CARBON NANOTUBES BUT NOT SPHERICAL NANOPARTICLES BLOCK AUTOPHAGY PROCESS BY A SHAPE-RELATED TARGETING OF LYSOSOMES IN MURINE MACROPHAGES

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Serenade Safe(r) Ecodesign Research and Education applied to nAnomaterial DEvelopment.
ANR Agence Nationale de la Recherche
nanoSAFE'16

Respiratory Exposure to manufactured Nanoparticles

- Pulmonary responses can be associated with NPs toxicity
  - Fibrosis

    Bermudez *et al.* 2004 ; Card *et al.* 2008 ; Mercer *et al.* 2011 ; Wang *et al.* 2013
  - Emphysema

    Chen *et al.* 2006

- Toxicological mechanisms
  - Oxidative stress
  - Inflammation
  - Genotoxicity

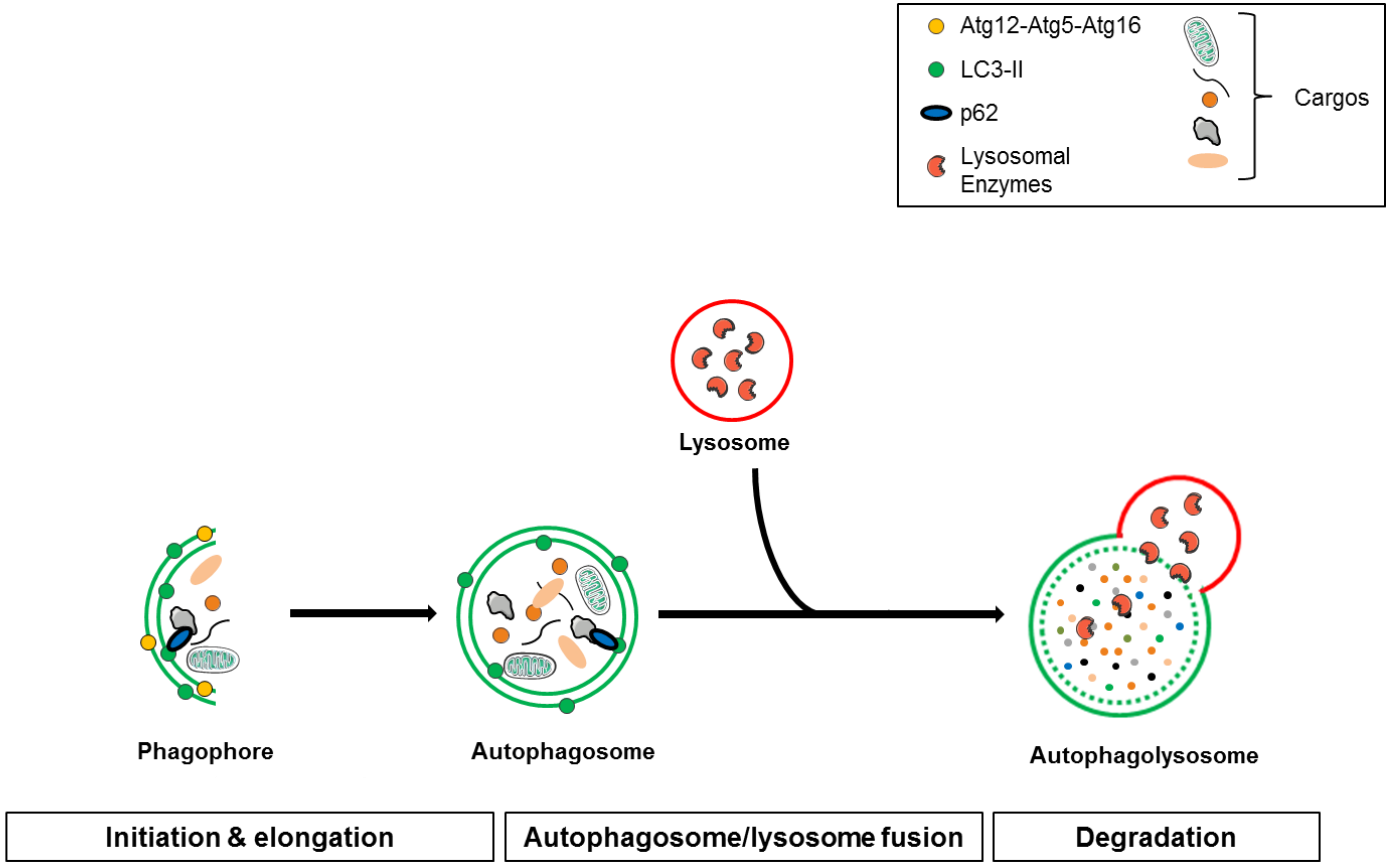
Dependent on the physicochemical characteristics of the NPs

Boczkowski *et al.* 2012 ; Shi *et al.* 2013 ; Manke *et al.* 2013

Autophagy?

Autophagy

- Physiological process for the degradation of cellular components
  ⟹ Regulation of cellular homeostasis



Link between autophagy and NPs toxicity?

Autophagy, oxidative stress and inflammation

- Autophagy can inhibit the production of reactive oxygen species (ROS)

Chen et al. 2009 , Scherz-Shouval et al. 2007

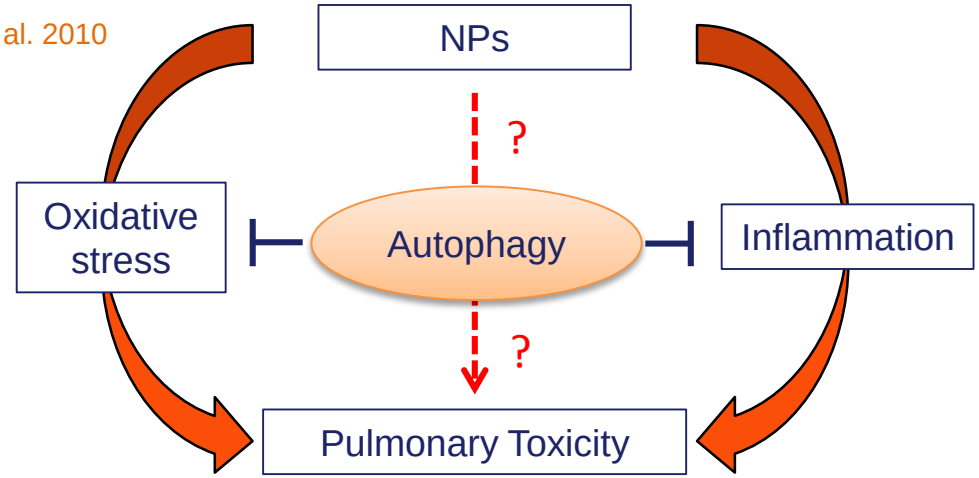
- Autophagy can suppress inflammation

Schroder *et al*. 2010, Shi *et al*. 2012

Autophagy and NPs toxicity

- NPs found in lysosome

Yang et al. 2010



Hypothesis

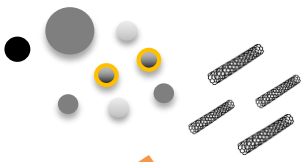
A defective autophagy could be a new mechanism explaining NPs effects

Aim

- Do the NPs causes a dysfunction of autophagy?
- Do the physicochemical parameters of NPs modulate this process?

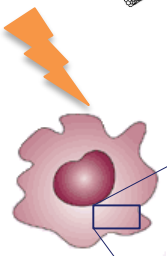
Experimental protocol

NPs:
TiO_2 et CNT



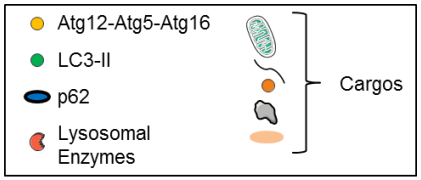
50 µg/mL

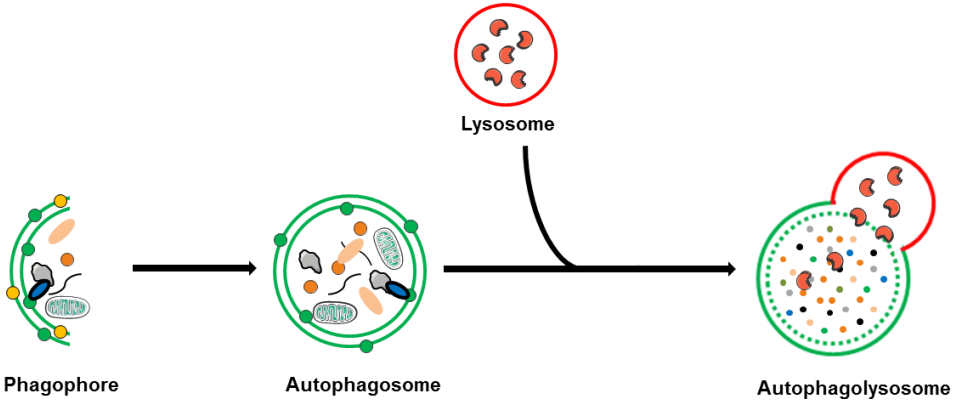
6h



Mouse macrophages (RAW264.7)

NPs effects on autophagy





a. Autophagic flux

b. Autophagosome formation

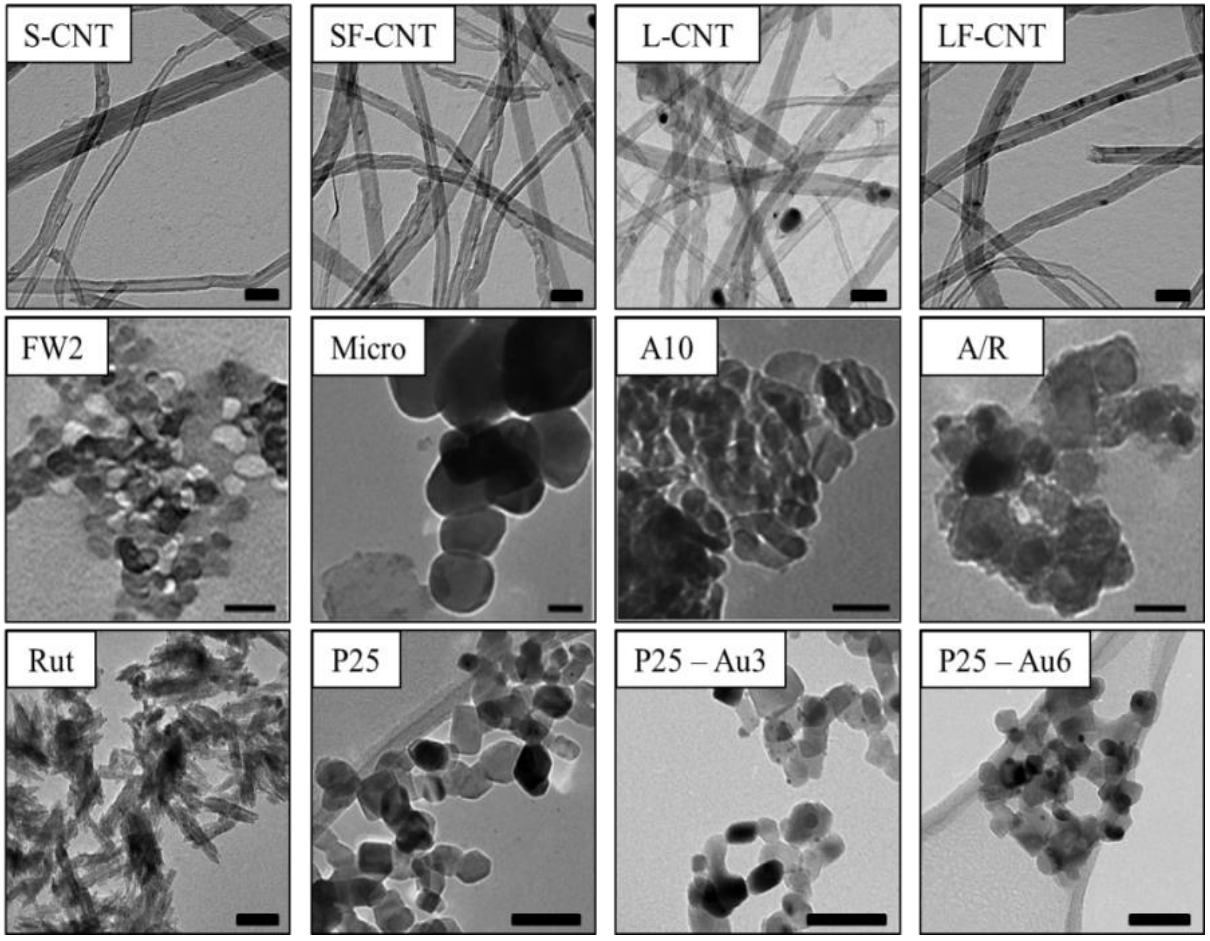
c. Fusion

d. Lysosome function

Nanoparticles characterization

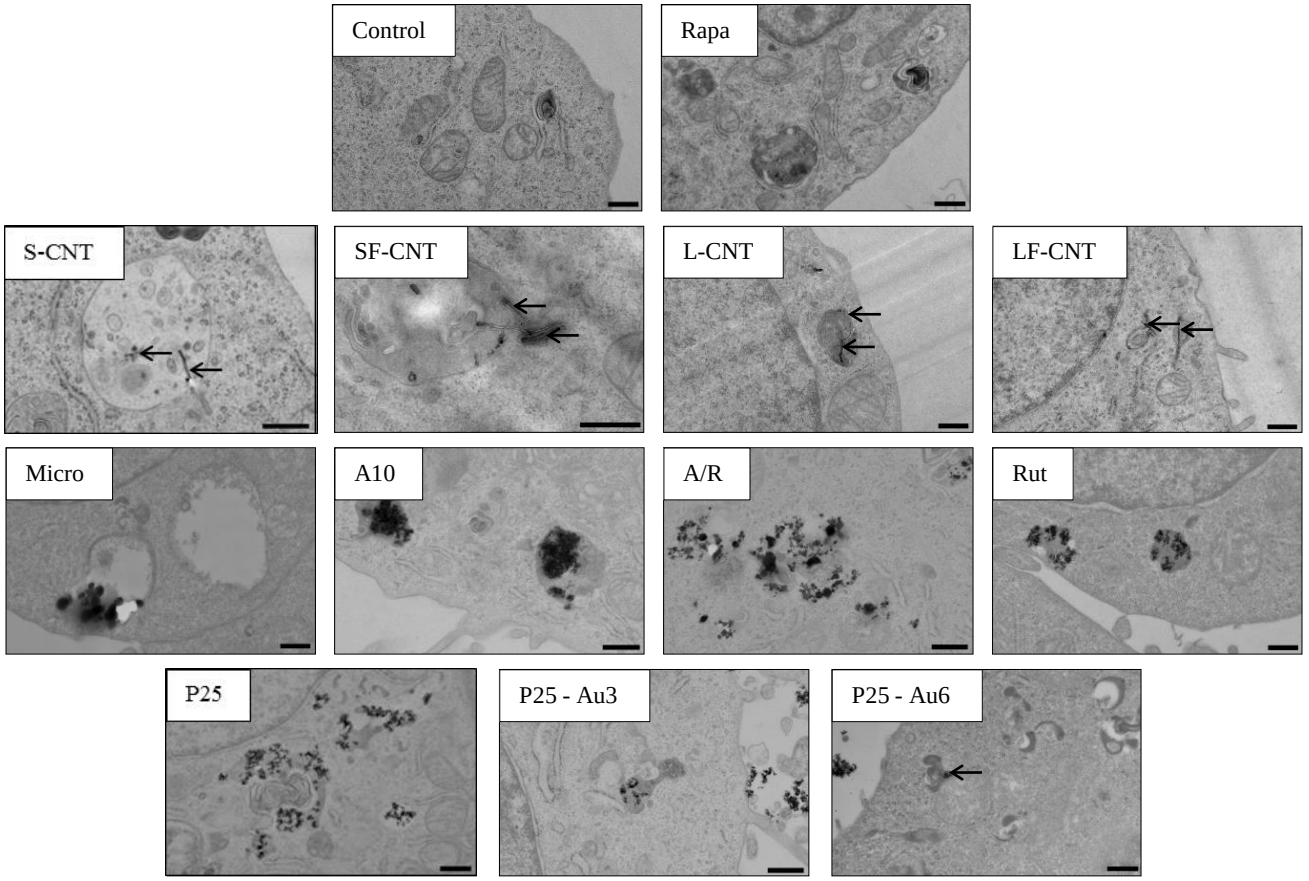
NPs	Composition	Crystal phase	Shape	Dimension (nm)	Specific surface area (m^2/g)	Zeta potential (mV)	Hydrodynamic diameter (nm)	Endotoxin level	Intrisic ROS production
S-CNT	Carbon	Amorphous	Nanotube	26×2000	Not determined	Not determined	Not determined	Not detected	+
SF-CNT	Carbon	Amorphous	Nanotube	25×2000	Not determined	Not determined	Not determined	Not detected	Not detected
L-CNT	Carbon	Amorphous	Nanotube	24×7000	Not determined	Not determined	Not determined	Not detected	+
LF-CNT	Carbon	Amorphous	Nanotube	25×9000	Not determined	Not determined	Not determined	Not detected	+
FW2	Carbon Black	Amorphous	Spherical	13	373 ±18	-11,9	1033 ±88	Not detected	Not detected
Micro	TiO_2	Anatase	Spherical	200	8 ±0,04	0,4	874 ±149	Not detected	+
A10	TiO_2	Anatase	Spherical	10	96 ±2,3	-2,1	1020 ±146	Not detected	Not detected
A/R	TiO_2	65 % Anatase + 35 % Rutile	Spherical	25-75	27 ±1,5	0,2	715 ±104	Not detected	Not detected
Rut	TiO_2	Rutile	Needle-like	5×20	160	-39,8	55 ±20	Not detected	+
P25	TiO_2	80 % Anatase + 20 % Rutile	Spherical	30	50	-7,3	48 ±19	Not detected	+
P25-Au3	TiO_2 + 1 % Au	80 % Anatase + 20 % Rutile	Spherical	30	57	-21,1	175 ±103	Not detected	+
P25-Au6	TiO_2 + 1 % Au	80 % Anatase + 20 % Rutile	Spherical	30	57	-21,9	54 ±34	Not detected	+

Nanoparticles characterization



NPs observed by TEM
Scale bar : 50 nm

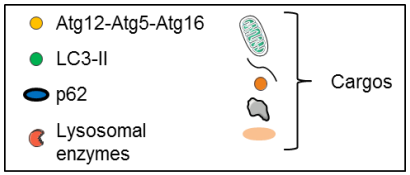
Nanoparticles internalization in macrophages

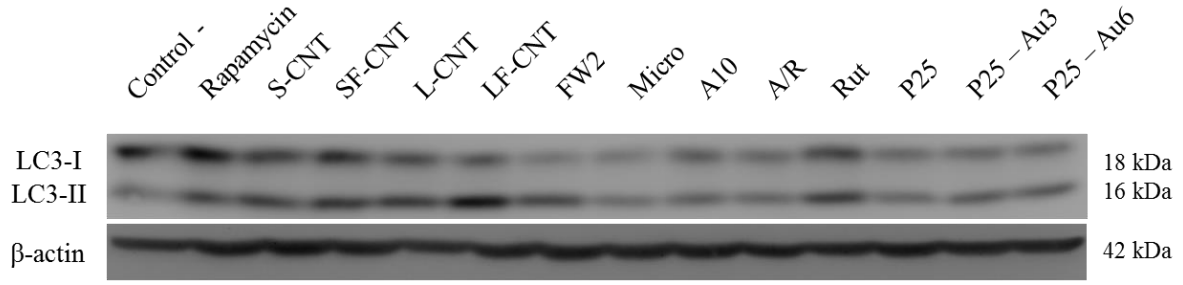


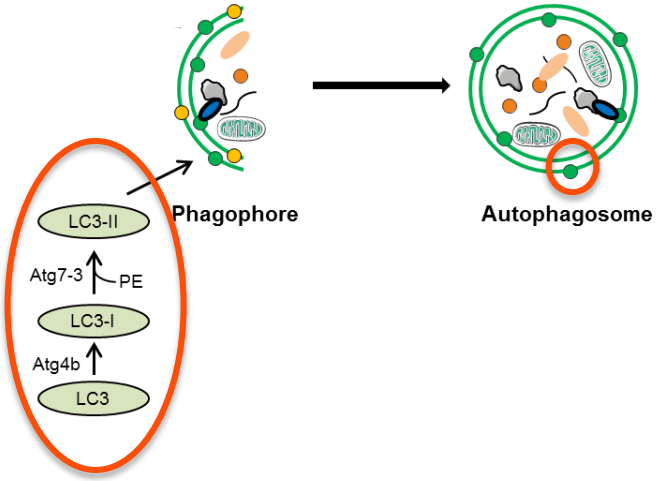
Pictures of macrophages exposed to NPs observed by TEM
Scale bar : 500 nm

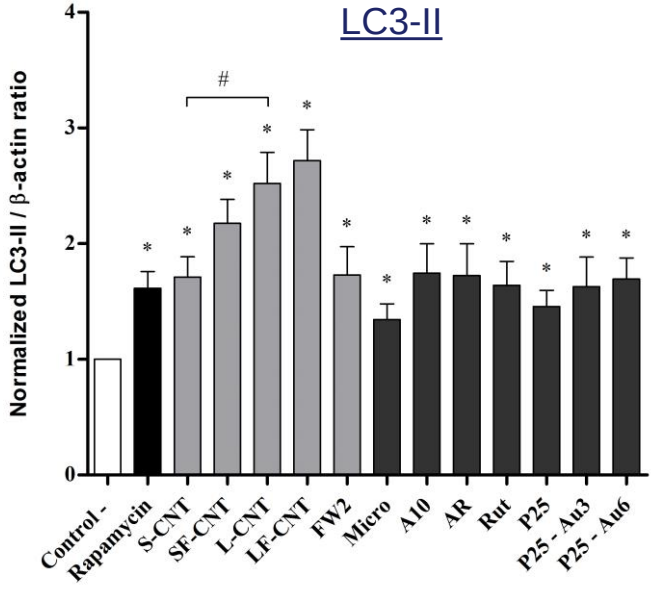
NPs could be internalize in macrophages, in autophagic-like structures

Autophagosome formation



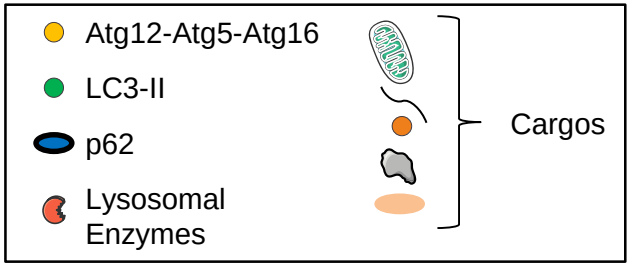


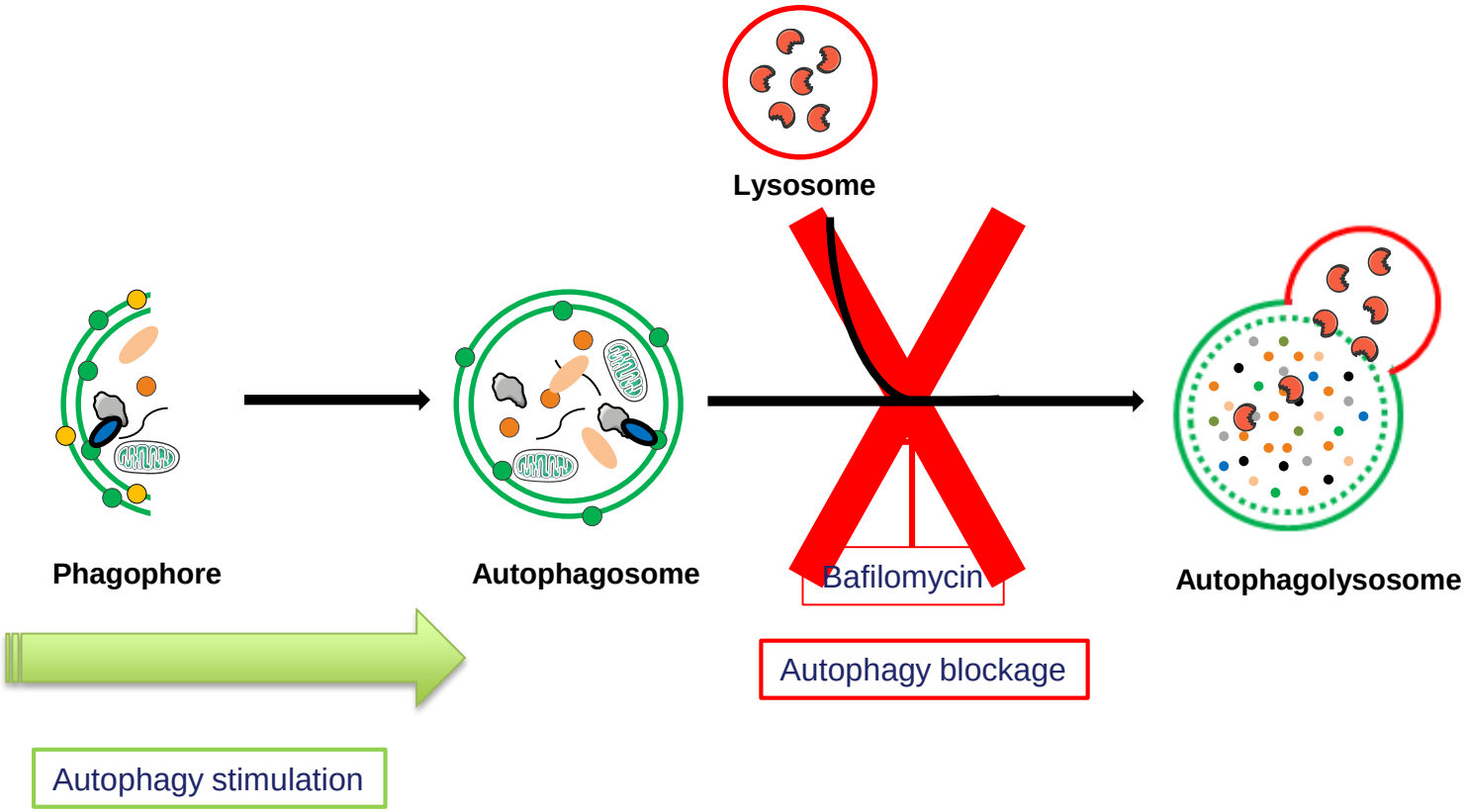




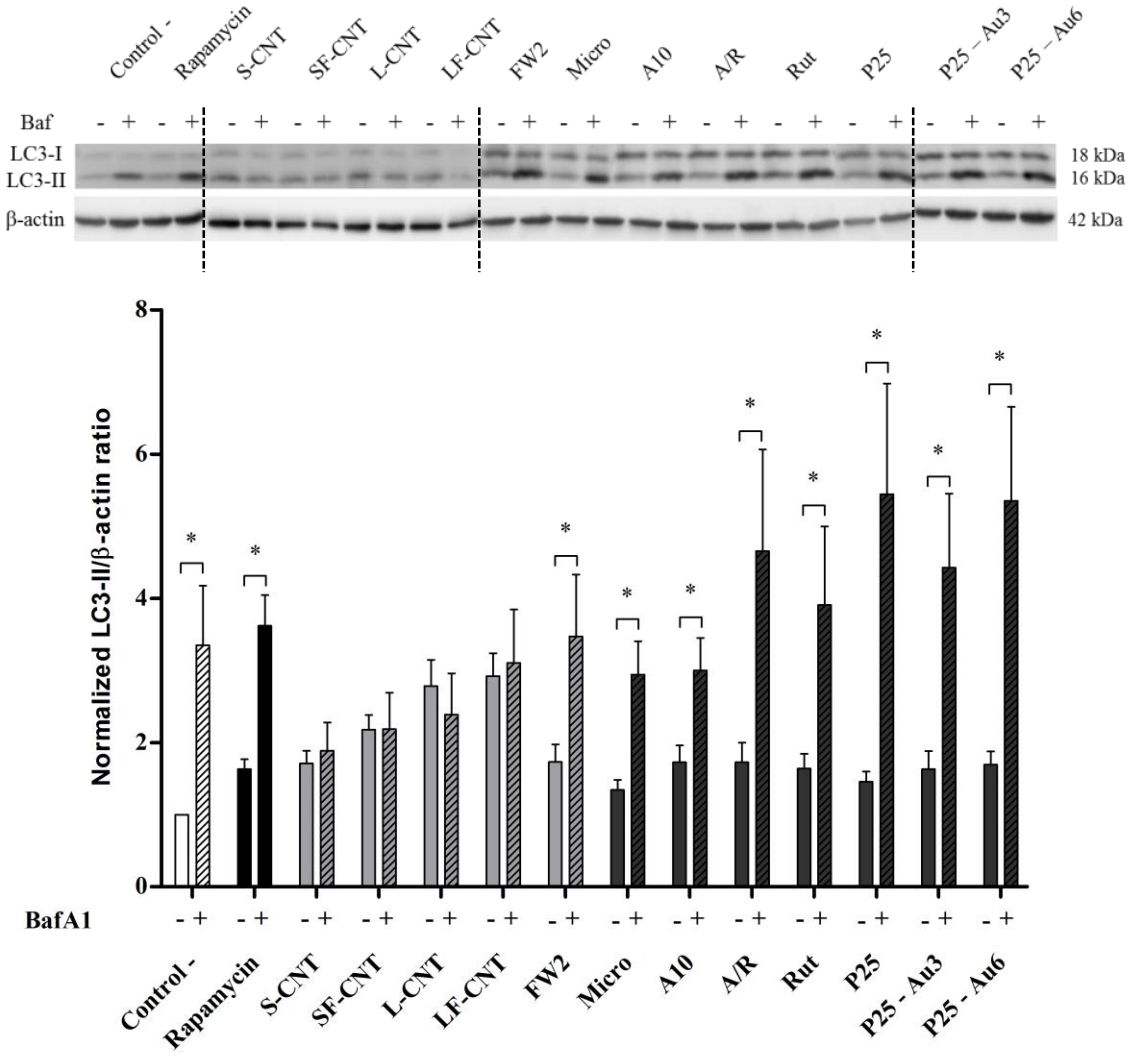
Accumulation of autophagosome in response to all NPs

Autophagic flux



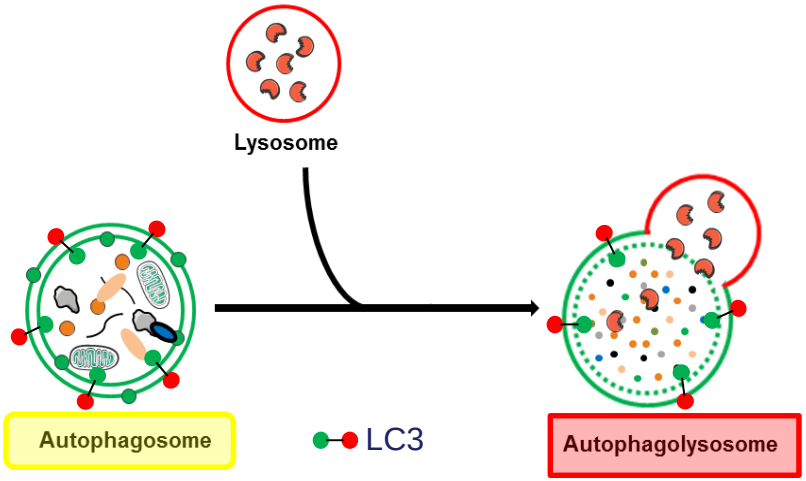


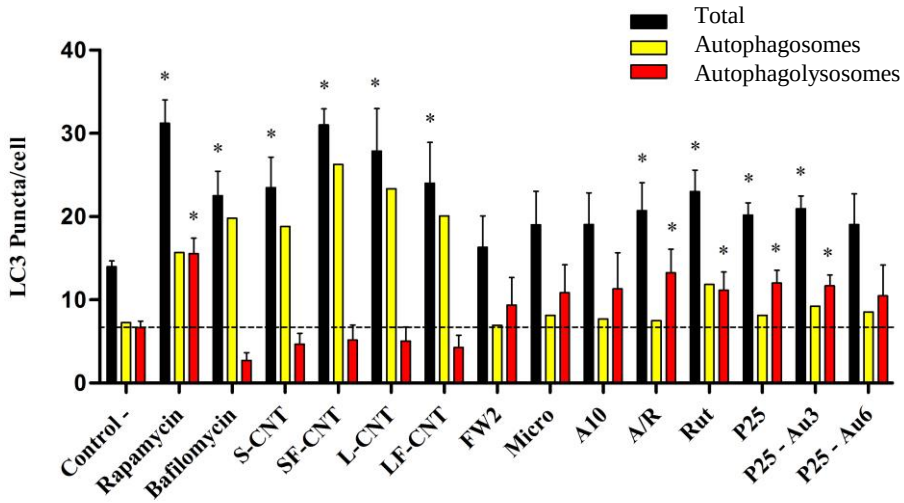
Autophagic flux

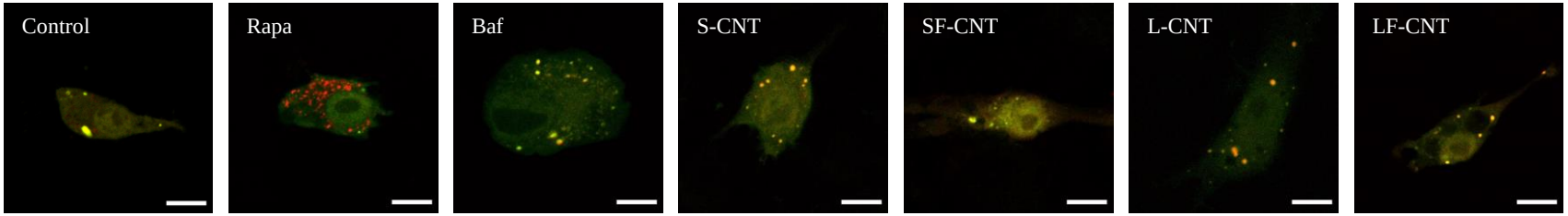


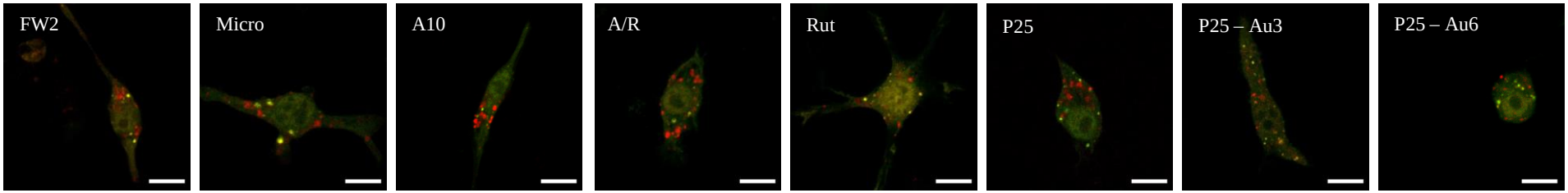
Blockage of autophagic flux with the 4 CNT but not spherical NPs

Autophagic flux



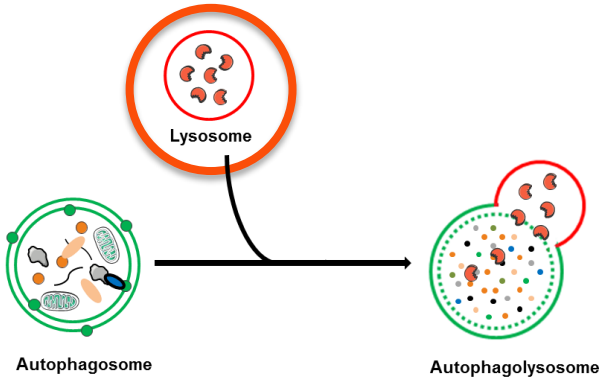


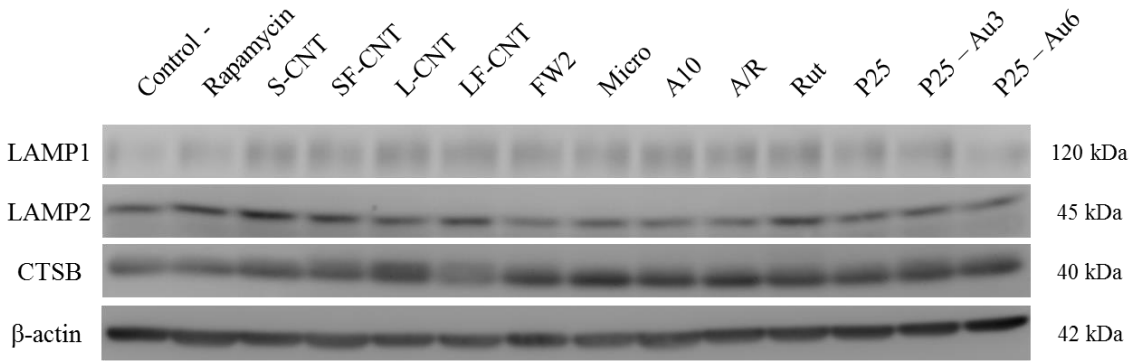




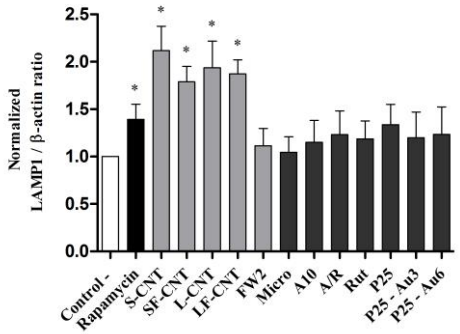
Blocking of autophagic flux with the 4 CNT but not spherical NPs

Lysosomal alterations

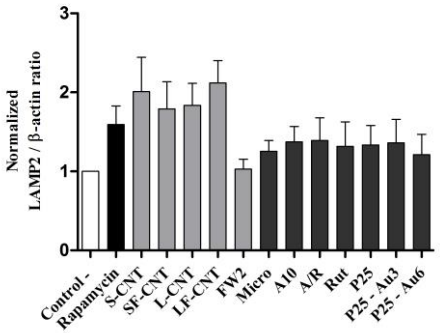




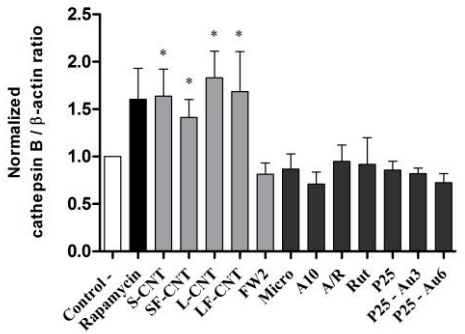
LAMP1



LAMP2

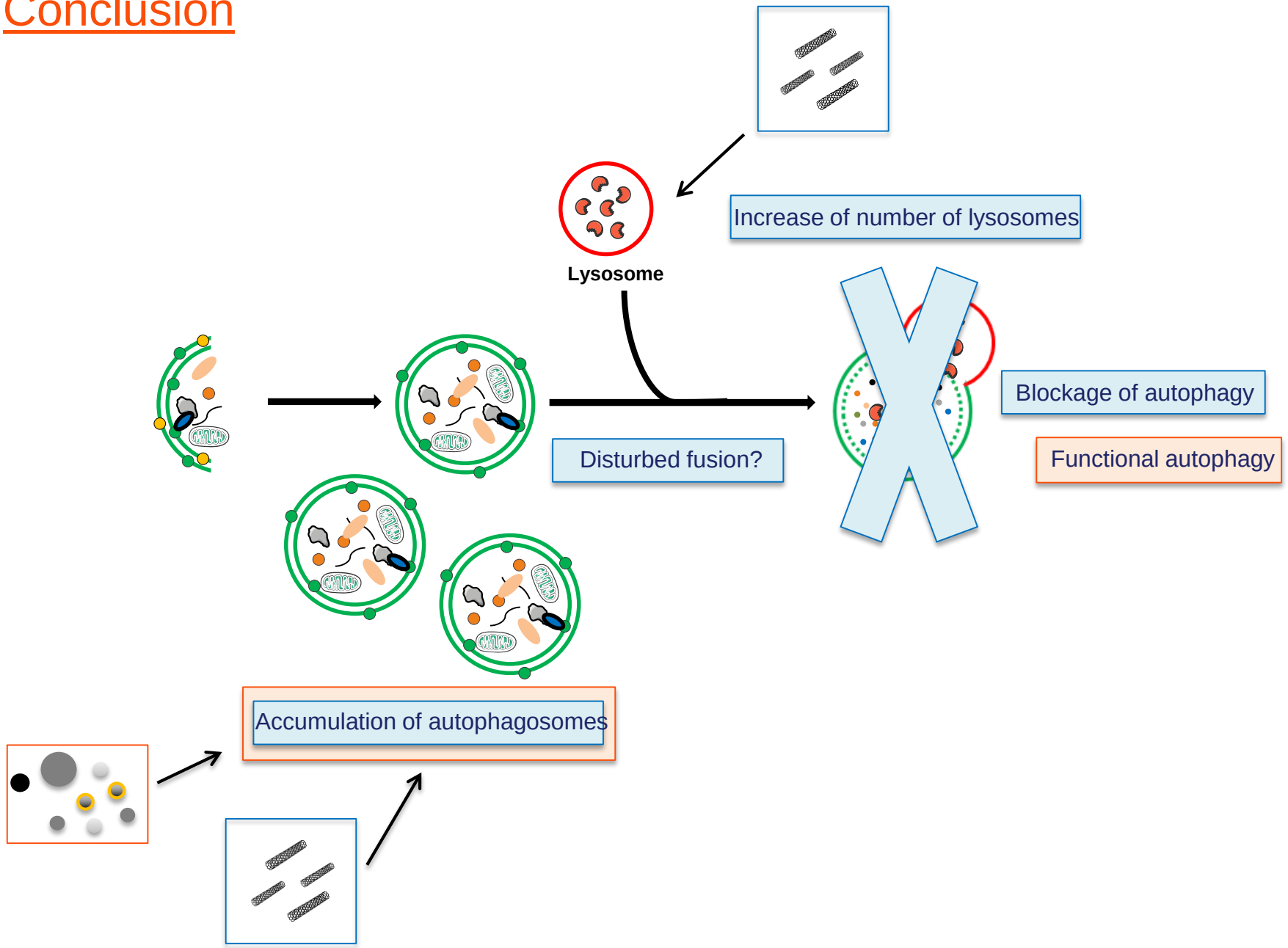


Cathepsine B



Increase of lysosome number in response to CNT

Conclusion


Increase of number of lysosomes
Lysosome
Disturbed fusion?
Blockage of autophagy
Functional autophagy
Accumulation of autophagosomes

Acknowledgments

Inserm U955 IMRB, team 4
Sophie Lanone
Jorge Boczkowski
Vanessa Cohignac
Stéphane Tchankouo
Emmanuel Paul
Shamila Vibhushan
Marie-Laur[illegible]
Benjamin [illegible]
Lucie Biza[illegible]
Philippe C[illegible]

And the other members of the team 4

Platform of IMRB
Elisabeth Marcos
Xavier Decrouy

CEA Saclay
Mathieu Pinault
Martine Mayne-L'Hermite
Nathalie Herlin-Boime
Adèle Gerdil
Baptiste Cottin

Hôpital Necker – Enfants malades
Patrice Codogno

Imagif Cell Biology Unit
Claire Boulogne
Cynthia Gillet

Thank you for your attention