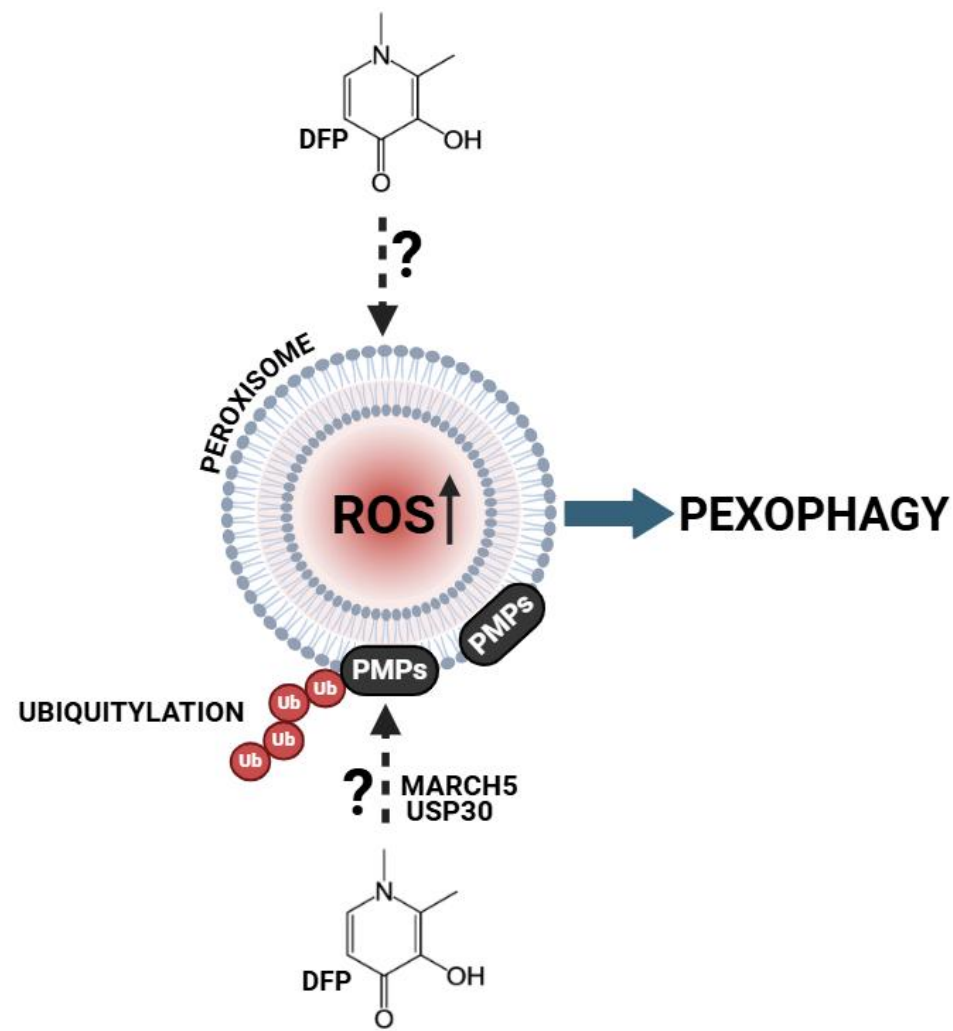


*in collaboration with Prof. Jorn Dengjel,
University of Fribourg, Switzerland

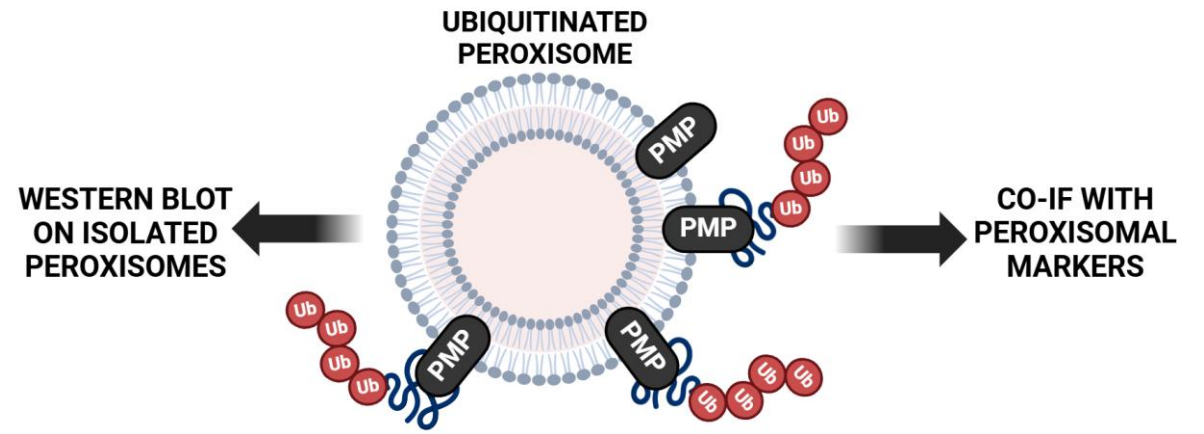
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AIM 2. UBIQUITINATION AND ROS PRODUCTION AT THE PEROXISOME

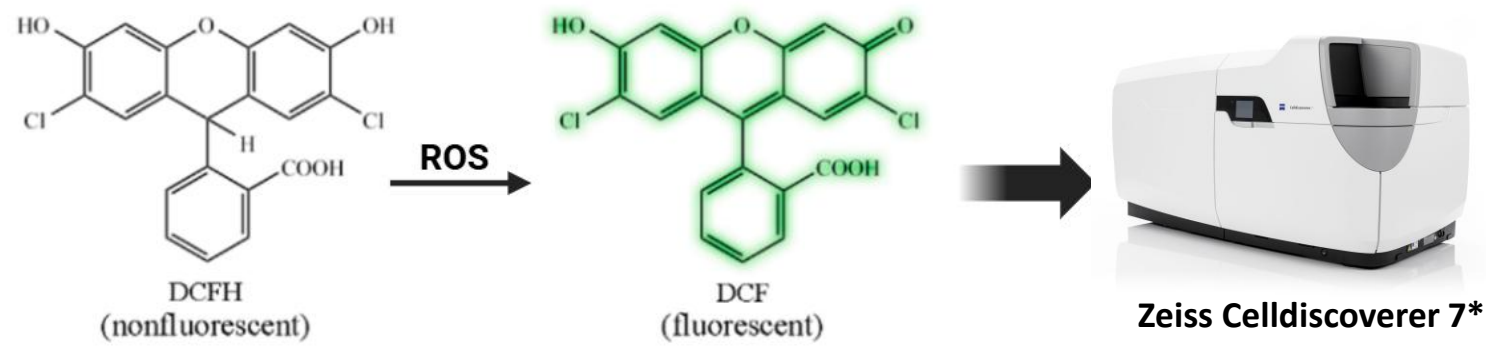
PMP: Peroxisome Membrane Protein



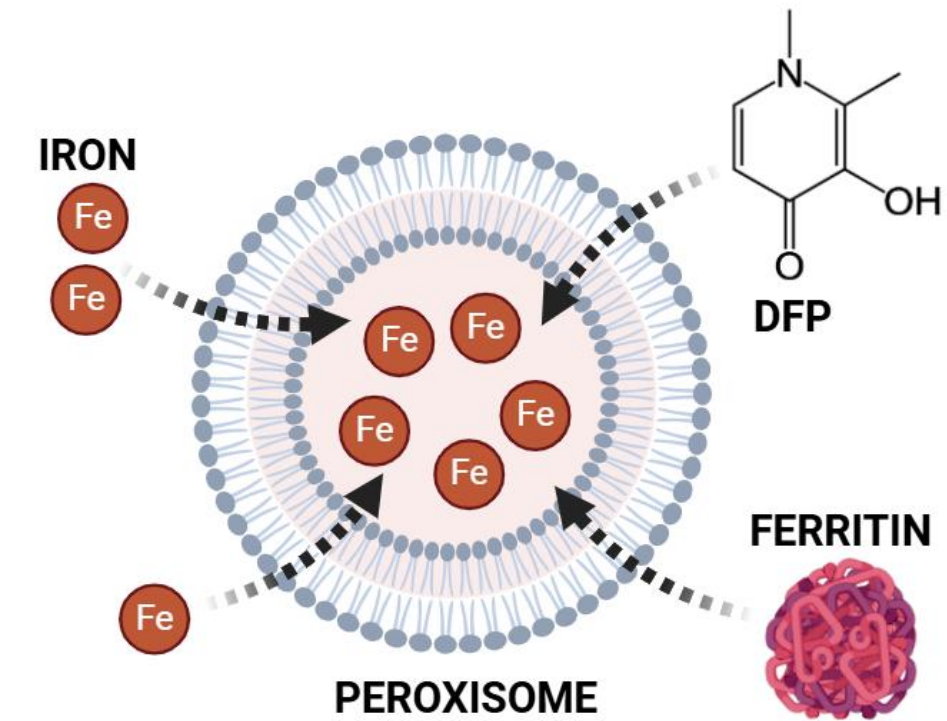
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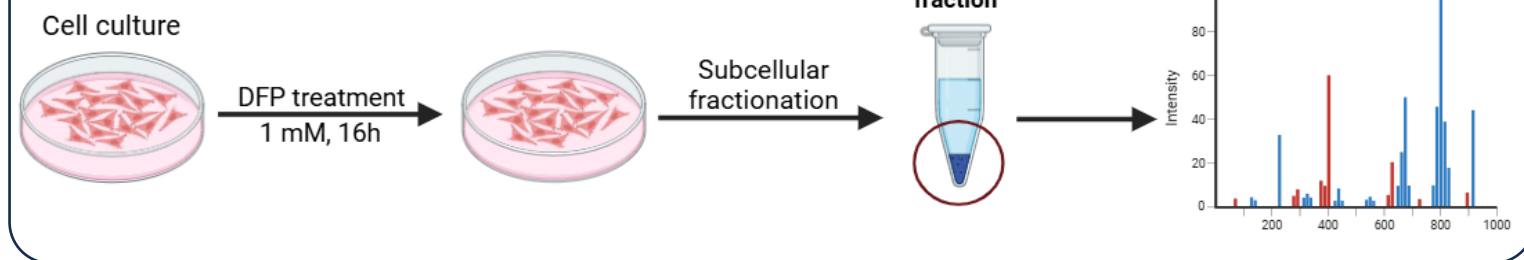
2.2



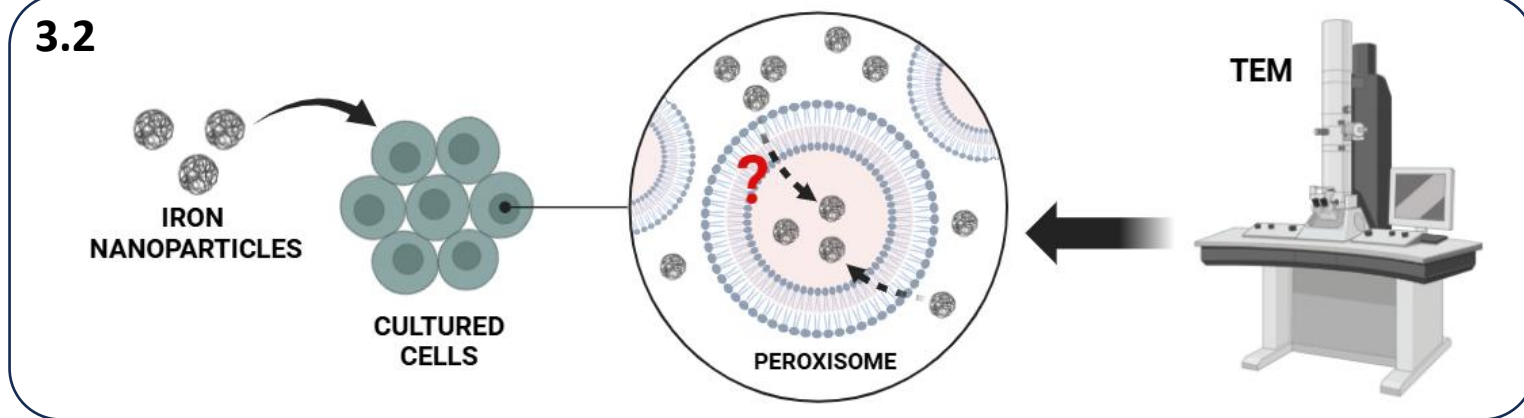
AIM 3. CHARACTERIZATION OF CYTOSOLIC AND PEROXISOMAL IRON DEPLETION AS A MECHANISM OF PEXOPHAGY INDUCTION



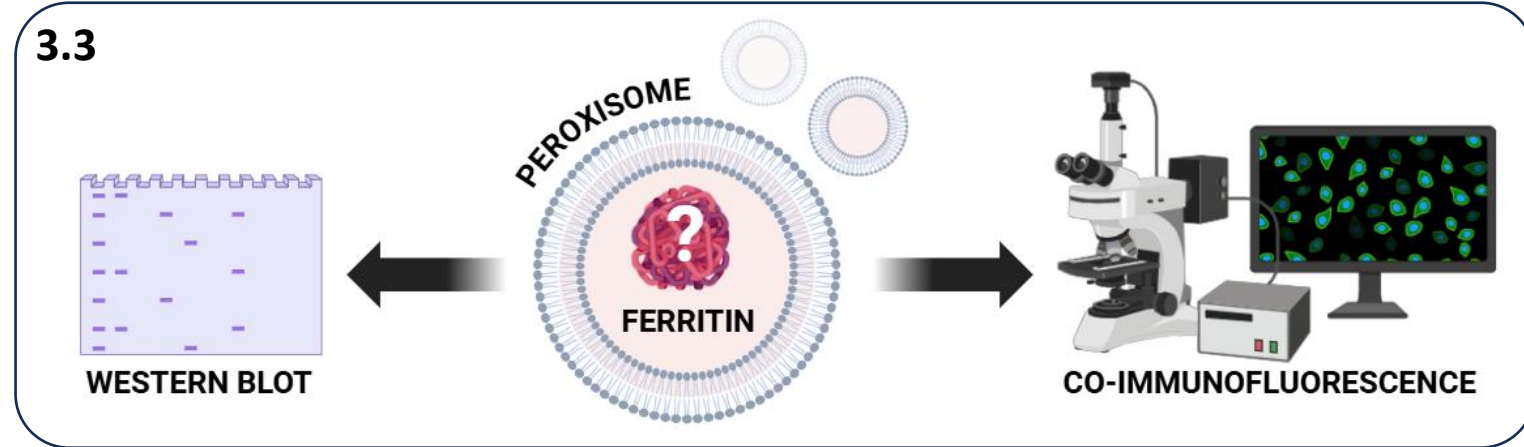
3.1



3.2



3.3



- Identification of **new phosphorylation sites and protein kinases** for the regulation of FLCN-FNIP1-GABARAP axis relevant to DFP-induced pexophagy;
- Characterization of peroxisomes as **functionally active organelles for iron storage** and DFP-unbalance of peroxisomal iron as a pexophagy inducer;
- Identification of **iron chelation as a cause of ROS production at the peroxisomes**, resulting into pexophagy induction;

ACKNOWLEDGEMENTS

Valentina Cianfanelli and all Lab members

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Prof. Francesco Cecconi Lab
Univ. Cattolica del Sacro Cuore – Italy



THANKS FOR YOUR ATTENTION

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**PRIN 2022 PNRR
Under 40**

Collaborators

Prof. Marco Colasanti, Prof.ssa Tiziana Persichini
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Prof. Jorn Dengjel
University of Fribourg – Switzerland

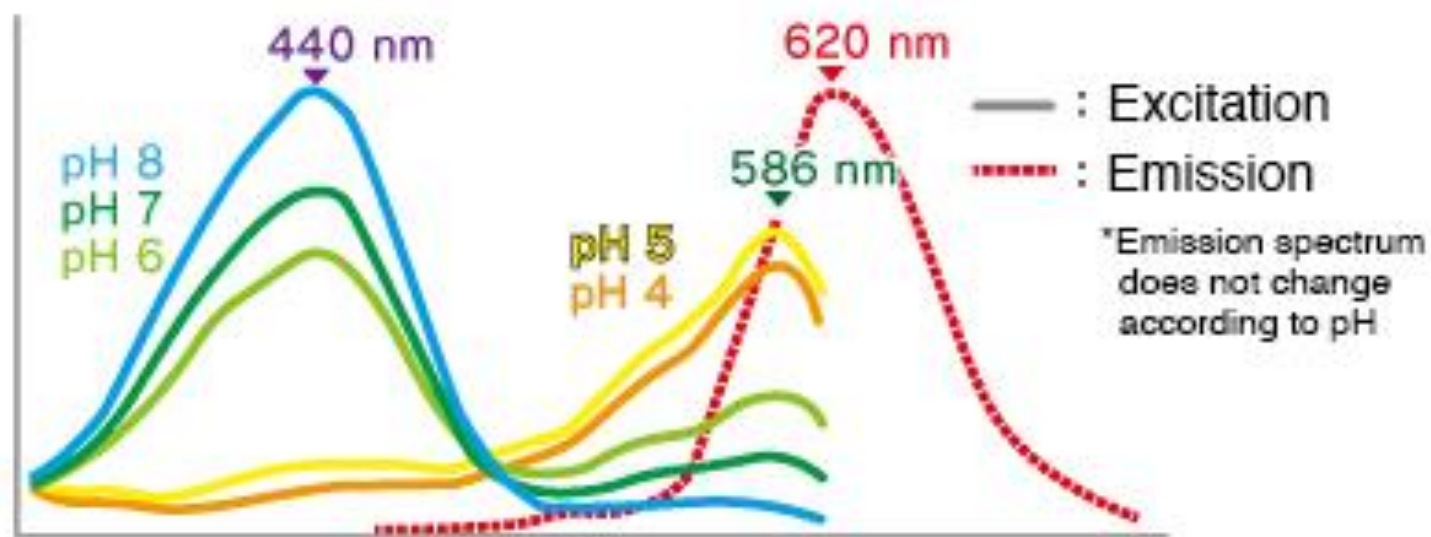
Chiara di Malta, PhD
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Giacomo Milletti, PhD
Danish Cancer Institute – Denmark

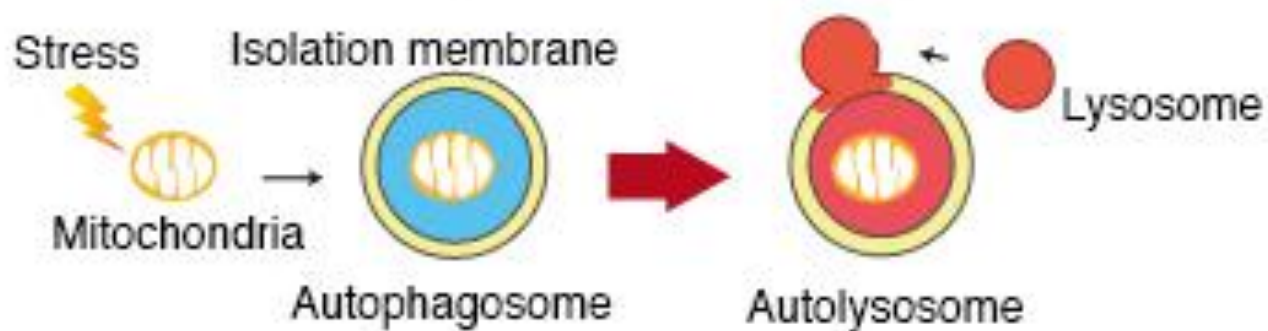
Prof. Paolo Porporato, Prof.ssa Simonetta Geninatti Crich, Simona Baroni, PhD
University of Turin - Italy

			MONTHS											
			0-3	3-6	6-9	9-12	12-15	15-18	18-21	21-24	24-27	27-30	30-33	33-36
AIM 1	Regulation of FLCN-FNIP1-GABARAP axis in DFP-induced pexophagy													
	Activity 1.1	Identification of DFP-induced phosphorylation sites on FLCN-FNIP1-GABARAP												
	Activity 1.2	Functional characterization of DFP-induced phosphorylation sites on FLCN-FNIP1-GABARAP in pexophagy												
	Activity 1.3	Characterization of ULK1 and TBK1 kinases in pexophagy regulation mediated by FLCN-FNIP1-GABARAP												
AIM 2	Characterization of cytosolic and peroxisomal iron depletion as a mechanism of pexophagy induction													
	Activity 2.1	Measurement of iron content in purified peroxisomes upon DFP treatment												
	Activity 2.2	Peroxisomal uptake of iron nanoparticles by TEM analysis												
	Activity 2.3	Investigation of ferritin presence at the peroxisome and its relevance in iron storage												
AIM 3	Characterization of iron depletion as a cause of ROS production at the peroxisome													
	Activity 3.1	Study of iron depletion impact on ROS production at the peroxisome												
	Activity 3.2	Investigation of the ubiquitin role in pexophagy induced by peroxisomal ROS, upon DFP												
	Activity 3.3	Functional characterization of E3 ligases/deubiquitinase in pexophagy induced by peroxisomal ROS, upon DFP												



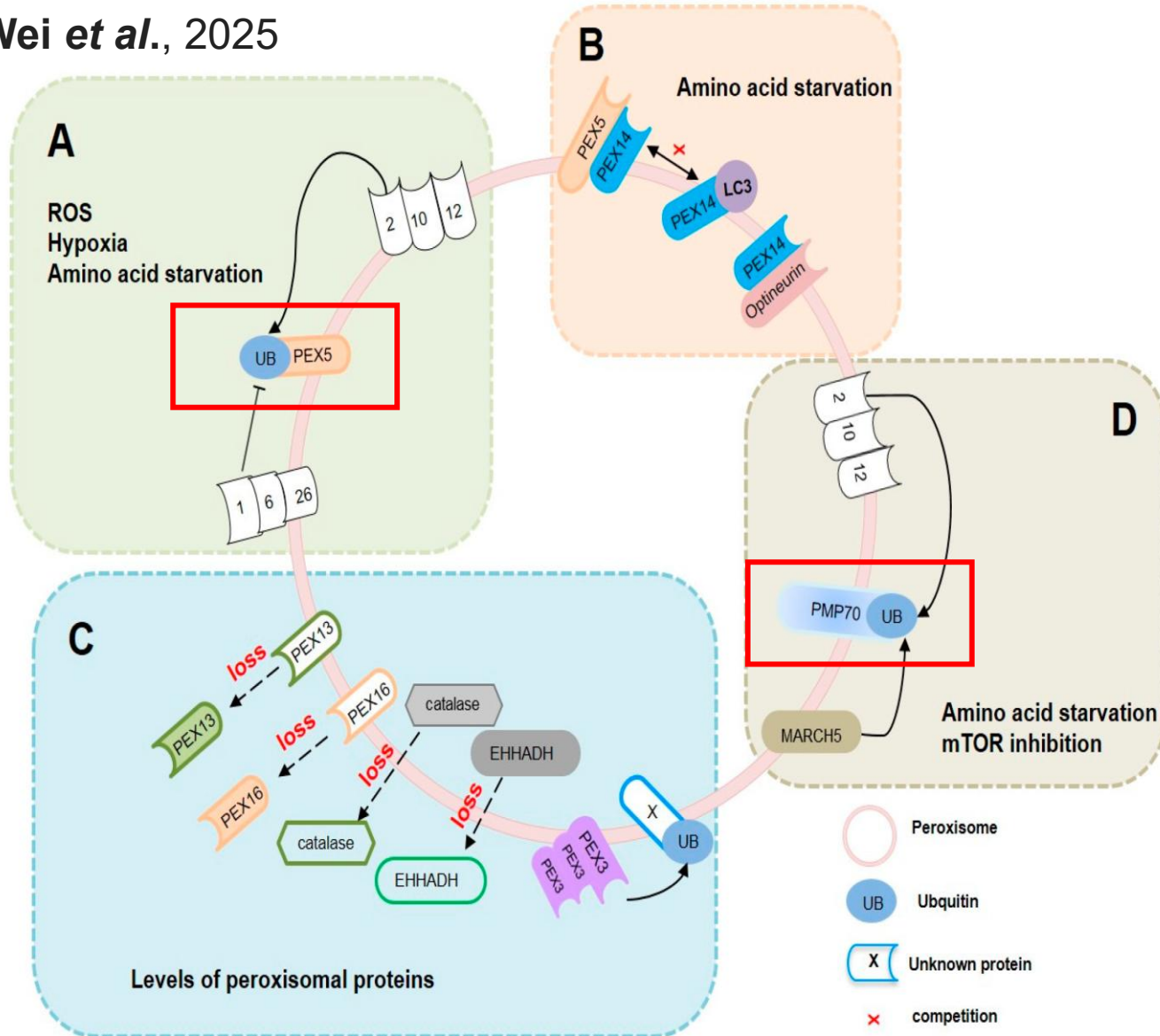
Change in
Excitation peak

440 nm	586 nm
Neutral (>pH 6)	Acidic (<pH 5)



Katayama, H. *et al.*, Chemistry & Biology 18, 1042-1052 (2011)

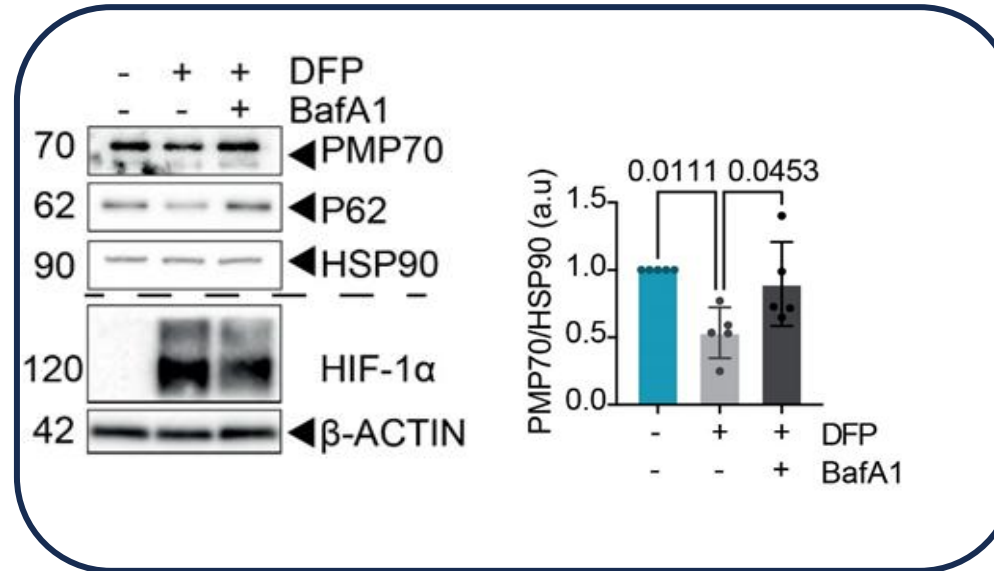
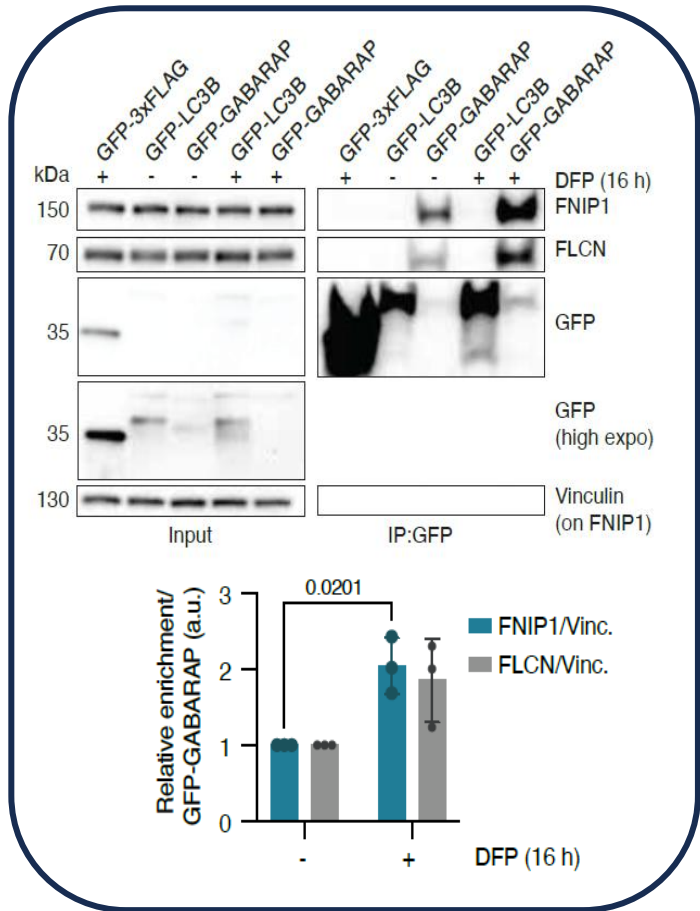
Wei *et al.*, 2025



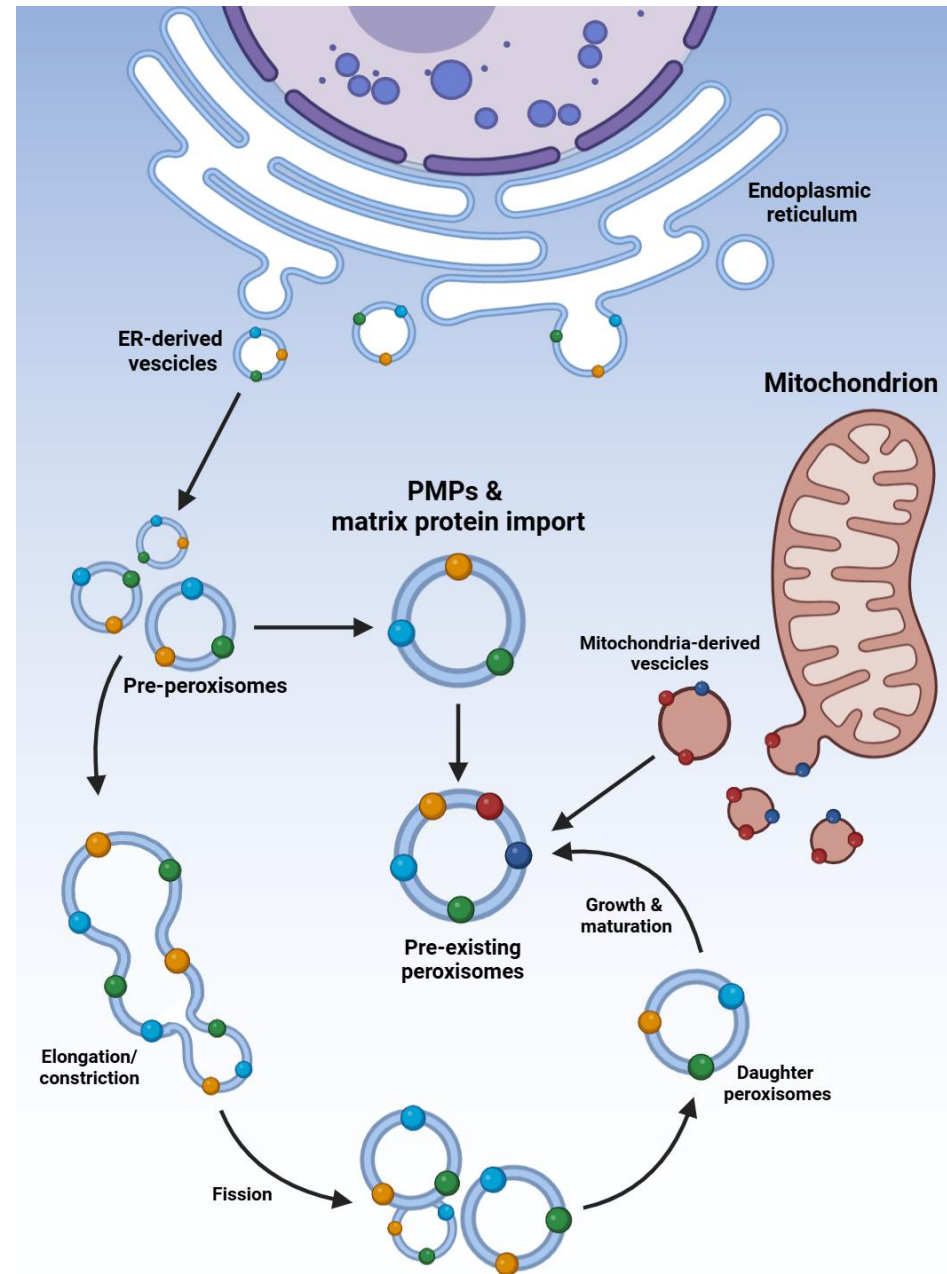
Different stimuli for pexophagy induction:

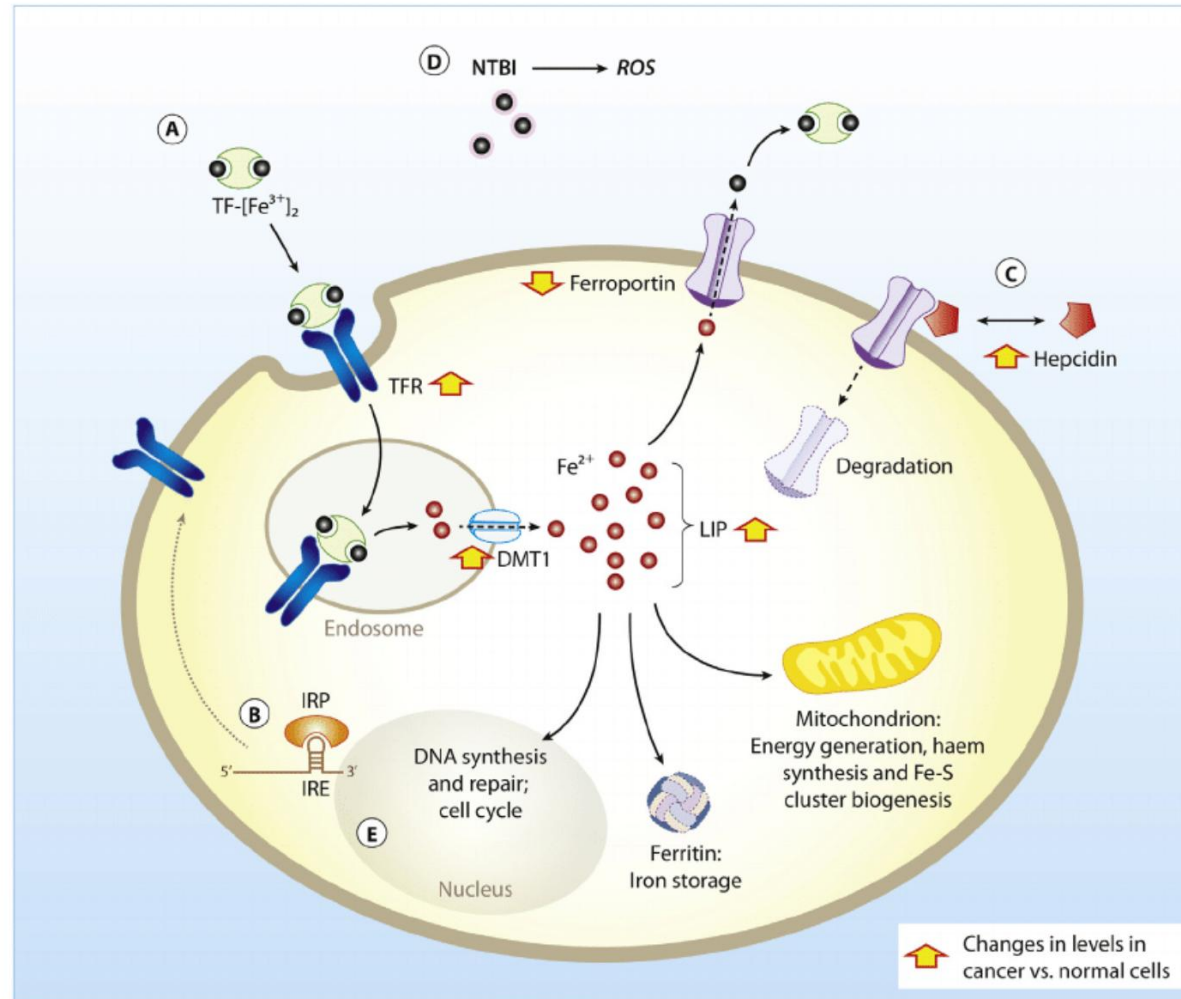
- Amino acid starvation
- mTORC1 inhibition
- Hypoxia
- Reactive Oxygen Species (ROS)

PRELIMINARY RESULTS

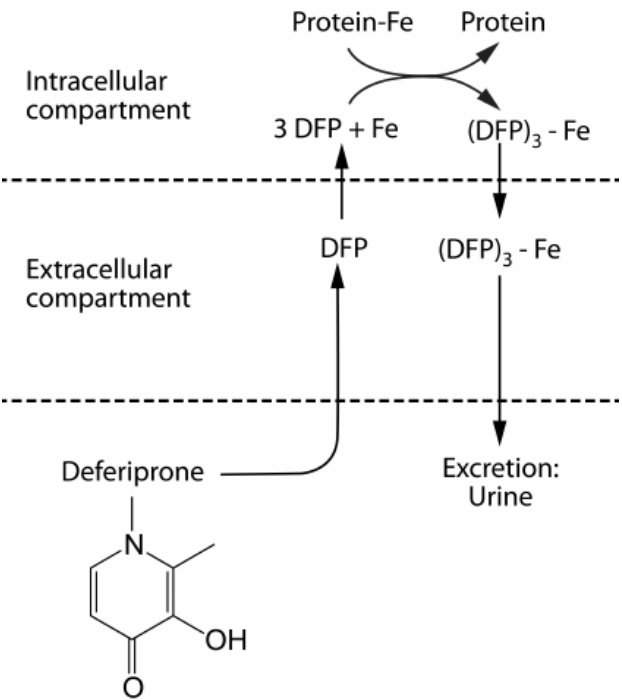
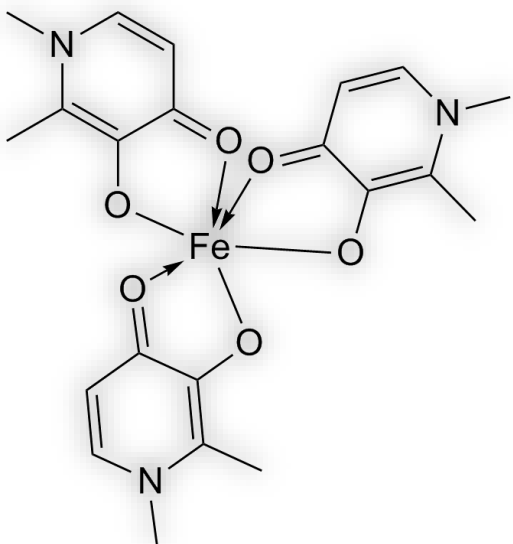


PEROXISOME BIOGENESIS



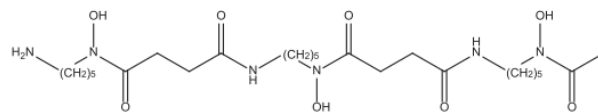


DEFERIPRONE (DFP)

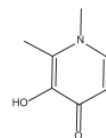


	“Ideal chelator”	Deferoxamine	Deferiprone	Deferasirox
Route of administration	Oral	Parenteral, usually subcutaneous or intravenous	Oral	Oral
Plasma half-life	Long enough to give constant protection from labile plasma iron	Short (minutes); requires constant delivery	Moderate (< 2 hours). Requires at least three times per day dosing	Long, 8-16 hours; remains in plasma at 24 h
Therapeutic index	High	High at moderate doses in iron overloaded subjects	Idiosyncratic side effects are most important	Probably high in iron-overloaded subjects*
Molar iron chelating efficiency; charge of iron (III complex)	High, uncharged	High (hexadentate); charged	Low (bidentate); uncharged	Moderate (tridentate); uncharged
Important side effects	None, or only in iron-depleted subjects	Auditory and retinal toxicity; effects on bones and growth; potential lung toxicity, all at high doses; local skin reactions at infusion sites	Rare but severe agranulocytosis; mild neutropenia; common abdominal discomfort; erosive arthritis	Abdominal discomfort; rash or mild diarrhea upon initiation of therapy. Mild increased creatinine
Ability to chelate intracellular cardiac and other tissue iron in humans	High	Probably lower than deferiprone and deferasirox	High in clinical and in-vitro studies	Insufficient clinical data available; promising in laboratory studies

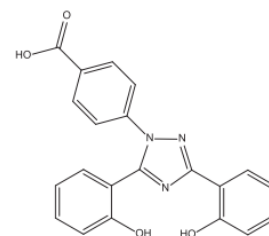
*Nephrotoxicity observed in non-iron-loaded animals has been minimal in iron-overloaded humans, but effectiveness in demonstrated only at higher end of tested doses, as discussed in text and ref 1.



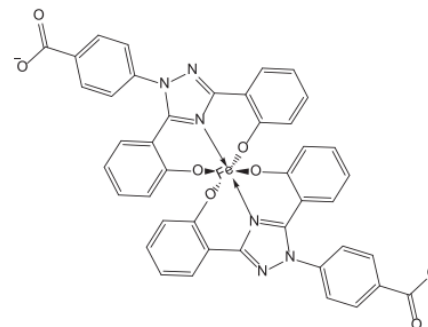
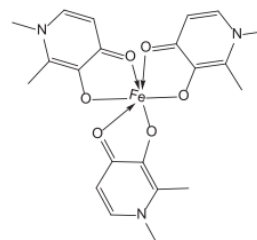
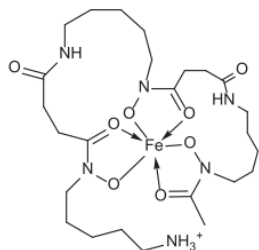
Desferrioxamine (DFO)



Deferiprone (L1)



Desferasirox (ICL-670)



Chelator property	Ideal chelator	DFO	L1	DFX
Cost	Affordable for patients in low income countries	Moderate	Moderate	Unaffordable and unavailable for most
Route of Administration	Oral	i.v injection or s.c infusion	Oral	Oral
Circulation $t_{1/2}$	Long enough to allow once-daily dosing and effective iron removal	Short (~20 min) requires all-day (8-12 h) delivery	Moderate; requires at least 3 times per day dosing	Ideal; 8-16 hours, requiring once-daily dosing
Therapeutic index	High	High at high doses in patients with high burden	Unpredictable	High
Toxicity	None	Neurotoxic, swelling at infusion sites, bone deformities	Agranulocytosis and mild neutropenia are common	Reversible kidney failure
Unsaturated Iron Binding Capacity	High: Long enough to prevent drastic fluctuations in LIP	None	Moderate	High
Ability to remove iron from heart, liver etc.	High	Low	High	High

