Unit of Human Pathologies in Model Organisms
Department of Molecular Biochemistry and Pharmacology

ISTITUTO DI RICERCHE FARMACOLOGICHE
MARIO NEGRI
IRCCS

Le giornate della salute e del benessere: Innovazione e Ricerca

Nanomateriali e nanotecnologie in alimenti e cosmetici

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FAST
Piazzale Morandi 1- Milano

TEST DI TOSSICITA' DI NANOMATERIALI

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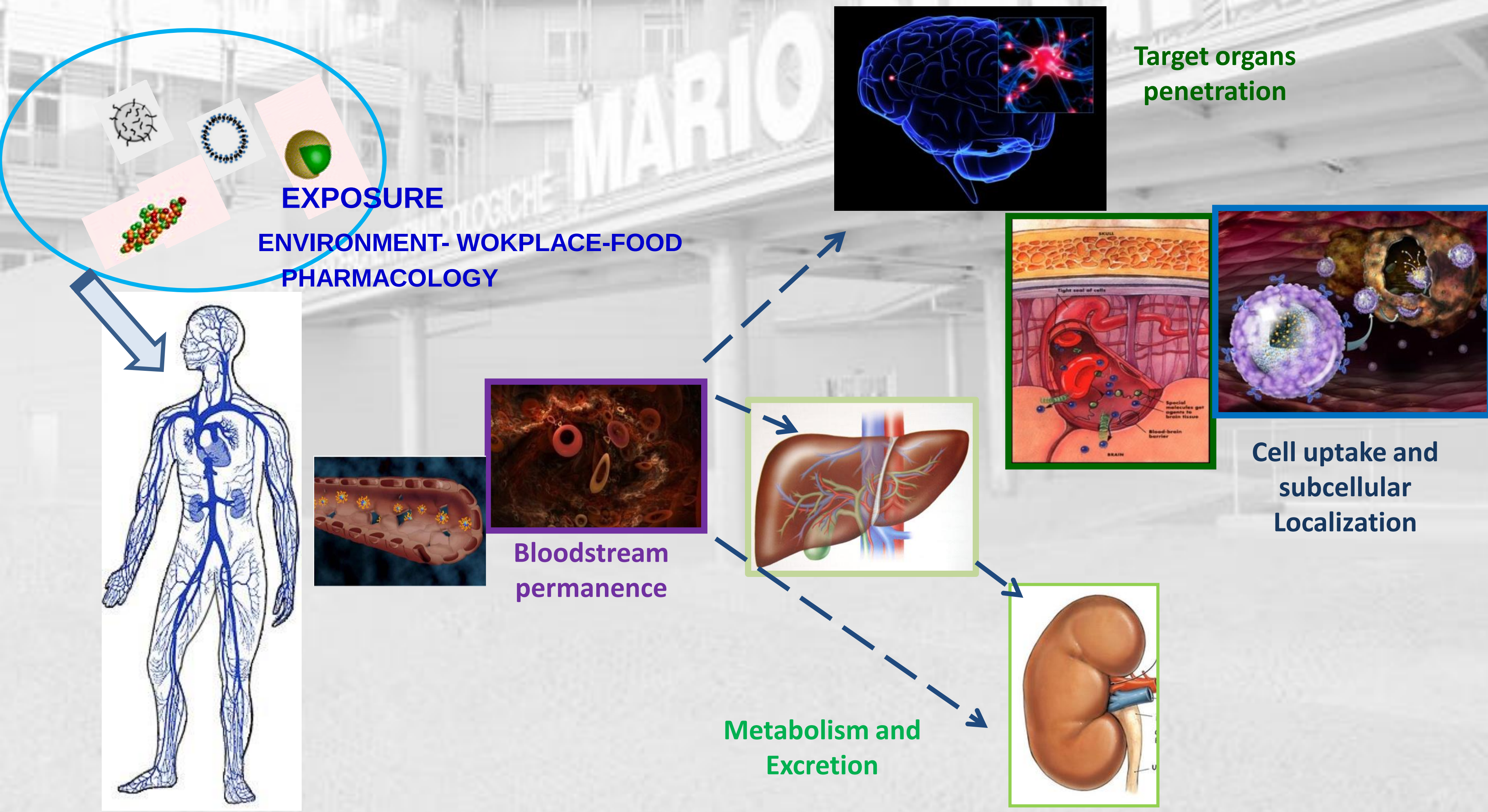
The toxicology of NPs

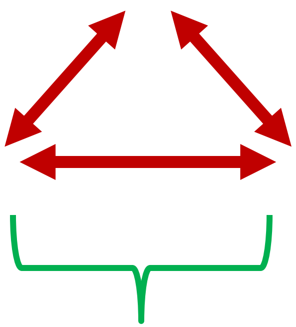
What adverse effects might we expect from exposure to NP?



Factors contributing to toxic response to nanoparticles

Intrinsic toxicity of the material

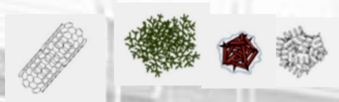
Exposure concentration



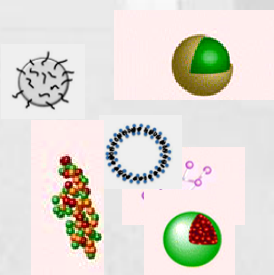
Length of exposure

Contribute to dose

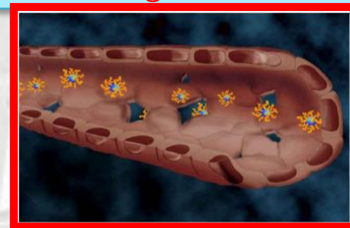
INTEGRATED PLATFORM to understand interaction nanomaterials-living organisms at different levels of complexity



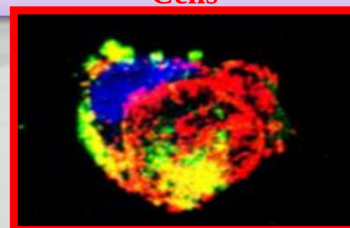
From the DOSE…



Biological fluids



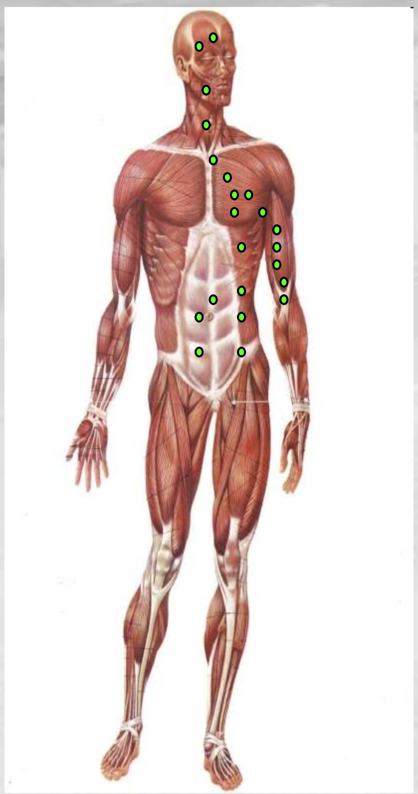
Cells



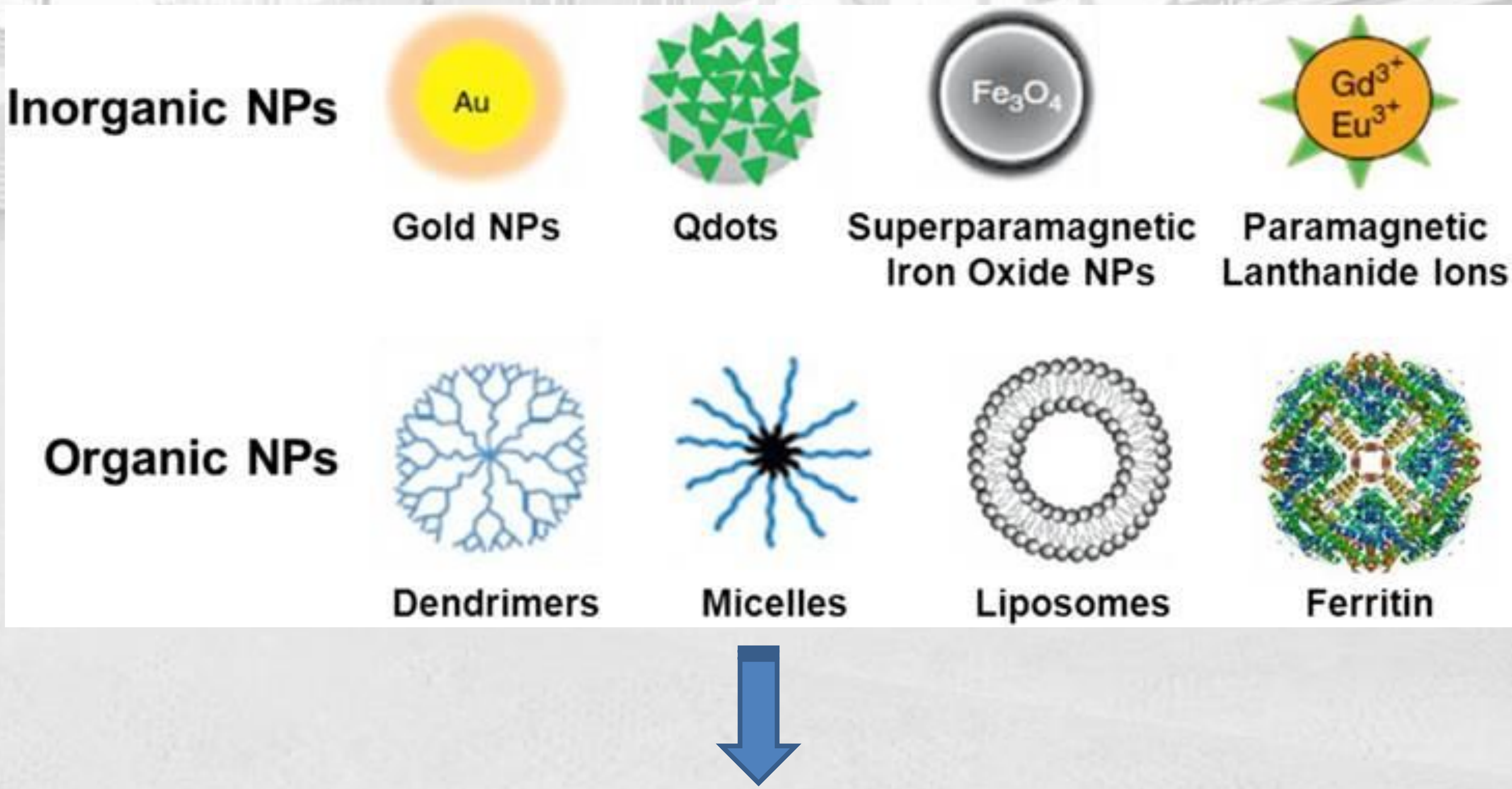
Imvertebrate animal Models

Vertebrate animals

…to the EFFECT on human health



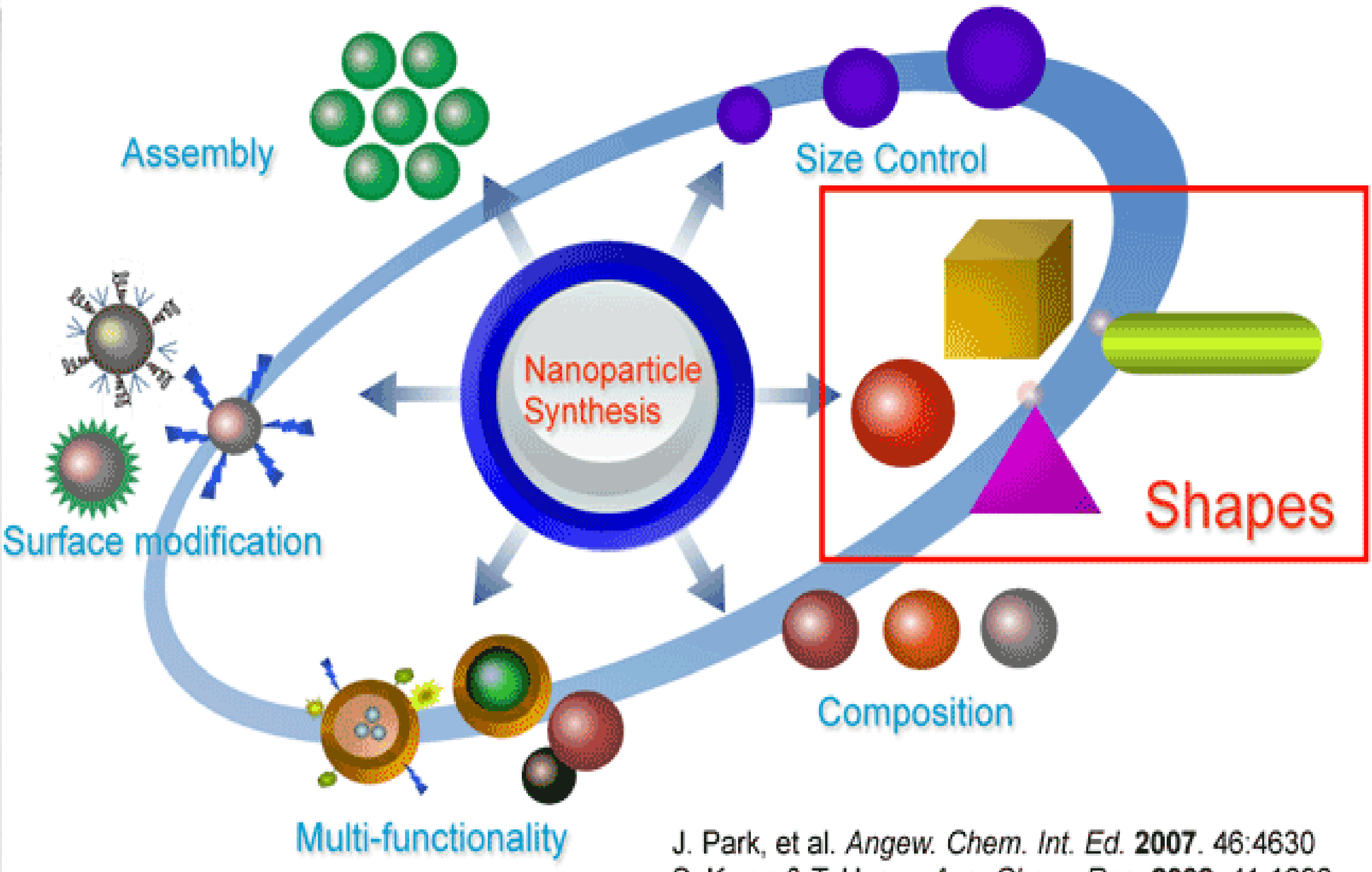
Which kind of nanomaterial?



BIO/NANO INTERACTION

Which features?

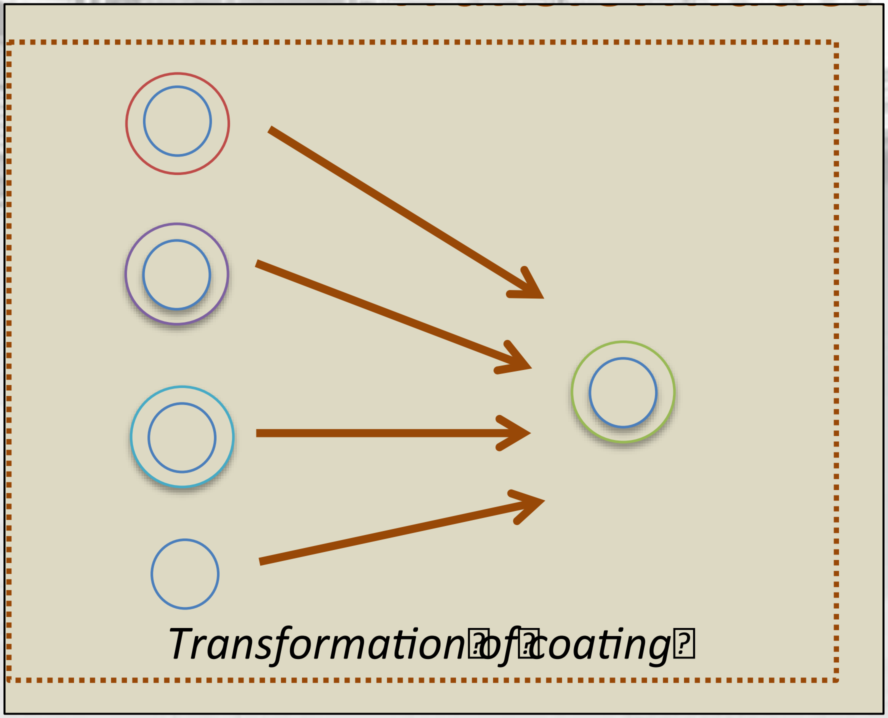
NP properties affect their biological effects

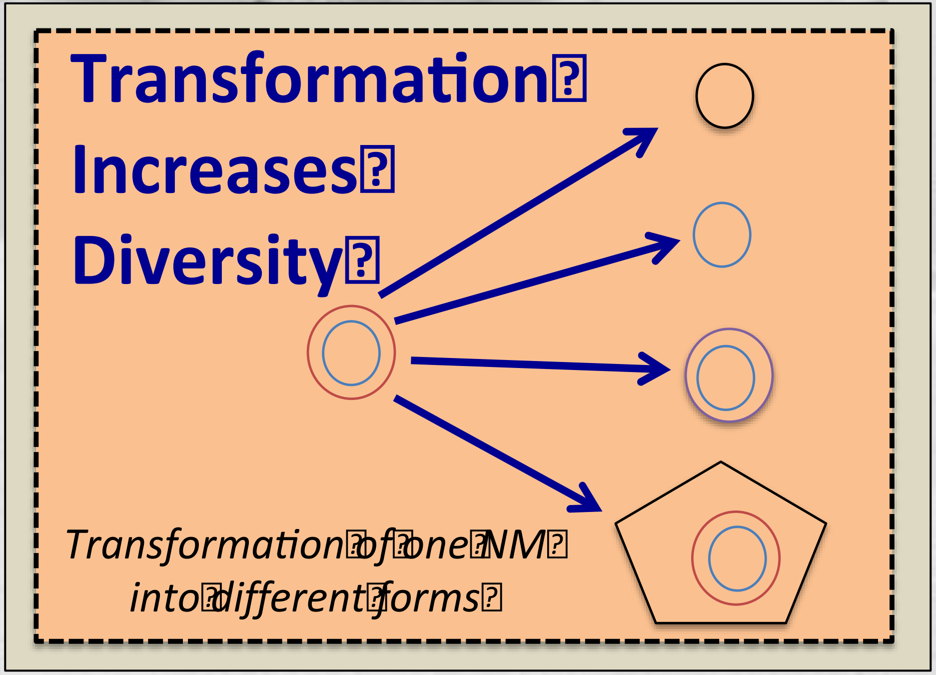


J. Park, et al. *Angew. Chem. Int. Ed.* **2007**. 46:4630
S. Kwon & T. Hyeon *Acc. Chem. Res.* **2008**. 41:1696

Environmental transformations can affect the similarity or increasing the diversity of nanomaterials

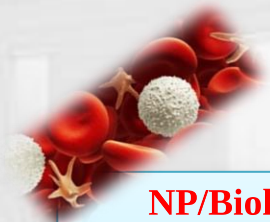
Transformation Increases Similarity

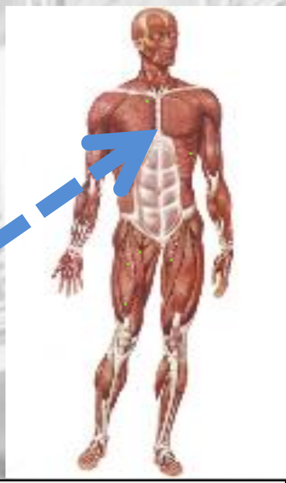




Mitrano et al., 2015

Different biological matrices with an increasing complexity





NP/Biological fluids

(medium, plasma, water, gastric juice, lung surfactant)

- Transmission Electron Microscopy
- Electron Spectroscopic Imaging
- Atomic Force Microscopy
- Binding experiments
- Surface plasmon resonance

NP/Cells

- Confocal Microscopy
- Transmission Electron Microscopy
- Super-resolution microscopy
- Cell segmentation, Automatic counting
- High throughput screenings (toxicity-physiology)
- FACS analysis.

NP/Simplified Animal Models

- Wild-type nematode *C. elegans*
- Transgenic *C. elegans* generation
- Treatment and behavioural trials
- Biochemical studies
- Histological and ultrastructural analyses

NP/ Rodents

- Specific Pathogen free animal facility
- Nude, immunocompetent and immunodeficient mice
- Tumor-bearing and transgenic and mice/rats
- *In vivo* imaging (MRI, MicroCT, Ultrasounds, Optix)
- Nanokinetics, histology, marker determination

Nanomaterials on the world market

SiO_2 and TiO_2 are the nanomaterials with the highest production volumes worldwide and are the most common in products as well

Estimated global production volumes of different nanomaterials.

Nanomaterial	Production volumes (in tons p.a.)	Year
SiO_2	1,590,000	2009
TiO_2	700-61,000 50,000 44.000 (only USA) 1,450 (only Japan)	2007/2008 2010 2008 2019
ZnO	20-10.000 480 (only Japan)	2007/2008 2009
CeO_2	10,000	2010
Al oxides	100	2003
ZrO_2	2,500	2010
Metals	20	2007
Silver	4-560	2005/2008
Quantum dots	< 100 kg	2001

Möller, M. et al., 2013,

Food grade titanium dioxide (E171) contains NPs

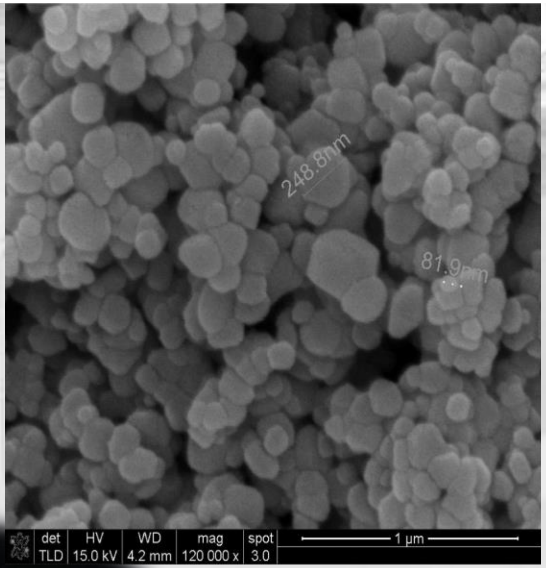

Environmental Science & Technology

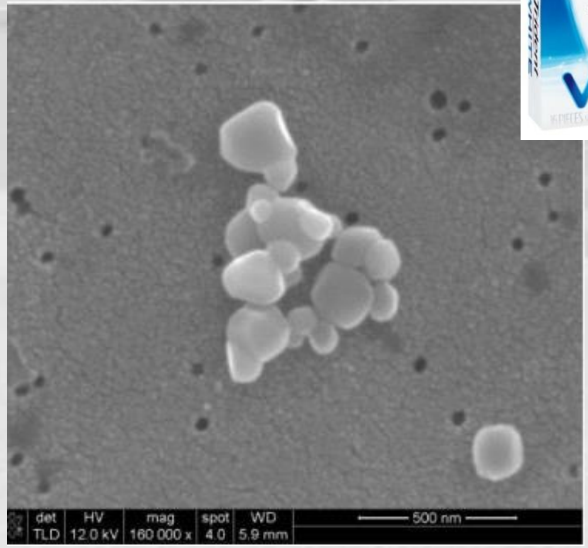
Article

pubs.acs.org/est

Titanium Dioxide Nanoparticles in Food and Personal Care Products

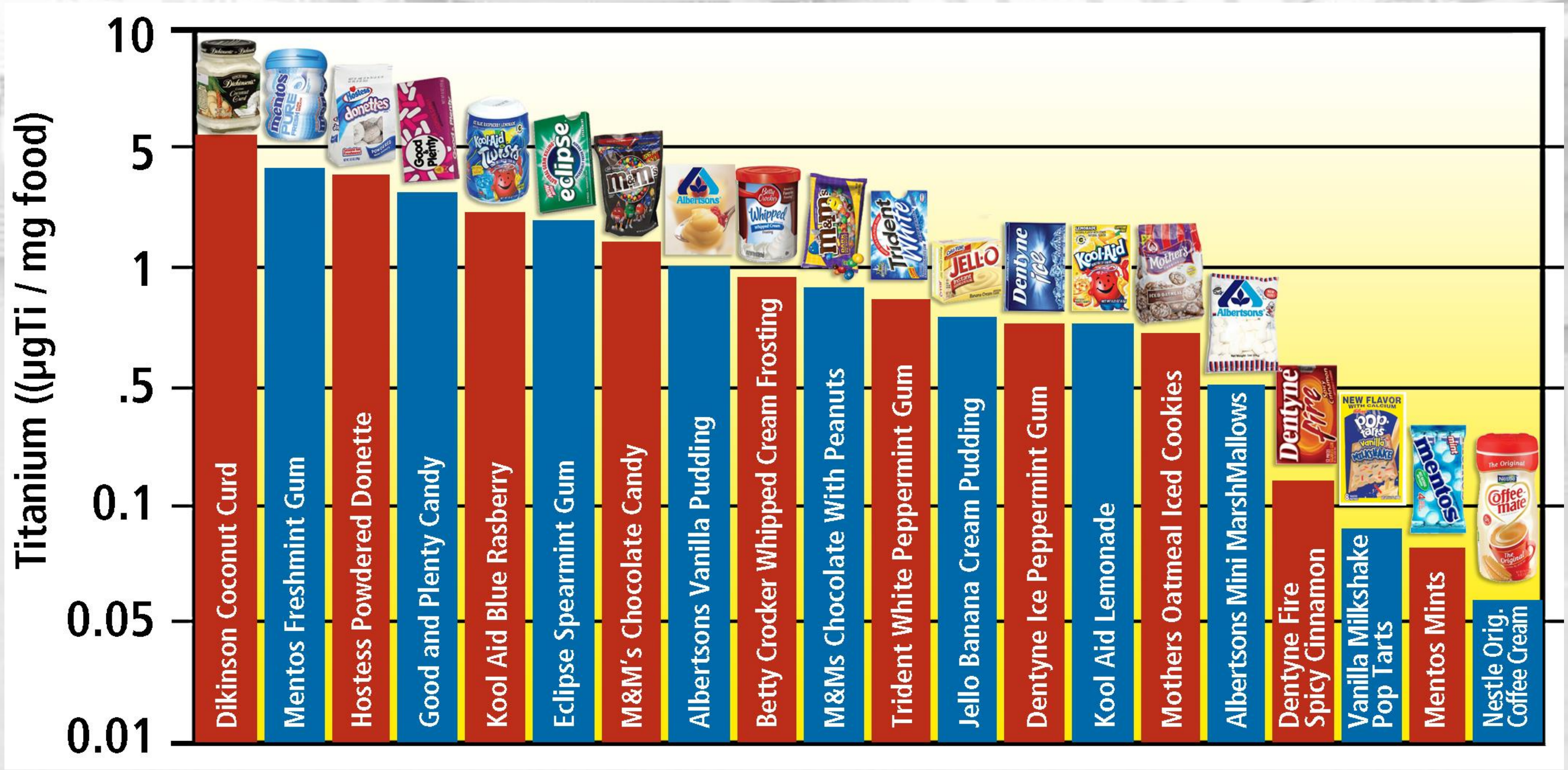
Alex Weir,[†] Paul Westerhoff,[*,†] Lars Fabricius,[‡,§] Kiril Hristovski,[∥] and Natalie von Goetz[‡]





Weir et al., 2012

Nano-TiO_2 is present in different foods



Weir et al., 2012

TiO_2 exposure (E171) largely depends on dietary habits

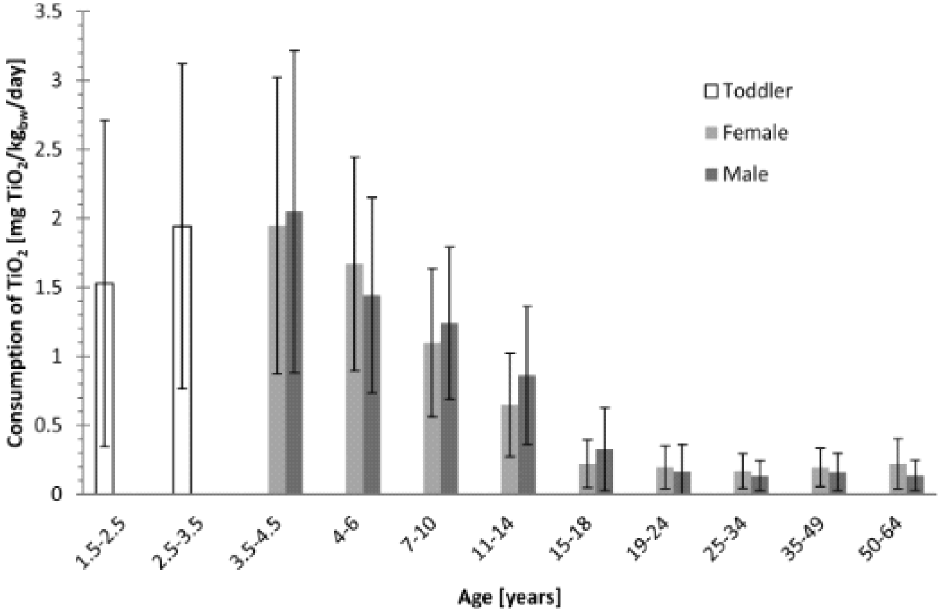


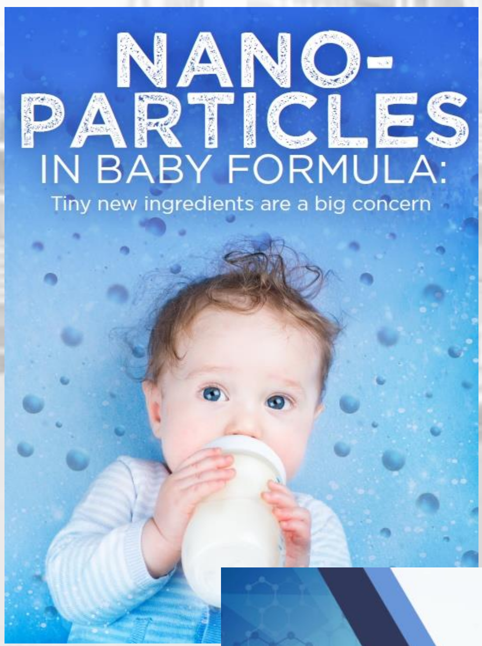
Figure 3. Histogram of the average daily exposure to TiO_2 for the US population (Monte Carlo simulation). Error bars represent the upper and lower boundary scenarios.

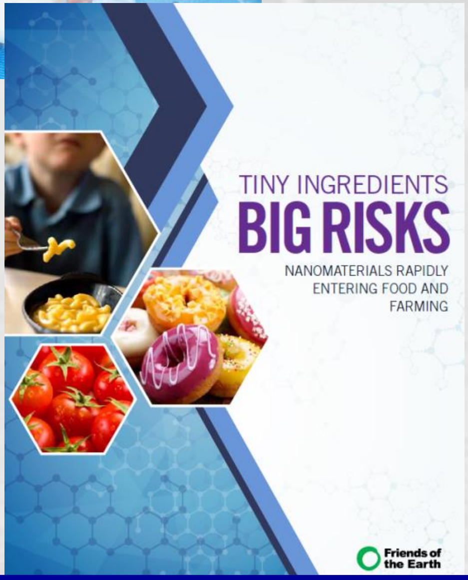
TiO_2 exposure ranges from 0.1-0.3 g/kg body weight /day.

Children are exposed around twenty-fold higher than adults

Weir et al., 2012

What do consumers think about TiO_2?





2. HEALTH CONCERNS: WHY NANOMATERIALS AND NANOFOODS POSE NEW RISKS

- They can be more chemically reactive and more bioactive than larger particles of the same chemicals.
- Due to their very small size, nanoparticles also have much greater access to our bodies, so they are more likely than larger particles to enter cells, tissues and organs.
- Greater bioavailability and greater bioactivity may introduce new toxicity risks.
- They can compromise our immune system response.
- They may have long-term pathological effects.

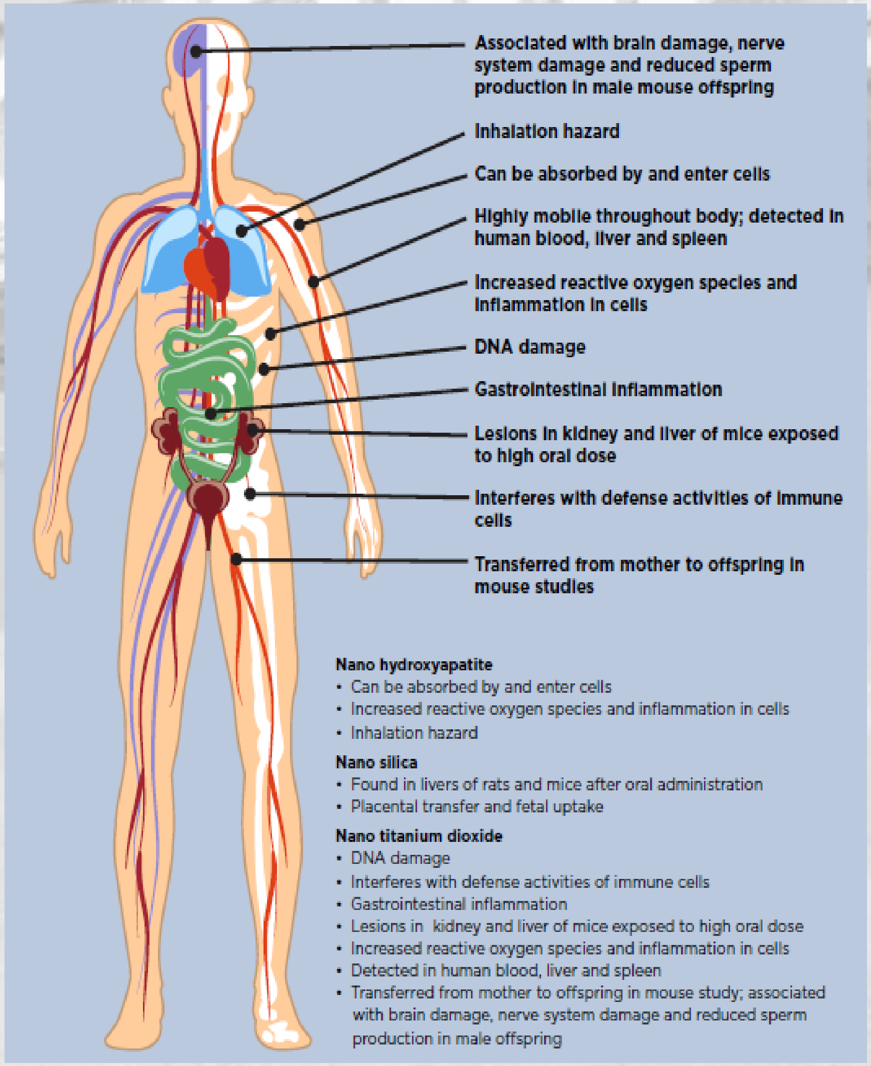
Friends of the earth, 2014, 2016

Il Salvagente, 2012

Which is the effect of ingested TiO_2 nanoparticles?

In the last decade a large body of evidence has been generated about sub-pathological and pathological effects of TiO_2 NPs *in vitro* and *in vivo.*

However, very often, these results were mismatching and controversial.



Friends of the earth, 2016

Which is the nature of E171?

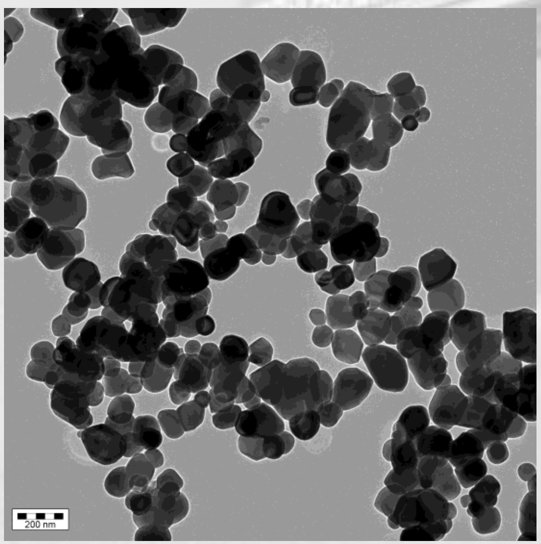
TRANSMISSION ELECTRON MICROSCOPY

Size distribution

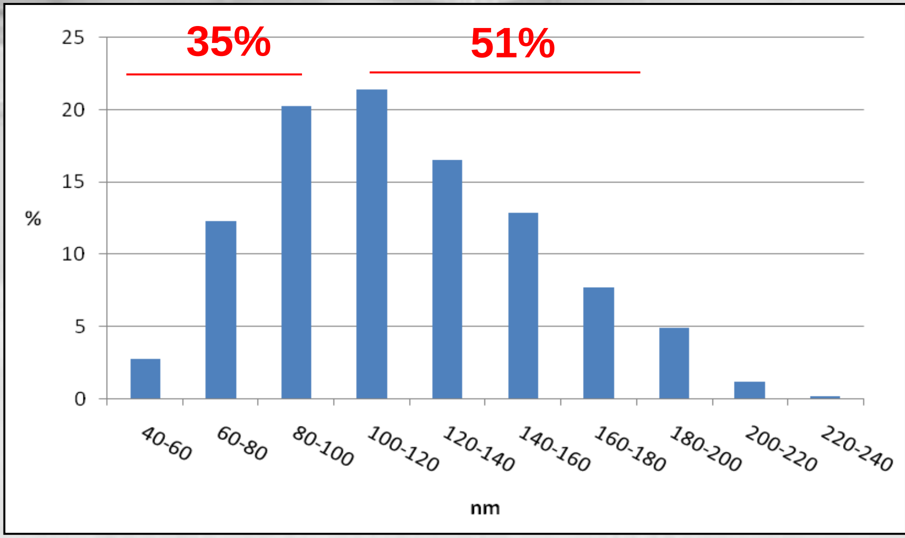
Shape

Colloidal stability

Food grade TiO_2 (E171 Pretiox –Faravelli)



Mean diameter distribution



The percentage of nanoparticles with sizes below 100 nm is 35.
The 51% of the nanoparticles have a diameter between 100 and 160 nm.

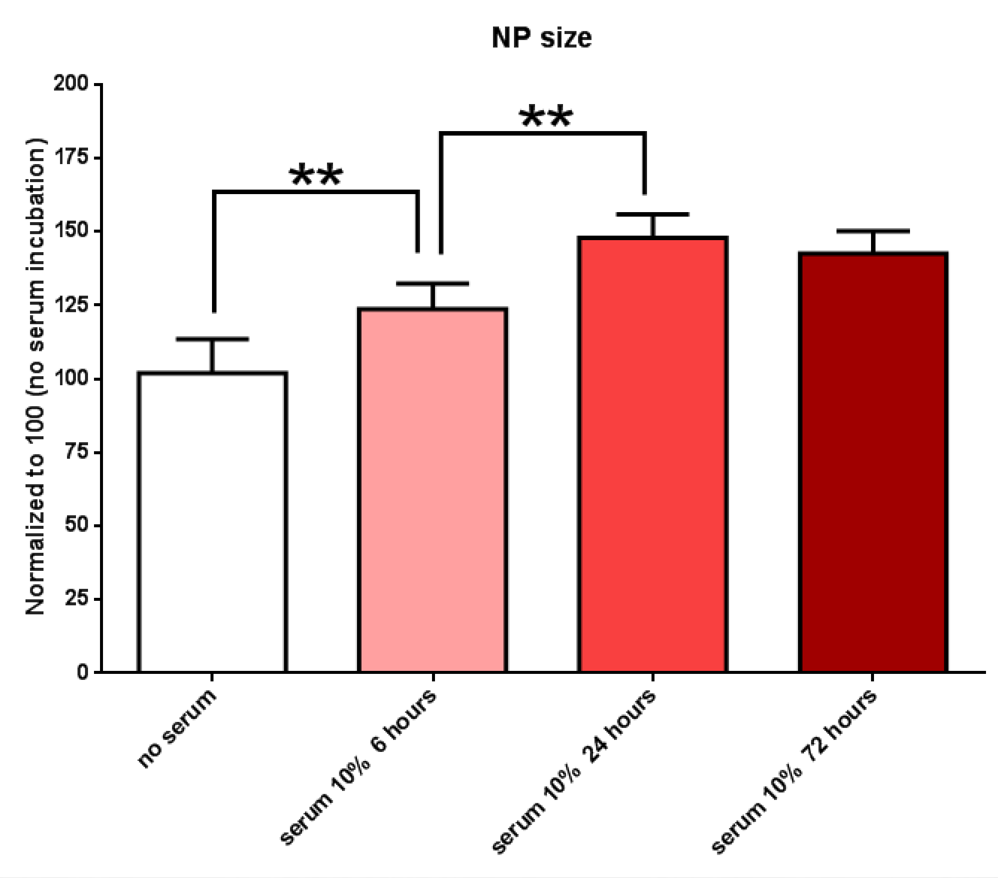
Fabio Fiordaliso, unpublished

Is it influenced by fluids?

NANOSIGHT

Size distribution

Colloidal stability



Serum incubation leads to a little but significant increase of the mean diameter of E171. This slow increase in size seems to be more related to a phenomenon of protein corona formation rather than aggregation.

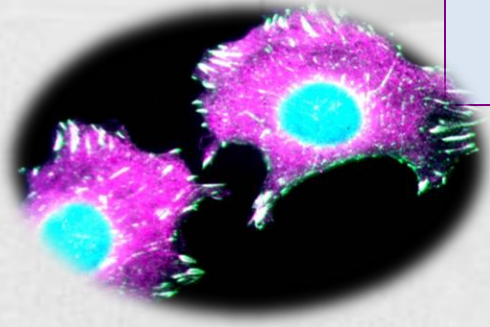
Paolo Bigini & Inge Nelissen, unpublished

Which is the effect ?

Caenorhabditis elegans:

an invertebrate animal model organism for the study of molecular genetic-related human diseases.

A bridge between cells and mammals.



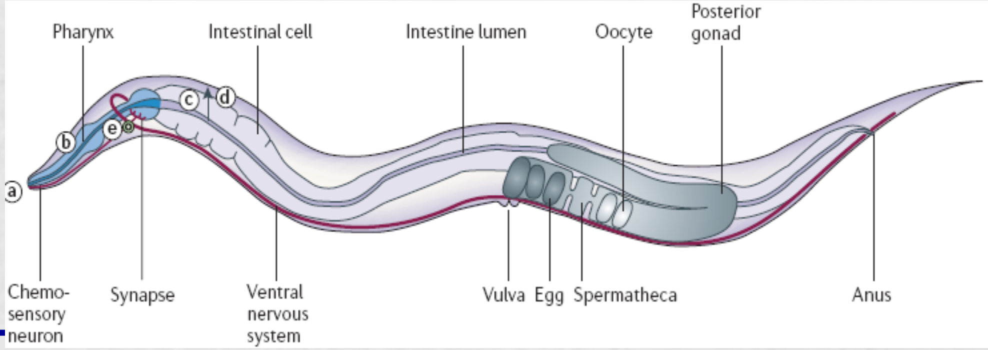
Why *C. elegans*?

Although evolutionarily far from vertebrates, 65% of its genes have human homologues and many human stress pathways are conserved. It is therefore a rapid and versatile system for easily recapitulating the key molecular mechanisms underlying complex toxicological features.

It is ease to use and relatively inexpensive (high-throughput assays on whole organisms).

A wide range of genetic tools are available permitting investigation of mechanisms and genetic sensitivity.

It has well-characterized anatomical and toxicological features, allowing easy correlation between the organ-specific bio-accumulation of NPs and their biological effects.



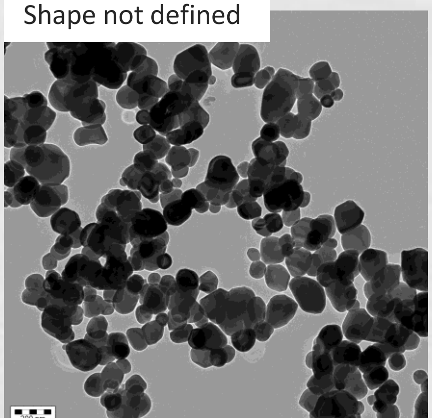
The effect of E171 was compared to that of TiO_2 NPs with well-defined dimensions.

0.2 mg/ml

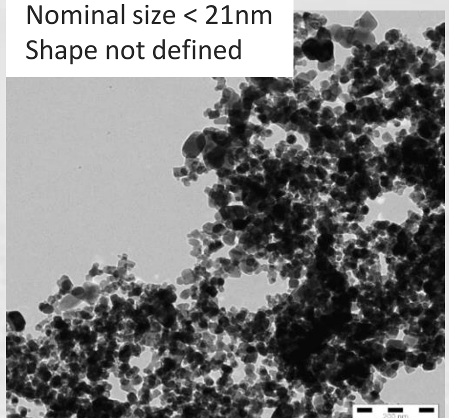
Behavioral alterations

Bio-accumulation (RAMAN Imaging)

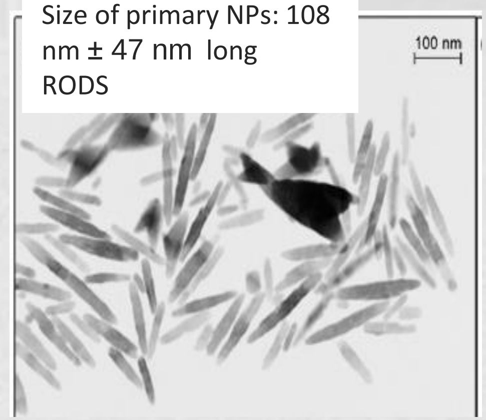
E171
Shape not defined



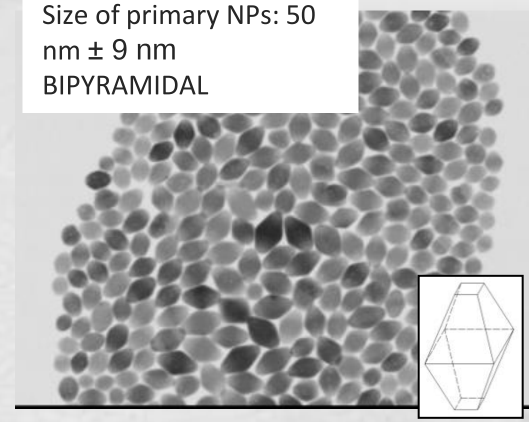
Sigma-Aldrich
Nominal size < 21nm
Shape not defined



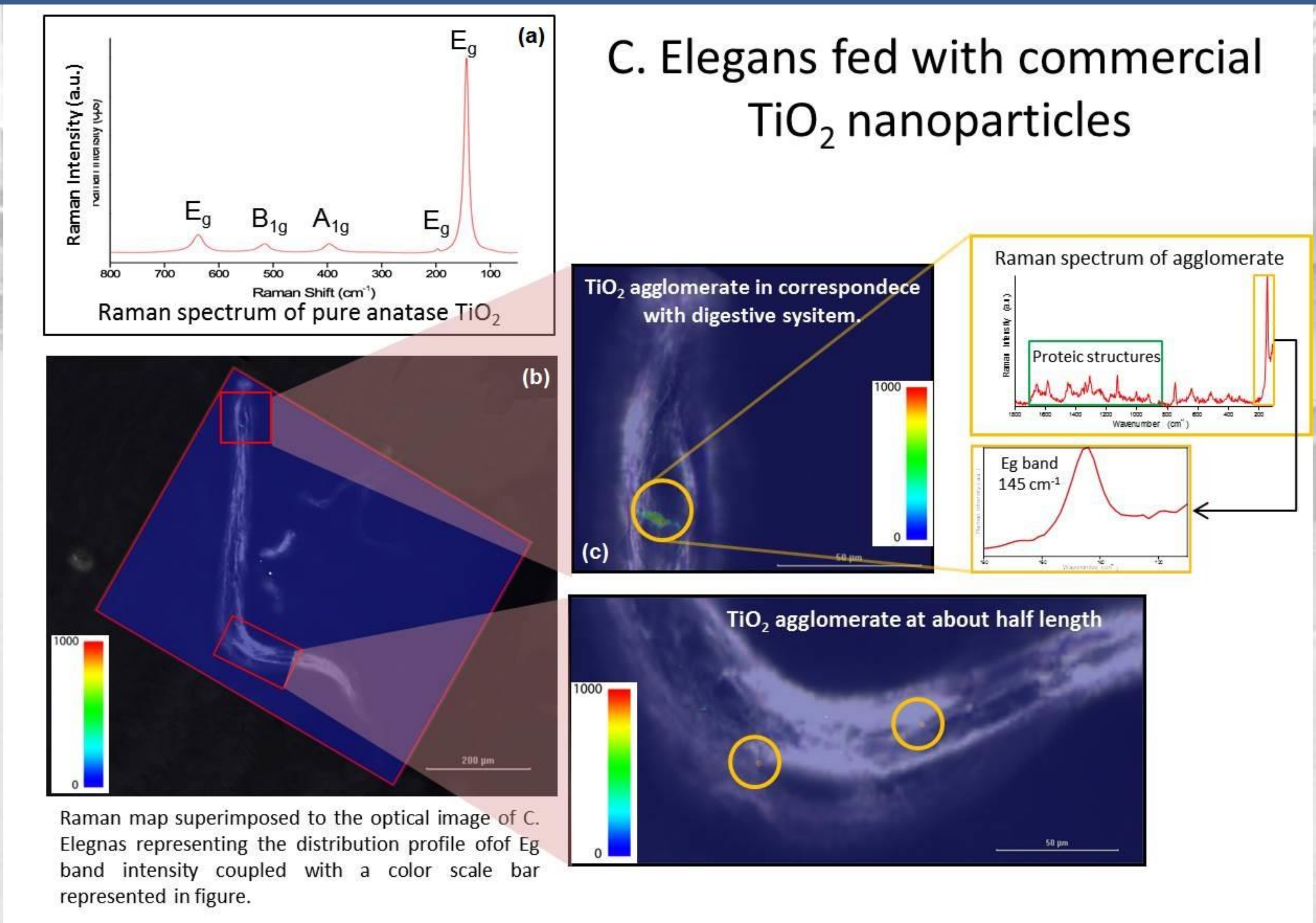
SETNanoMetro 1
Size of primary NPs: 108 nm ± 47 nm long
RODS



SETNanoMetro 2
Size of primary NPs: 50 nm ± 9 nm
BIPYRAMIDAL



TiO_2 preferentially accumulated in the pharynx and reproductive area



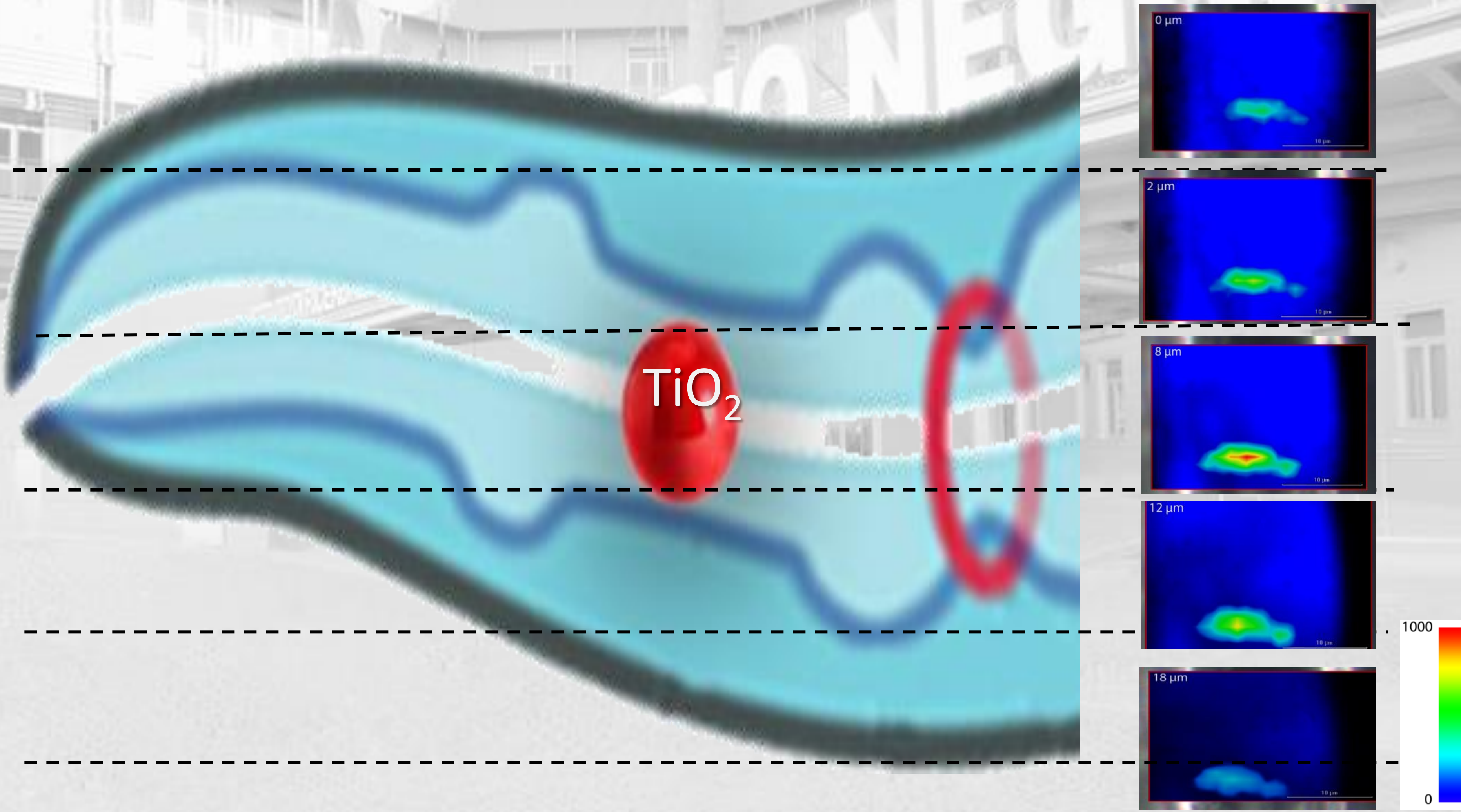
a) Raman spectrum of TiO_2; (b) Raman mapping of *C. elegance* worm fed with TiO_2; (c) Raman mapping particular showing TiO_2 Agglomerate detected in *C. elegans* model system.

Iannarelli et al., submitted

TiO_2 accumulates in the pharynx

Raman Mapping Depth Profiling

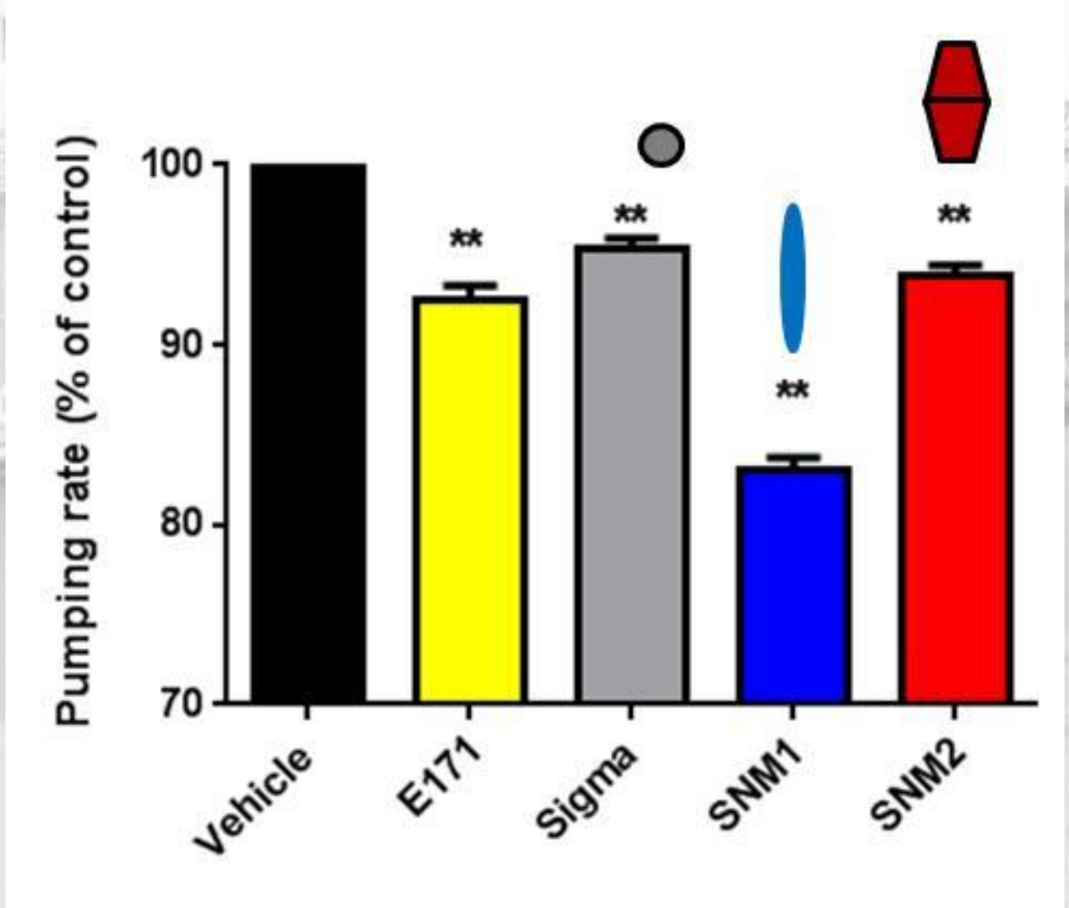
Raman Section on z axis



Colour scale, from blue to red, is related to the peak height of charachteristic anatase 145 cm^{-1} signal
Blue areas, are related to charachteristic signals of *C. elegans* in the region from 2800 to 3100 cm^{-1}

Iannarelli et al., submitted

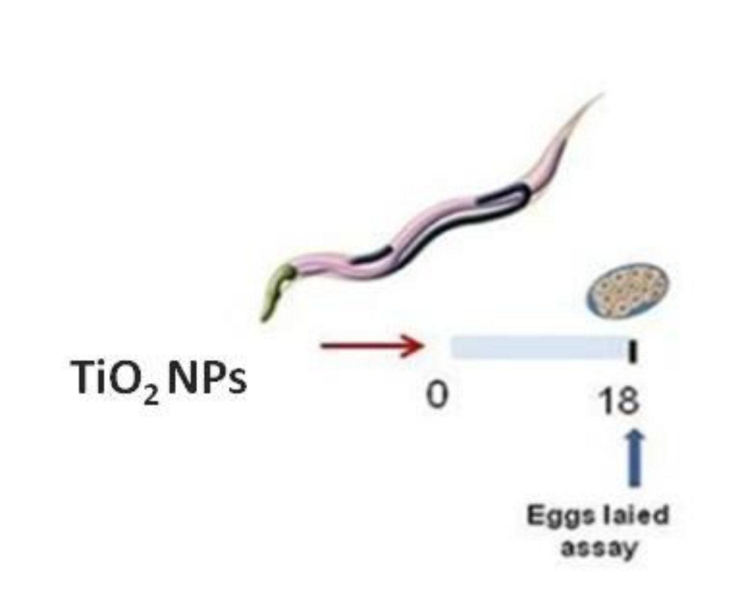
TiO_2 permanently affects the feeding behavior of worms

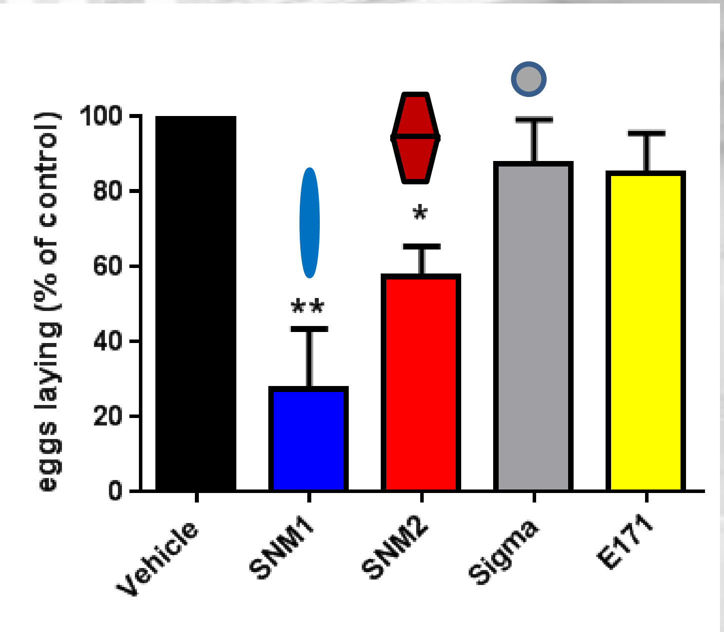


Data are expressed as the mean ± standard error (SE) (N=40 worms/group).
****p< 0.01 *vs.* Vehicle, one-way Anova and Bonferroni *post hoc* test analysis.**

Iannarelli et al., submitted

Once ingested, TiO_2 NPs pass the intestinal barrier and reach the reproductive system of worms, affecting the eggs deposition.



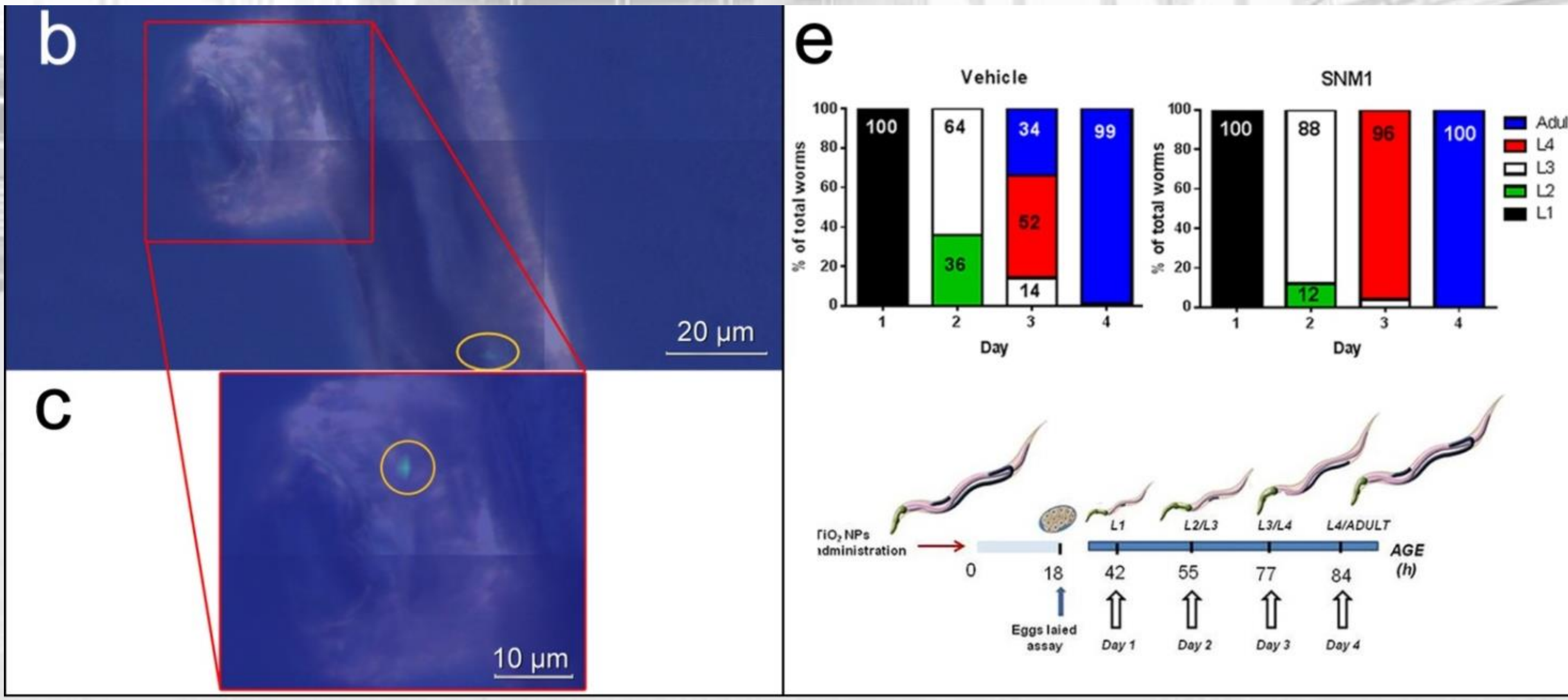


*p<0.05 and ** p<0.01 vs. Vehicle according to one-way Anova and Bonferroni *post hoc* test analysis.

The shape and the agglomeration state of NPs influence their effect on the worm's reproduction.

Iannarelli et al., submitted

Only rods TiO_2 NPs affect the larval development



Iannarelli et al., submitted

The Raman-nematode approach as a first *in vivo* screening method

This combined Raman-nematode approach, is rapid and inexpensive enough to be applied as first screening for the ability of NPs to biodistribute and exert toxicological properties *in vivo*.

In line with the three Rs guiding principles on the humane use of animals in scientific research, this alternative approach also offers the advantage of avoiding ethical issues involving the use of vertebrates and, by guiding the design of tissue-specific toxicological evaluation, helps minimize the number of animals needed.

It is important to use nanosized reference materials with known identity and quantity to establish the relationships between the size, shape and agglomeration state of NPs, and their ability to biodistribute, pass through biological membranes, accumulate in specific tissues, and exert a toxic effect.

The toxicology of nanoparticles is a mature science.

We know what to do but…

- Testing and assessment: methods and strategies for NM safety.
- Does NP as a grouping make sense?
- Exposure measurement and mitigation guidance development for occupational setting, human exposure for consumers and enviromental exposure.
- Life cycle considerations.
- Risk assessment and regulatory programmes.

Thanks to.....

Department of Molecular Biochemistry and Pharmacology

Unit of Human Pathologies in Model Organisms

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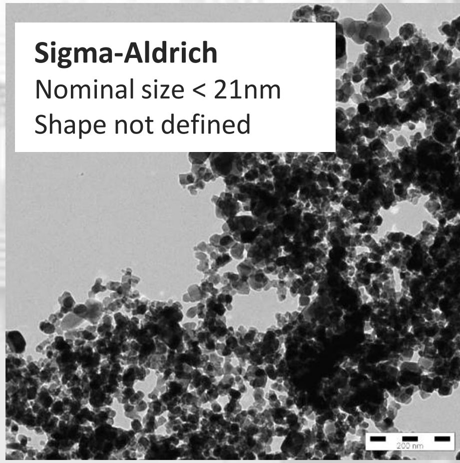
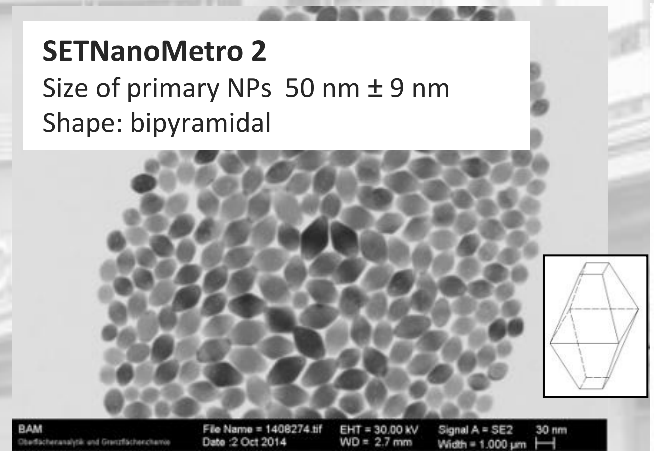
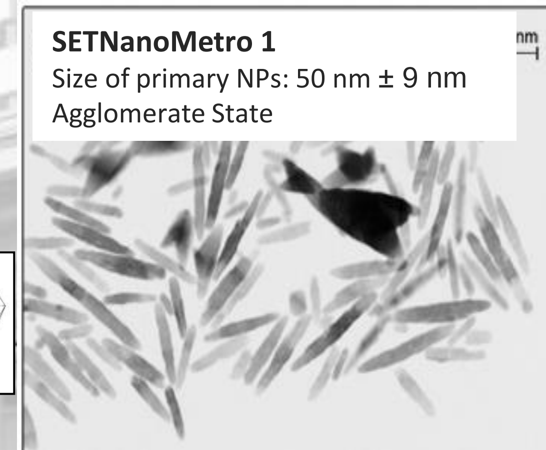
Andrea M. Rossi
Luca Iannarelli
Andrea Giovannozzi

Transmission Electron Microscopy (TEM)

Primary particles size







Dynamic Light Scattering (DLS)

Agglomeration state

in accordance with **ISO 22412:2008(E)**