Assemblée Générale GDR Mi2B

3-4 septembre 2015, Marseille, France

Caractérisation *in vitro /in vivo* des réponses biologiques induites par des nanoparticules d'oxydes métalliques (projets TITANIUMS & PROTON)

H. SEZNEC

CENBG/IN2P3, *INC, INSB*

*i*RiBio, *ionizing* Radiation interactions and Biology

TITANIUM DIOXIDE – TiO_2

CHARACTERISTICS
- ❑ Semiconductor material
- ❑ White color
- ❑ UV filter
- ❑ Photo-catalytic properties
- ❑ …

APPLICATIONS
- ❑ Pigments/opacifier (paint, plastic goods,…)
- ❑ Cosmetics (sun-screen, Tooth past...)
- ❑ Food additive (E171, sweets,…)
- ❑ Self-cleaning materials

***IN VIVO* SYMPTOMS**
- ❑ Chronic inflammation
- ❑ Fibrosis
- ❑ ...

IN VITRO OBSERVATIONS
- ❑ Oxidative stress
- ❑ DNA damages, apoptosis
- ❑ ....

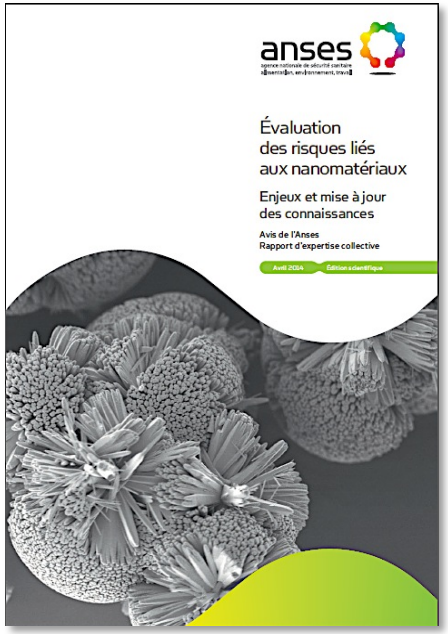
CONCERNS?

2006 - International Agency for Cancer Research [IARC]
- ❑ Macroscopic scale : **BIOLOGICALLY INERT**
- ❑ Nanometric Scale : **ACCUTE SURFACE REACTIVITY + NANOMETRIC SIZE + WIDESPREAD USE**

POTENTIALS OF DERMAL PENETRATION, INGESTION, INHALATION, *INJECTION*

STRONG NEEDS FOR RISK EVALUATION
- ❑ **Need to standardization** (physico- chemical properties, surface reactivity , biological models , ...)
- ❑ **Require specific methods** (harmonization of toxicological models / ecotoxicological detection / measurement , ...)
- ❑ **MULTIDISCIPLINARITY : CHEMISTRY, BIOLOGY, PHYSICS**

DETECTION	TRACKING	LOCALISATION	QUANTIFICATION



Agence nationale de sécurité sanitaire, de l'alimentation, de l'environnement et du travail – Report April2014

OUTLINE

1st Part	**TiO_2 Nanoparticles** ❑ Hydrothermal Synthesis **(P25, NF, NA)** ❑ Physicochemical characterization in physiological environment ❑ Functionalization
2nd Part	**TiO_2 Nanoparticles** and ***in vitro* experiments** *(primary cells, immortalized cells)* ❑ Multi-elemental Micro-analysis ❑ Intracellular localization – conventional microscopies ❑ Intracellular localization - correlative & multimodal microscopies ❑ Micro-imaging et quantitative micro-analysis at the subcellular scale ❑ Physico-chemical characteristics of NPs and cellular toxicity : ?
3rd Part	**TiO_2 Nanoparticles** and ***in vivo* experiments** (***C. elegans)*** ❑ Detection, *in situ* et *in vivo* tracking – localization ❑ *in vivo toxicity*

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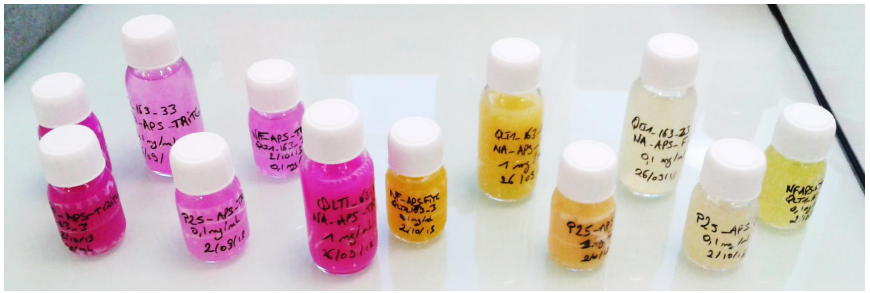
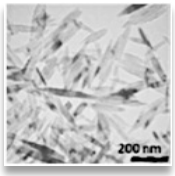




TITANIUMS –NANOPARTICLES (NP) : HYDROTHERMAL SYNTHESIS

P25

(DEGUSSA / EVONIK)
30 nm

NaOH 10N, 130°C, 20H

Nano-SHEETS
(Titanate Nanosheets)

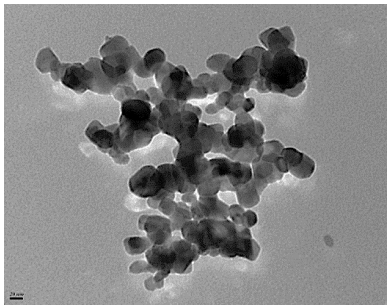
pH = 11, 140°C, 72H

Nano-NEEDLES TiO_2
100 < L< 500 nm

+ TRI-ETHANOLAMINE

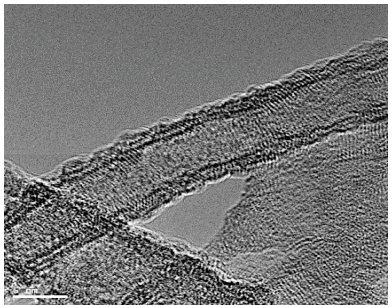
INPs (Sugimoto) TiO_2
40< L< 50 nm

P25



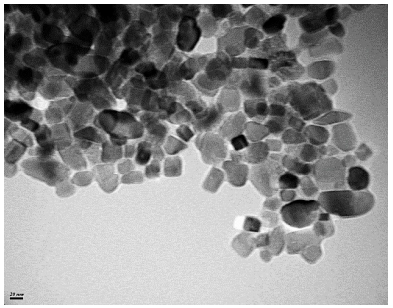
150 nm

Nano-Sheets



5 nm

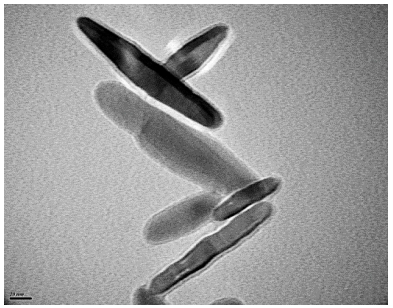
INPs



150 nm

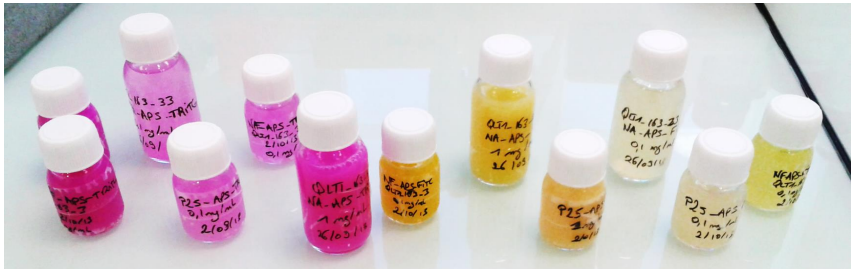
100 % ANATASE

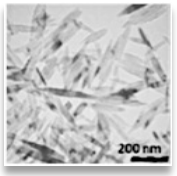
Nano-Needles



80 nm

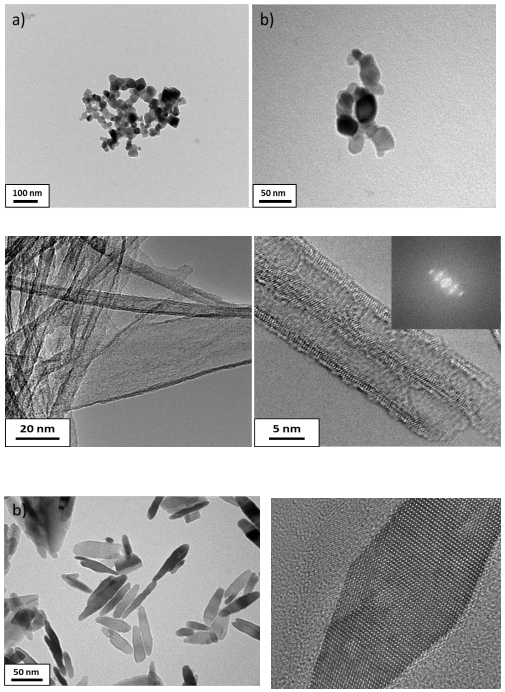
100 % ANATASE





TITANIUMS –NANOPARTICLES CHARACTERIZATION IN PHYSIOLOGICAL MEDIA (pH 7.4)

Properties	AEROXIDE P25 (*Evonik*)	Titanate Nanosheets	NanoNeedles	INPs
Morphology	Various shape, Sphere, isotropic	"Tubes", folded sheets	Elongated	Isotropic NPs
Length, nm [a]	15-50	100-500	10-80	10-60
average	*24*	*178*	*45*	*29*
Width, nm [a]	15-50	-	15	29
Thickness, nm [a]	-	6-8	-	-
Aspect ratio	1	19	3	1
Phase Composition [b]	87 % A [c] 13 % R [c]	100 % HT	96 % A [c] 4 % B [c]	100 % A [c]
Hydro. Diameter, nm [d]	250 +/-79	364 +/-90	41 +/- 19	196 +/- 61
Zeta potential, mV, pH 7.4	- 16,6	-29,6	-33,9	-
Surface Charge pH 7,4	Negative	Negative	Negative	Negative
Specific Surf. Area, m^2/g [e]	50	360	86	49
Coating	None	None	None	None
Aggregation	++++	++++	+	++
Acronym	P25	TNs	NNs	INPs



Submitted data

SUMMARY
1st Part

4 TYPE of NPS WITH SPECIFIC PROPERTIES

- ❑ **P25** : commercially available NPs (reference)
- ❑ **INPs** (isotropic nanoparticles)
- ❑ Nano-Sheets (**TNs**) with important specific surface
- ❑ Nano-needles (**NN**)

BEHAVIOR IN SOLUTION & PHYSIOLOGIC pH

- ❑ NEGATIVELY CHARGED NPS
- ❑ **P25** & **TNs**: STRONG TENDENCY TO AGGLOMERATION

FUNCTIONALIZATION

- ❑ MODIFICATION OF THE SURFACE REACTIVITY
- ❑ CELL IMAGING AND *IN SITU* MULTI-CELLULAR TRACKING, REAL TIME ANALYSIS

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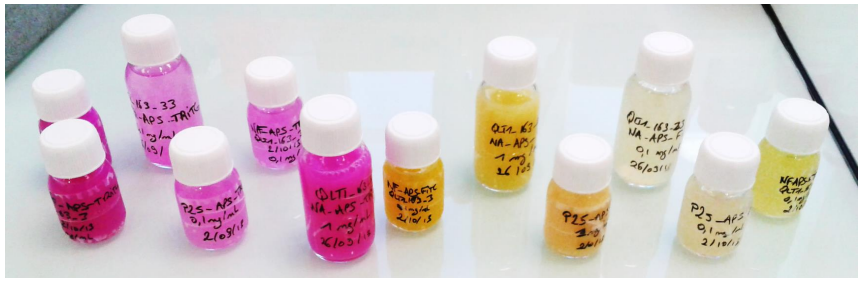

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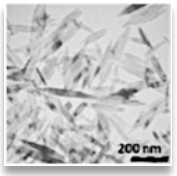
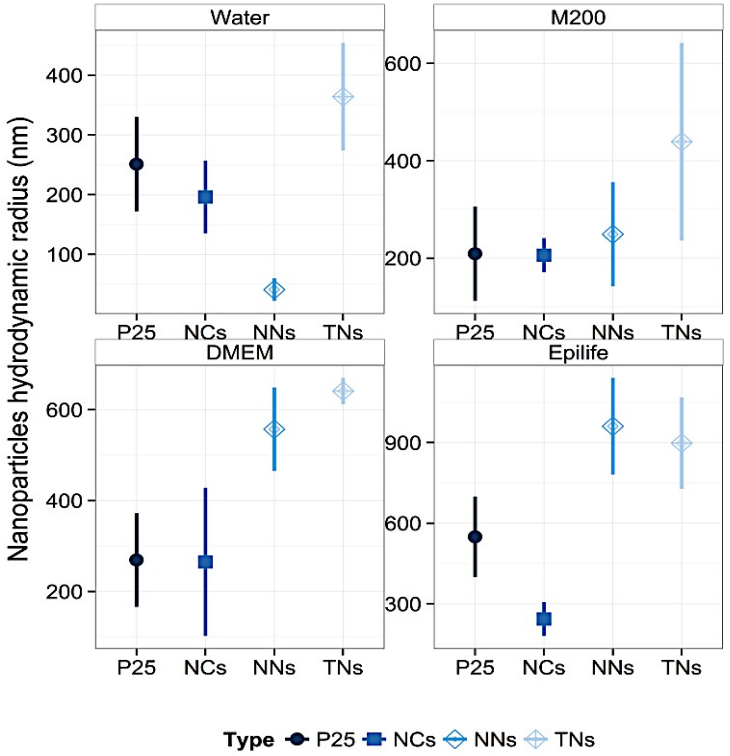


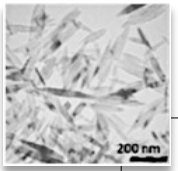
NPs CHARACTERIZATION IN PHYSIOLOGICAL MEDIA (pH 7.4) - **INFLUENCE OF THE CULTURE MEDIUM**



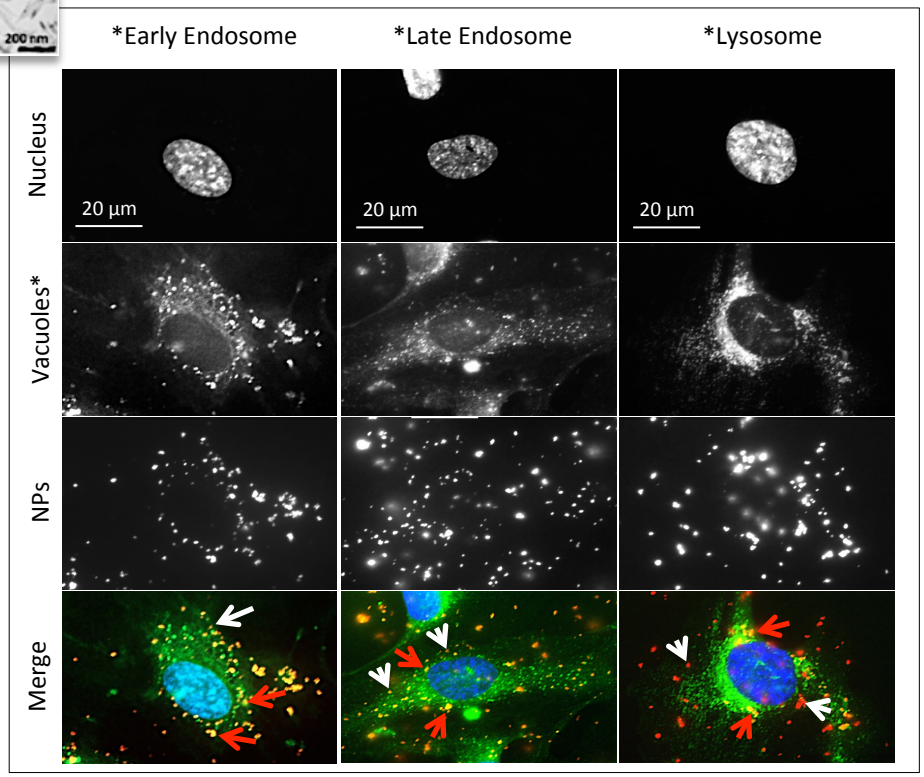
Variation of hydrodynamic radius (nm) of TiO_2 NPs in culture medium.

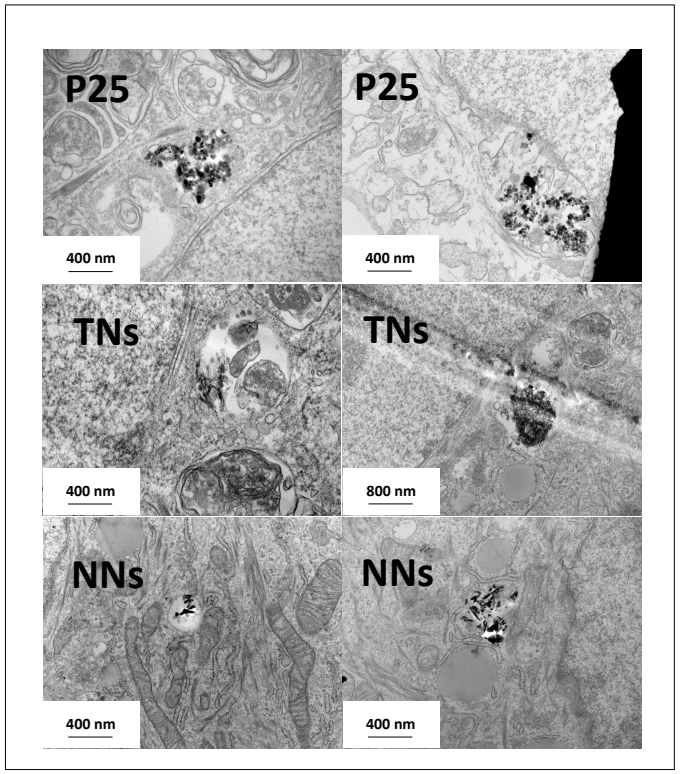
NPs behavior has been evaluated in the synthesis solution (**water**) and in biological medium **M200**, (Huvec), **DMEM** (Hela) and **Epilife** (HEKn)

Submitted data



Human umbilical Vein Cells HUVEC
P25-APS-TRITC (2 µg.cm^{-2})
8 hours of exposures

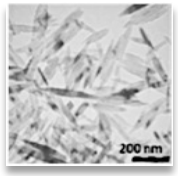




NANOPARTICLES LOCALIZATION : EXCLUSIVELY in the CYTOPLASME (FREE + ENDOSOMES / LYSOSOMES) – RELATED TO THE TIME OF EXPOSURE

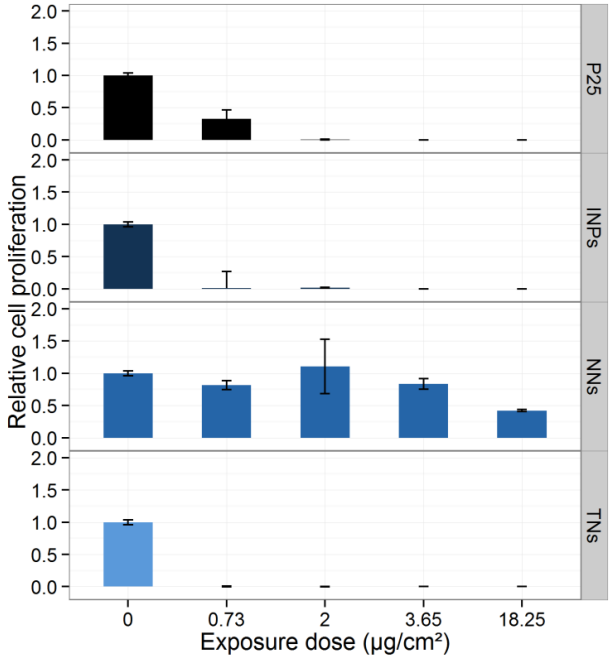
NO SPECIFIC LOCALIZATION	❑ **P25, TNs, NNs** ❑ **FUNCTIONALIZATION (+TRITC, +FITC)** ❑ **CELL TYPES** (HUVEC, Keratinocytes, HaCat, HeLa)

Le Trequesser, *et al* – JOCB 2015

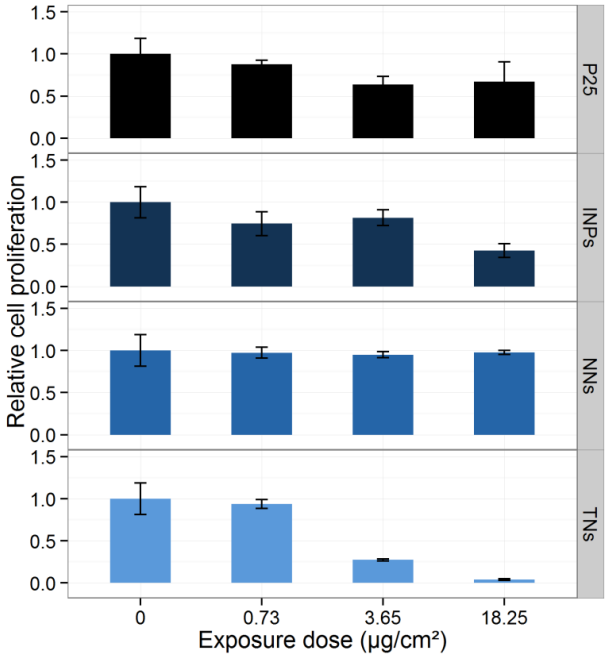


IN VITRO TOXICOLOGY – NPs, DOSE RESPONSE and CELL TYPE
HUVEC *versus* HeLa

HUVEC
PRIMARY ENDOTHELIAL CELLS



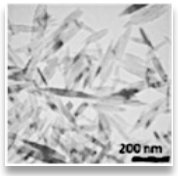
Hela
IMMORTALIZED AND CANCEROUS CELL LINE



Relative cell proliferation (mean +/- *sd*) measured 8 days after exposure to TiO_2 NPs.
Exposure doses (µg/cm²): 0, 0.73, 2.0, 3.65, 18.25

TOXCITY RELATED TO CELL TYPE (IMMORTALIZED *versus* PRIMARY,....)
TOXICITY RELATED TO THE TYPE OF NPs, TO THE DOSE

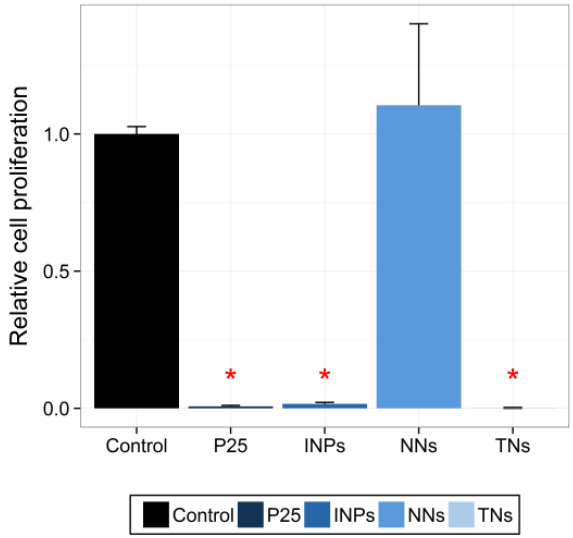
Submitted data



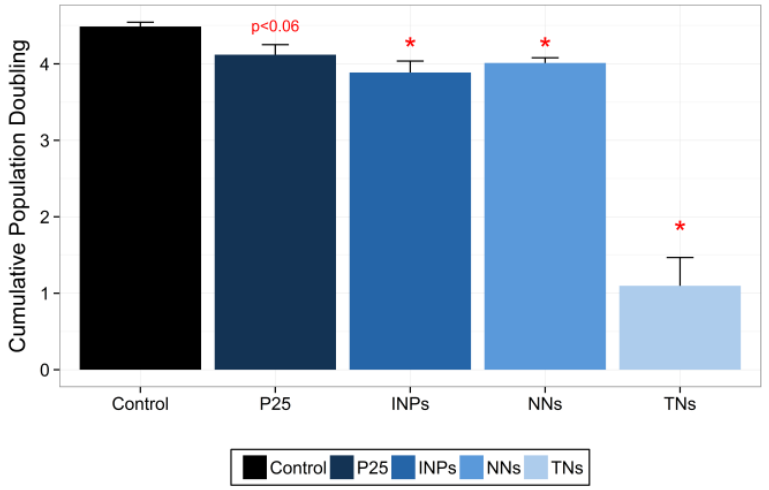
IN VITRO TOXICOLOGY – NPs, DOSE RESPONSE, CELL TYPE & CELL FUNCTION

HUVEC versus HEKn

Huvec
PRIMARY ENDOTHELIAL CELLS
"NUTRITION" FUNCTION



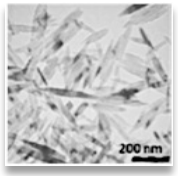
HEKn
PRIMARY KERATINOCYTES CELLS
BARRIER FUCNTION



Relative cell proliferation (mean +/- *sd*) measured 8 days after exposure to TiO_2 NPs.
Exposure doses (µg/cm²): 2.0

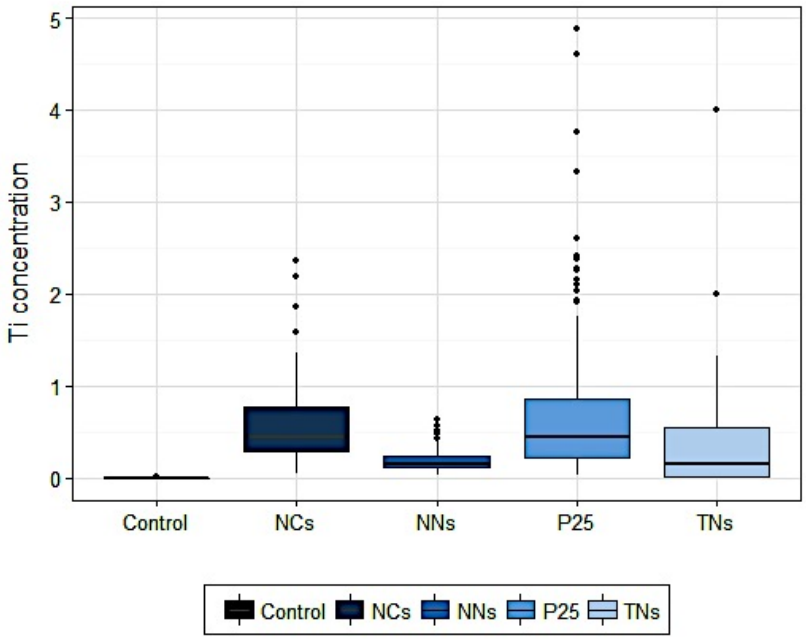
TOXCITY RELATED TO CELL TYPE AND/OR CELL FUNCTION, TO THE TYPE OF NPS, TO THE BEHAVIOR OF THE NPs in BIOLOGICAL MEDIUM

Submitted data

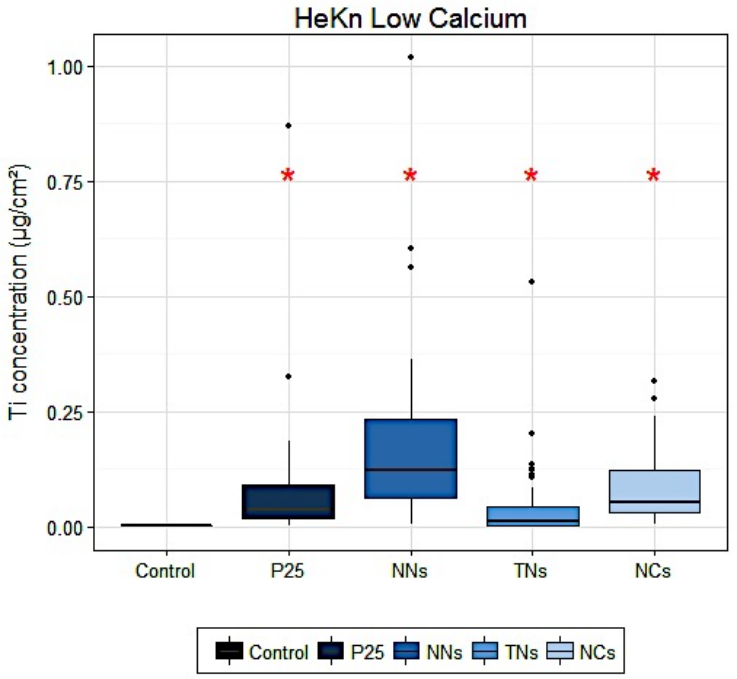


IN VITRO TOXICOLOGY – **TITANIUMS INTRACELLULAR CONTENT**

PRIMARY ENDOTHELIAL CELLS (HUVEC)

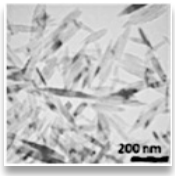


PRIMARY KERATINOCYTES (HEKn)



in vitro NANOPARTICLES TOXICITY	
TOXICITY	❑ DOSE & TIME-DEPENDANT ❑ NANOPARTICLES ❑ CELL LINES (HEKn, Huvec, Hela, HaCat) – PRIMARY CELLS ❑ PHYSIOLOGICAL MEDIUM ❑ **QUANTITY of INTERNALIZED NANOPARTICLES** (Huvec > HEKn) ❑ ***INTRACELLULAR CONCENTRATION THRESHOLD***

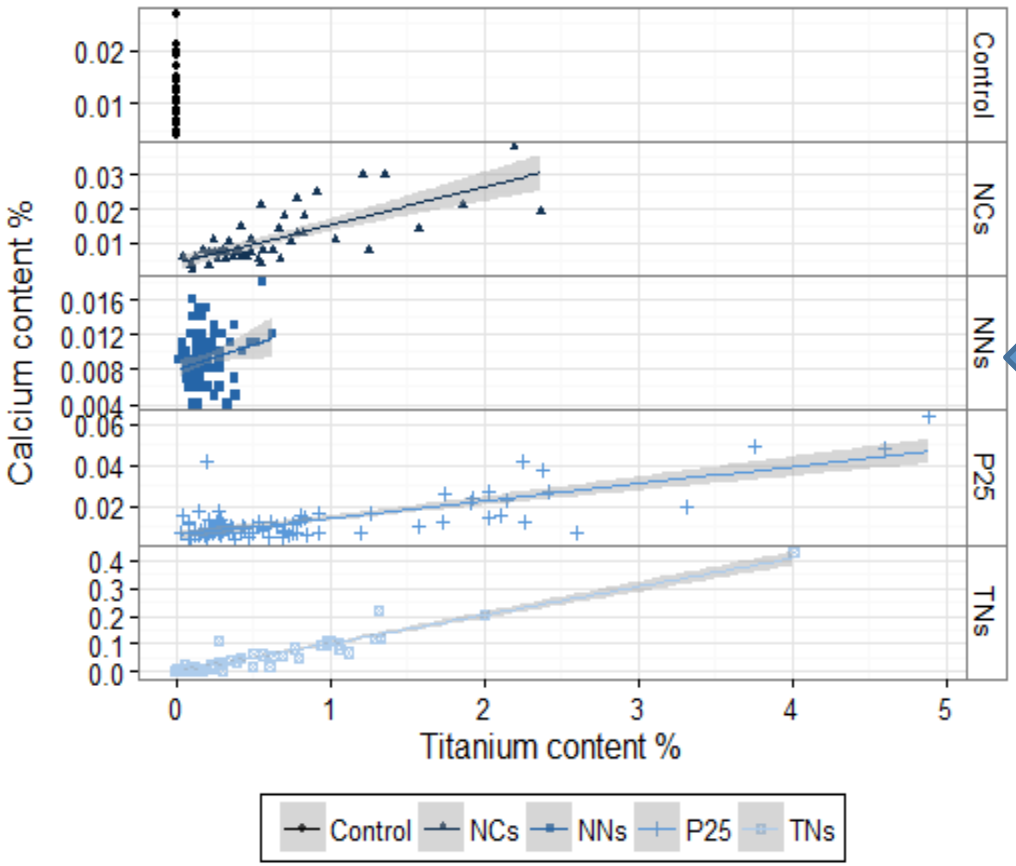
Submitted data



IN VITRO TOXICOLOGY – CORRELATION BETWEEN **TITANIUM AND CALCIUM INTRACELLULAR CONTENTS**

Simon *et al* 2011 *Nanotoxicology*

HUVEC
PRIMARY ENDOTHELIAL CELLS

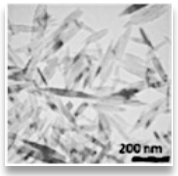


NO CORRELATION
between Ca & Ti (*r=0.2*)

Correlation between Ti and Ca content in single Huvec cells exposed to different types of TiO_2 NPs.
Exposure doses (µg/cm²): 2.0 - Exposure time : 16- 20 h

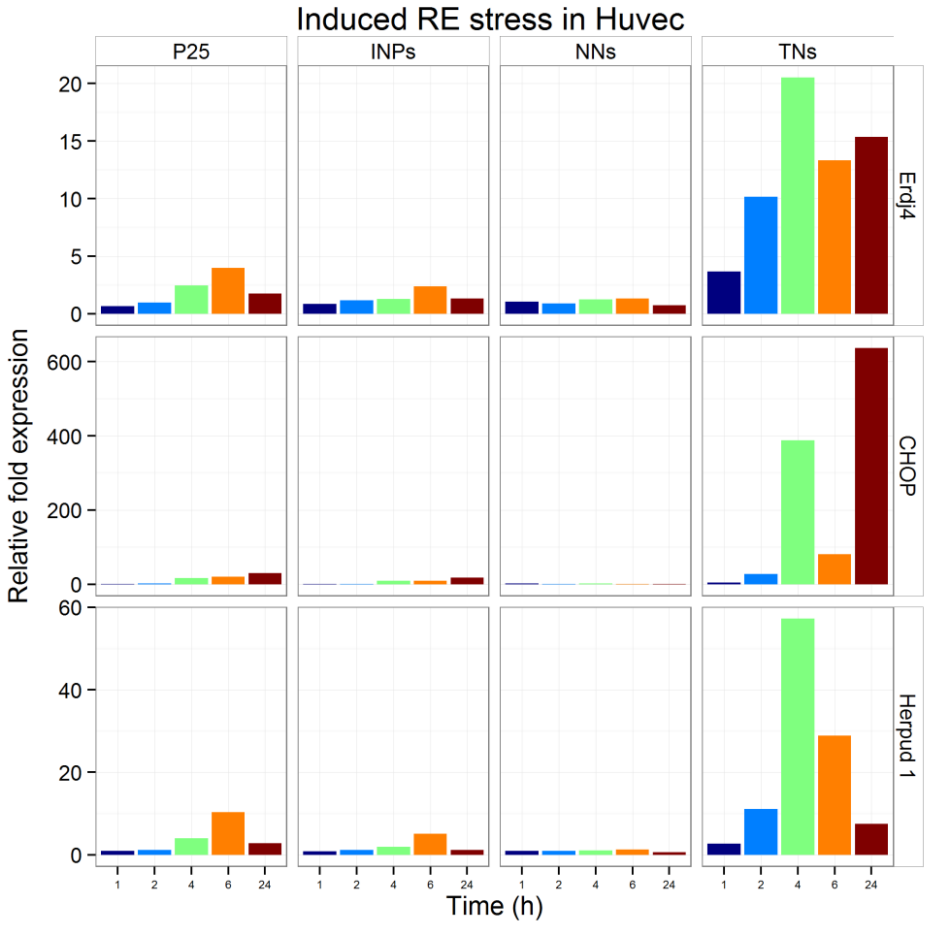
Intracellular TiO_2 content threshold *(to be confirmed)*
P25: +/- 6µg/cm²; **INPs**, +/- 5 µg/cm²; **TNs**, +/- 5 µg/cm²; NNs : *na*

Submitted data



IN VITRO TOXICOLOGY – **CALCIUM HOMEOSTASIS and ER STRESS PATHWAYS**

Huvec :
GENES EXPRESSION –
METABOLIC PATHWAYS
RT-qPCR, 16h



Relative fold expression of three ER STRESS PATHWAYS after exposure to different types of TiO_2 NPs.
Exposure doses (µg/cm²): 2.0 - Exposure time : 16- 20 h

CORRELATION BETWEEN TiO_2 NPs TOXICITY, DOSE, TIME of EXPOSURE in all cell types tested
ER STRESS INDUCTION ASSOCIATED WITH Calcium homeostasis, Mitochondrial defects, ROS

Submitted data

SUMMARY
2nd Part

TiO_2 Nanoparticles & *Human cells (in vitro)*

- ❑ **3 DIFFERENT CELL TYPES** (primary cells *versus* immortalized cell lines)
- ❑ **4 TYPES of NANOPARTICLES** (+ natives *versus* functionalized)

Micro-imaging and quantitative micro-analysis at the cellular level

- ❑ Detection, tracking of NPs at the subcellular scale
- ❑ NPs Intracellular localization
- ❑ Intracellular quantification and relation with physicochemistry of the NPs

***in vitro* toxicity of the NPs** are function with

- ❑ NPs TYPE (shape, surface reactivity, behavior in solution,...)
- ❑ CELL TYPE (primary *versus* immortalized)
- ❑ DOSE and *threshold*
- ❑ QUANTITY of the INTERNALIZED NPs
- ❑ **Correlation with the INTRACELLULAR CALCIUM HOMEOSTASIS**
- ❑ **Correlation with METABOLIC PATHWAYS IMPAIRED (RE Stress)**

(TIME-& DOSE-DEPENDANT)

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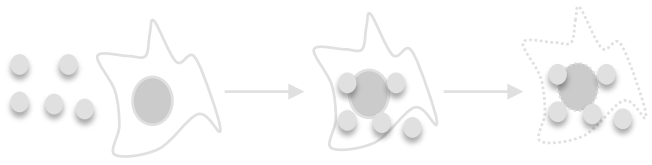
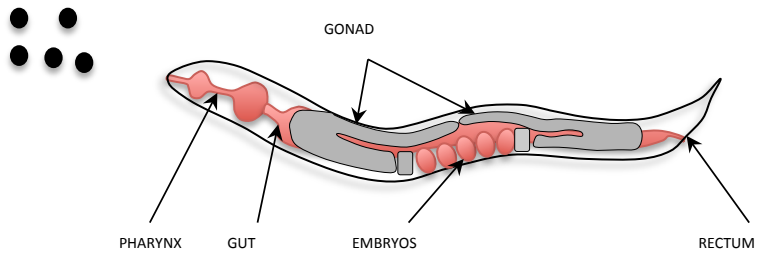
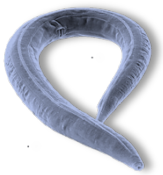
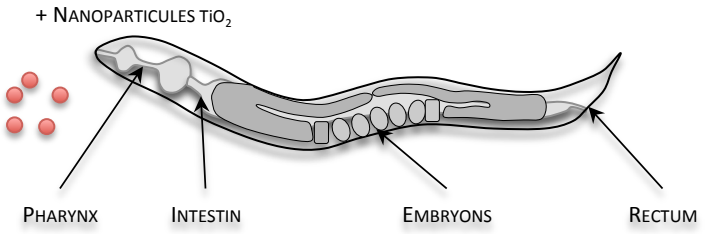
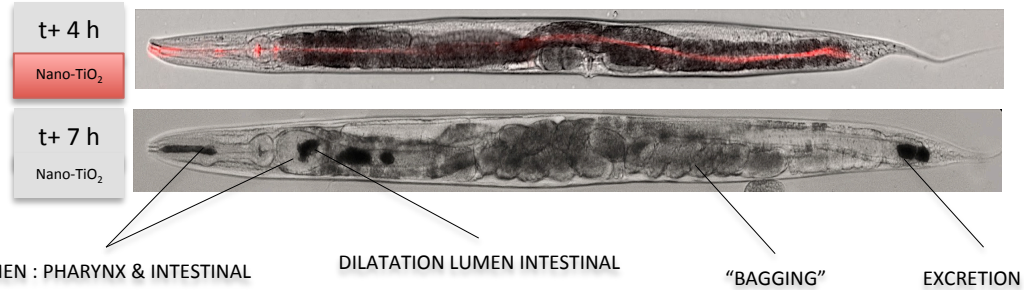


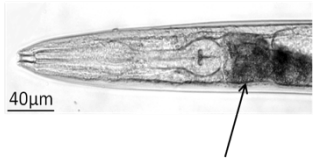


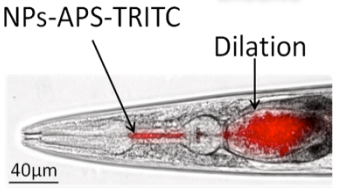


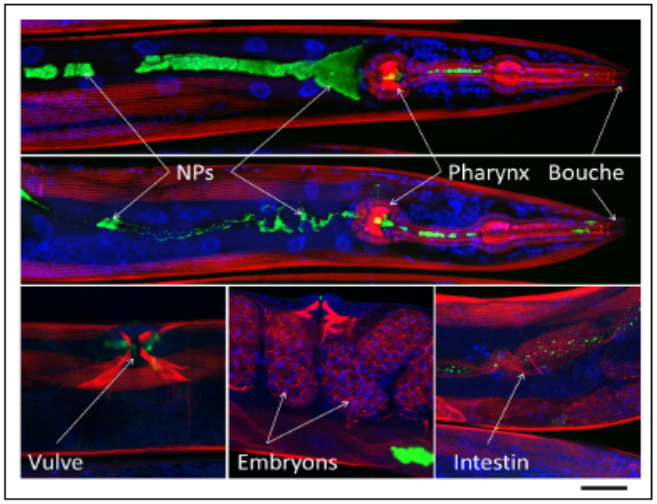
IN VIVO TOXICOLOGY : INTRACELLULAR LOCALISATION & CONVENTIONAL MICROSCOPIES

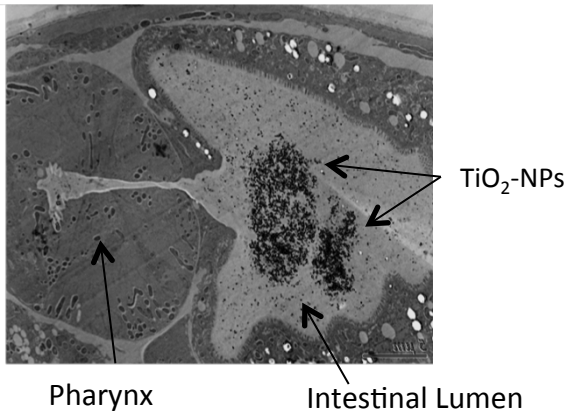








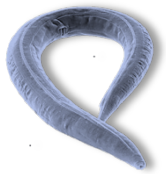




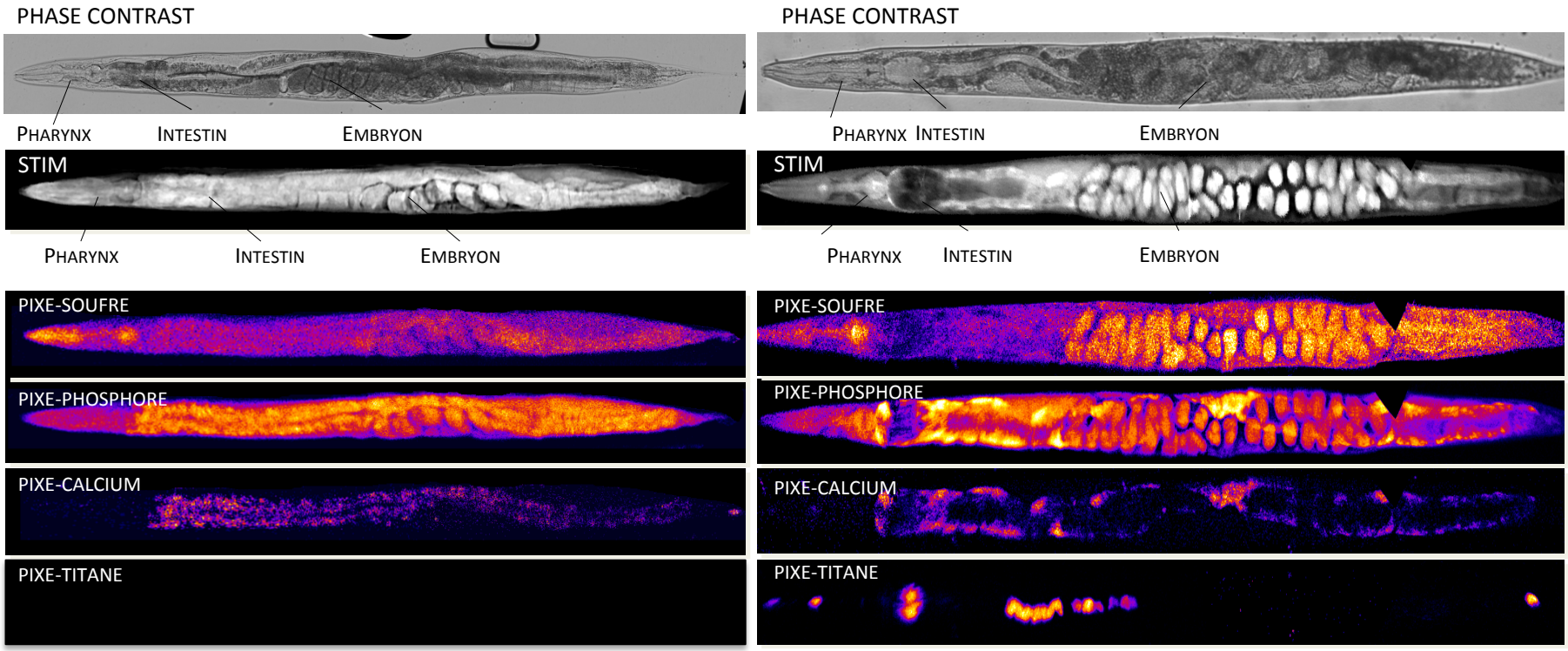
NANOPARTICLES : INGESTION AND EXCRETION

NO CELLULAR INTERNALIZATION (Confocal & Electron Microscopies)
DRAMATIC EFFECT ON MAIN PHYSIOLOGICAL FUNCTIONS
EGG LAYING, LARVAL DEVELOPMENT, BODY GROWTH, MORTALITY,...

Submitted data



IN VIVO TOXICOLOGY : INTRACELLULAR LOCALISATION & IBA-2D



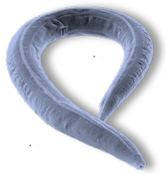
CONTROL

EXPOSED – P25 TiO_2

in vivo LOCALIZATION

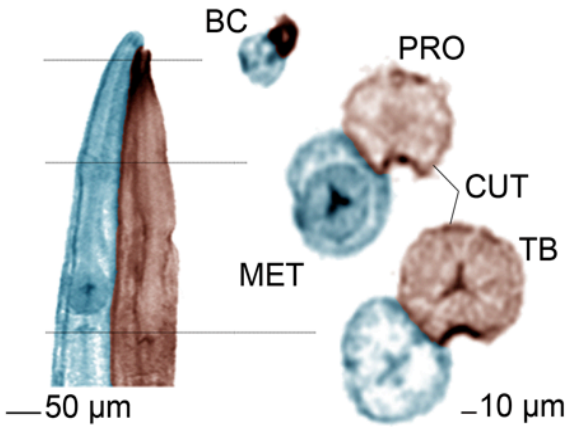
LOCALIZATION : DIGESTIVE LUMEN	❑ **NATIVES NANOPARTICLES** ❑ **LARVAL STAGE L4, YOUNG ADULTE** ❑ **HOMEOSTSIS ALTERATION – CALCIUM, POTASSIUM** ❑ **STRESS RE ...**

Submitted data

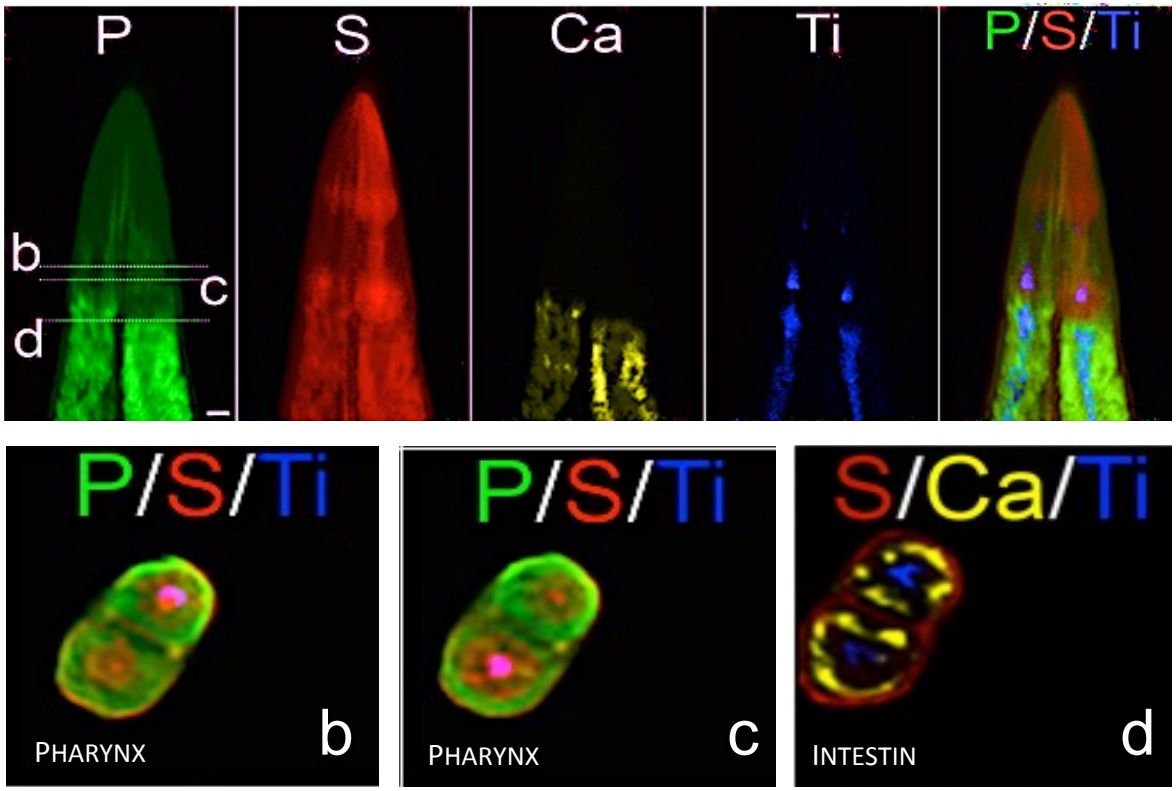


IN VIVO TOXICOLOGY : INTRACELLULAR LOCALISATION & IBA-3D

STIM & PIXE-TOMOGRAPHIES & *C. elegans*



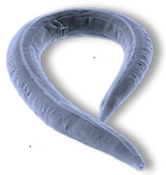
STIM-TOMOGRAPHIE



PIXE-TOMOGRAPHIE

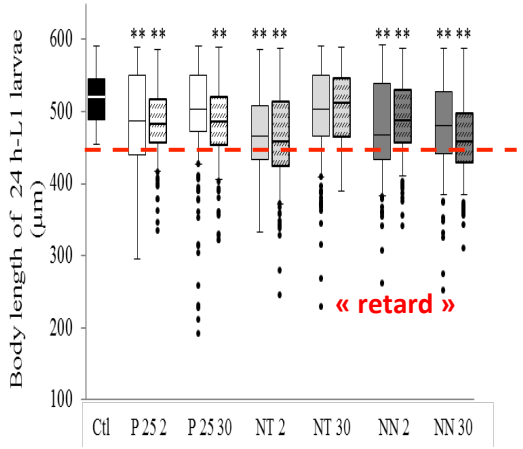
***in vivo* LOCALIZATION**	
LOCALIZATION : DIGESTIVE LUMEN	❑ **HOMEOSTSIS ALTERATION – CALCIUM, POTASSIUM** ❑ **NO <u>INTERNALIZATION IN CELLS</u>.**

Submitted data

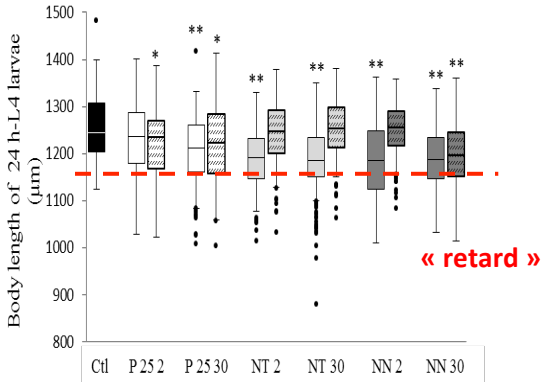


IN VIVO TOXICOLOGY : GROWTH (LARVAL DEVELOPMENT)

GROWTH (**L1 STAGE**)



GROWTH (**L4 STAGE**)



in vivo NANOPARTICLES TOXICITY
NO INTERNALISATION IN CELL
Animals maintained in the dark – limited photocatalysis

NO MAJOR EFFECT according to the **SHAPE** of the **NPs**
NO MAJOR EFFECT of the **CONCENTRATION**
(Marked effects at the lowest dose : bio-availability ?)

NO MAJOR EFFECT **on FOOD SOURCE : *E. Coli* OP50 *Gram(-)***
NO INTERACTIONS

GROWTH	Larval stage dependent Highest sensitivity of the L1 Larval stage
LETHALITY	Larval stage dependent Highest sensitivity of the L1 Larval stage
LIFE CYCLE	**2 days** EFFECTS OBSERVED THE FIRST 10 DAYS OF EXPOSURES
REPRODUCTION	*NO DRASTIC EFFECT*

Submitted data

PERPSPECTIVES

TiO_2 Nanoparticules & Human Cells

- ❑ CHARACTERIZATION OF THE SPECIFIC METABOLIC PATHWAYS INVOLVED
- ❑ CORRELATION WITH TiO_2 NPs TOXICITY
- ❑ DOSE CORRELATION
- ❑ CONSEQUENCES FOR LONG-TERM EXPOSURE
- ❑ *INTERESTS FOR FUTURE AND POTENTIAL THERAPIES : CANCER*

METAL AND METAL OXIDE NPs & Human Cells

TiO_2, HfO_2, GD_2O_3, Au, Pl, ...

- ❑ CHARACTERIZATION OF THE SPECIFIC METABOLIC PATHWAYS INVOLVED
- ❑ ROUTINE EVALUATION METABOLIC PATHWAYS **– collaboration: BMY Screen**

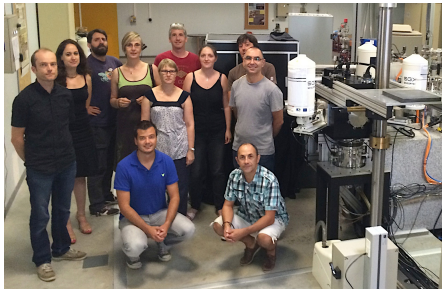
TiO_2 Nanoparticules & *C. elegans*

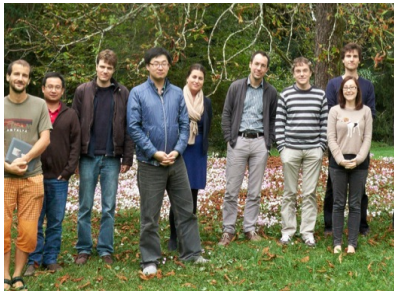
- ❑ CHARACTERIZATION OF THE SPECIFIC METABOLIC PATHWAYS INVOLVED
- ❑ UNDERSTANDING THE MECHANISMS OF ACTION
- ❑ SAME MECHANIMS THAN IN HUMANS - VALIDATION

Acronyme	**PROTON *(Défi Nano, Mi CNRS)***		
Titre	Evaluation Multi-échelle de nouvelles stratégies anti-cancéreuses associant PROTONs et Nanoparticules : de *l'in silico* à *l'in cellulo/in vivo*		
Durée	36 mois	Budget accordée 2015	40 k€
Consortium	CENBG (CNRS/IN2P3), ISMO (Univ. Paris Sud), ICMCB (CNRS/INC), Service Radiothérapie Oncologie Institut Bergonié Bordeaux Unité VINCO/INSERM, Institut Bergonié Bordeaux		

PROTON	**Consortium, Expertise & travaux antérieurs**	
ICMCB MH Delville	**Axe 1 -** Synthèse hydrothermale, caractérisation Nanoparticules,... TiO_2, HfO_2, Gd_2O_3, Fe_2O_3, Gd_2O_3/Eu, ...	CHIMIE – INC
CENBG iRiBio H. Seznec P. Barberet	**Axes 2-3-4-5** : Modélisation Monte Carlo (Geant4, Geant4DNA) Micro-irradiation cellulaire & Micro-analyse chimique multi- élémentaire Radiobiologie, nanotoxicologie, biologie cellulaire	PHYSIQUE, CHIMIE, BIOLOGIE IN2P3, INC, INSB
ISMO S. Lacombe	**Axes 3-4-5** : Micro-analyse chimique/Micro-imagerie Radiobiologie Proton, Hadron), Nano-Radiobiologie, biologie cellulaire	PHYSIQUE, CHIMIE, BIOLOGIE Univ. Paris Sud/Orsay

Service Clinique **Radiothérapie Oncologie** **Institut Bergonié** **Institut Curie/Hôpital**	**Plateformes** - AIFIRA - Synchrotrons - BIC, IBISA	***Collaborations avec différents Industriels :*** - *Nanobiotix* - *NanoH, CHeMatech* - *QuantumWise (Danemark)*





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THANK YOU FOR YOUR ATTENTION…