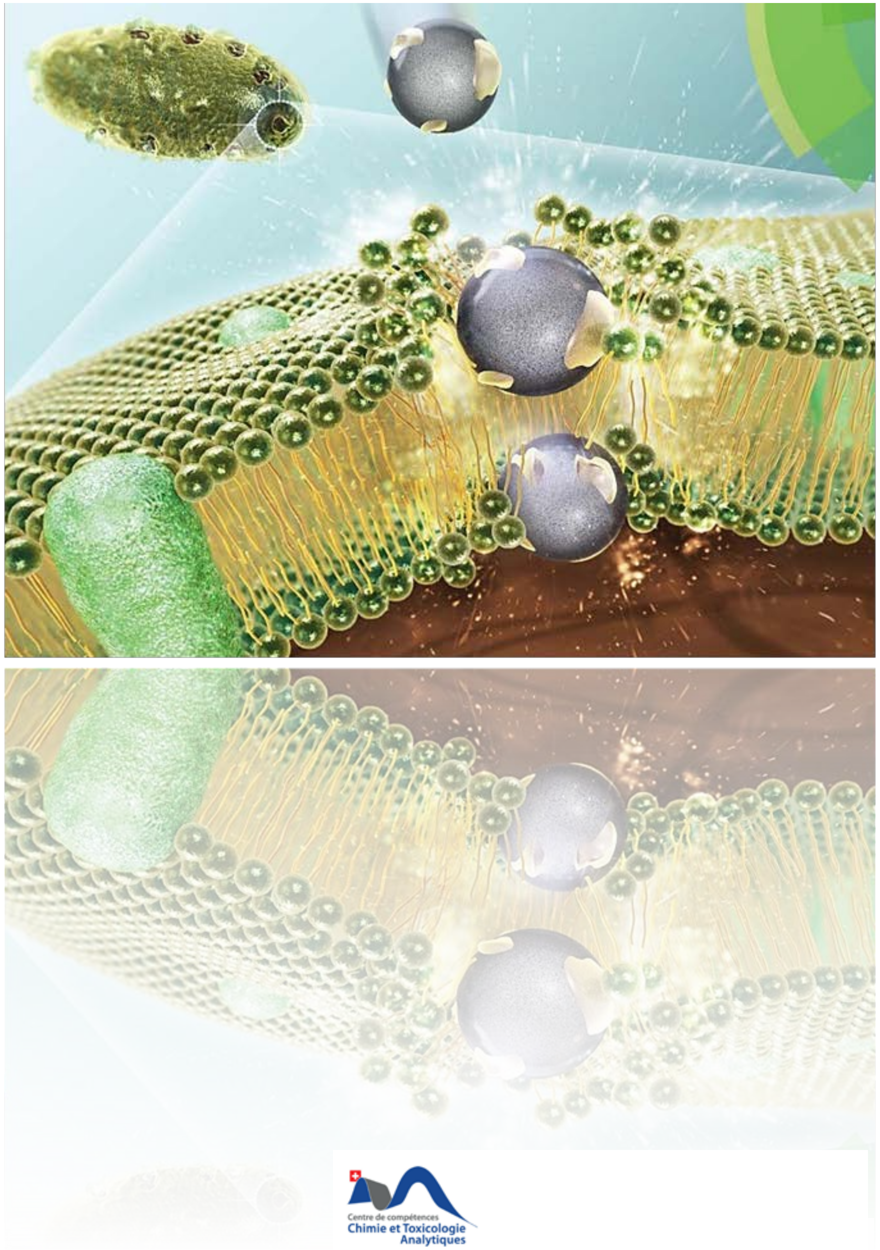
What microorganisms tell us about the impact of engineered nanoparticles in aquatic environment



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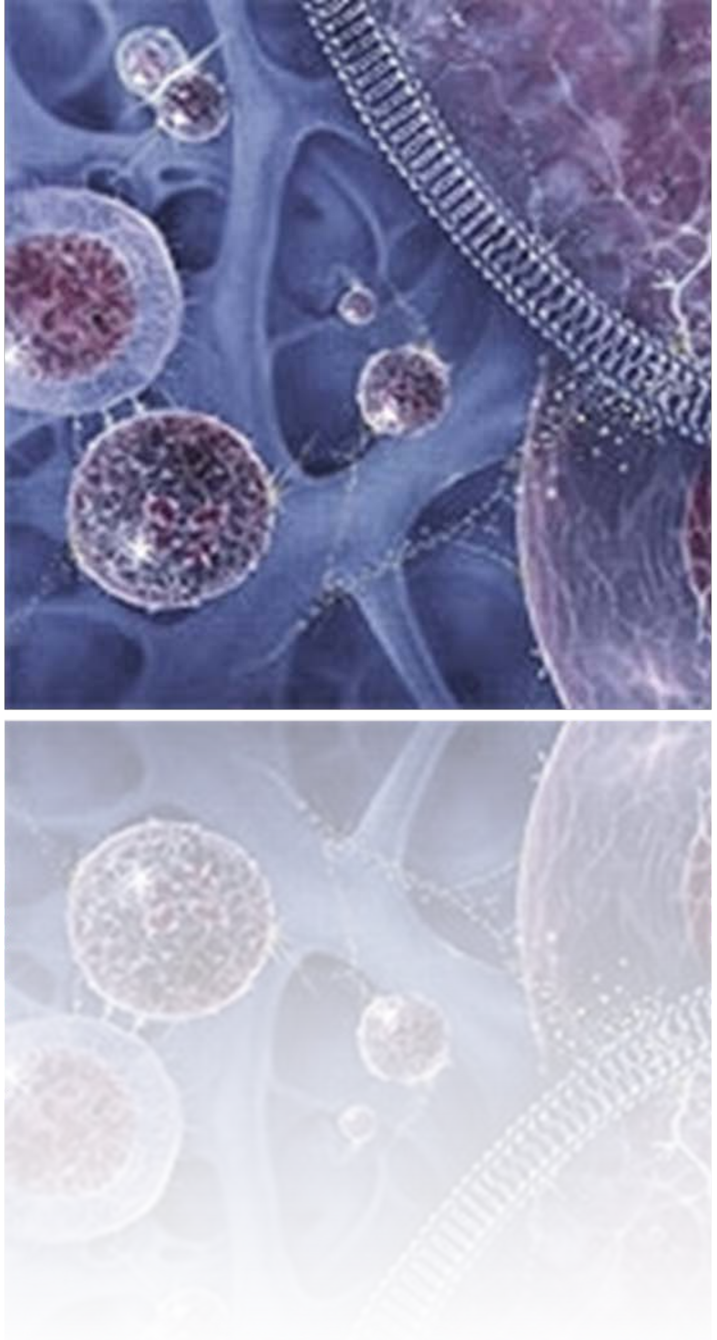
UNIVERSITÉ
DE GENÈVE

Centre de compétences Chimie et Toxicologie Analytiques
25èmes Journées Scientifiques
Notre environnement est-il toxique ?
jeudi 15 et vendredi 16 septembre 2022
Eurotel Victoria, Les Diablerets, VD

Outline

- Benefits over risk of nanomaterials
- Nanomaterial – aquatic microorganisms interaction and effects
    - Green algae
    - Particle-ingestive unicellular organisms
- Conclusion and outlook



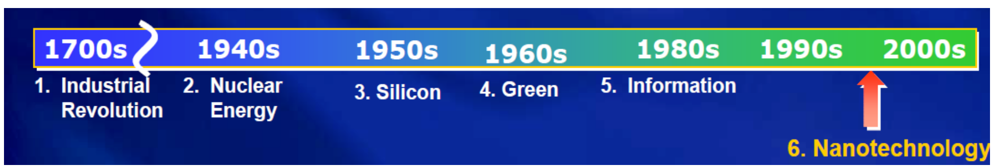
Nanomaterials are changing our world

Richard P. Feynman

“There's Plenty of Room at the Bottom: An Invitation to Enter a New Field of Physics” lecture at annual American Physical Society meeting at Caltech on December 29, **1959**

"arrange the atoms the way we want"

“Nanotechnology is the 6th truly revolutionary technology introduced in the modern world…” --D. Allan Bromley



Former Assistant for Science and Technology to the President George H. W. Bush (1989-1993)

- 6064 nanoproducts by 827 companies in 47 countries (2016)
- Global nanotechnology market expected to exceed US$ 125 Billion by 2024
- 6 million workers in nanotech by 2020

(Research and Markets , May 02, 2018)

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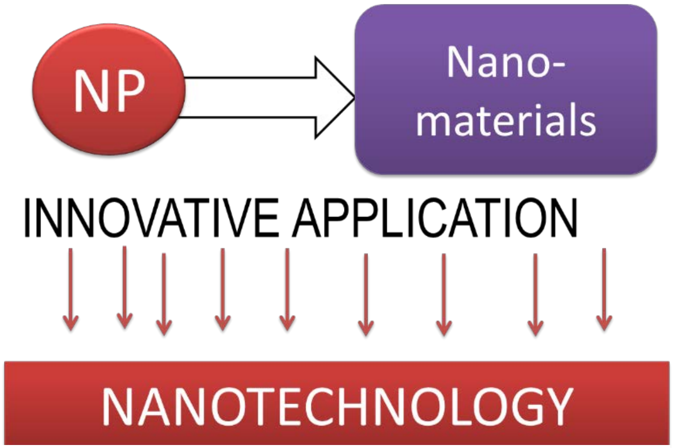
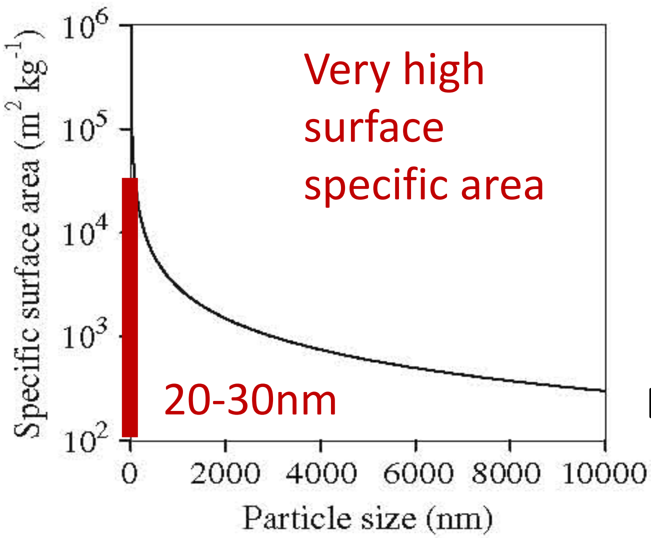
Nanotechnology is based on novel chemical entities: nanoparticles





Building blocks: from a few hundred atoms to millions of atoms

Novel properties at nanoscale:

- electrical
- mechanical
- optical
- chemical
- biological

Nano gold is red in collor
Bulk gold is yellow in collor

The Lycurgus, a 4th-century Roman glass cage cup

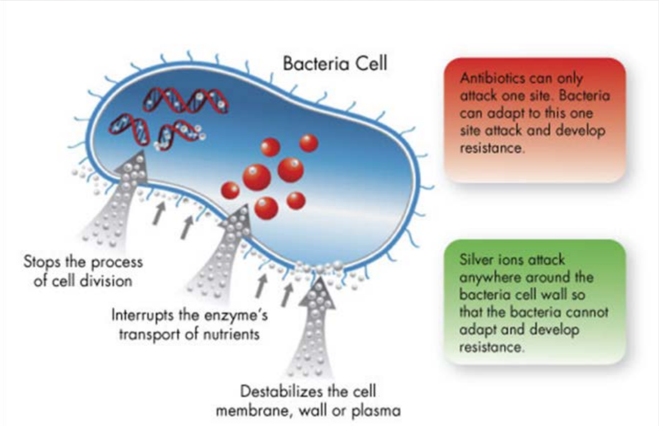
Nanos and the environment: Lots of promise

- "Nanos" that **outperform** "ordinary" materials are used for improved detection and removal of chemical substances and biological contaminants, such as viruses, bacteria, parasites (biocides) ….

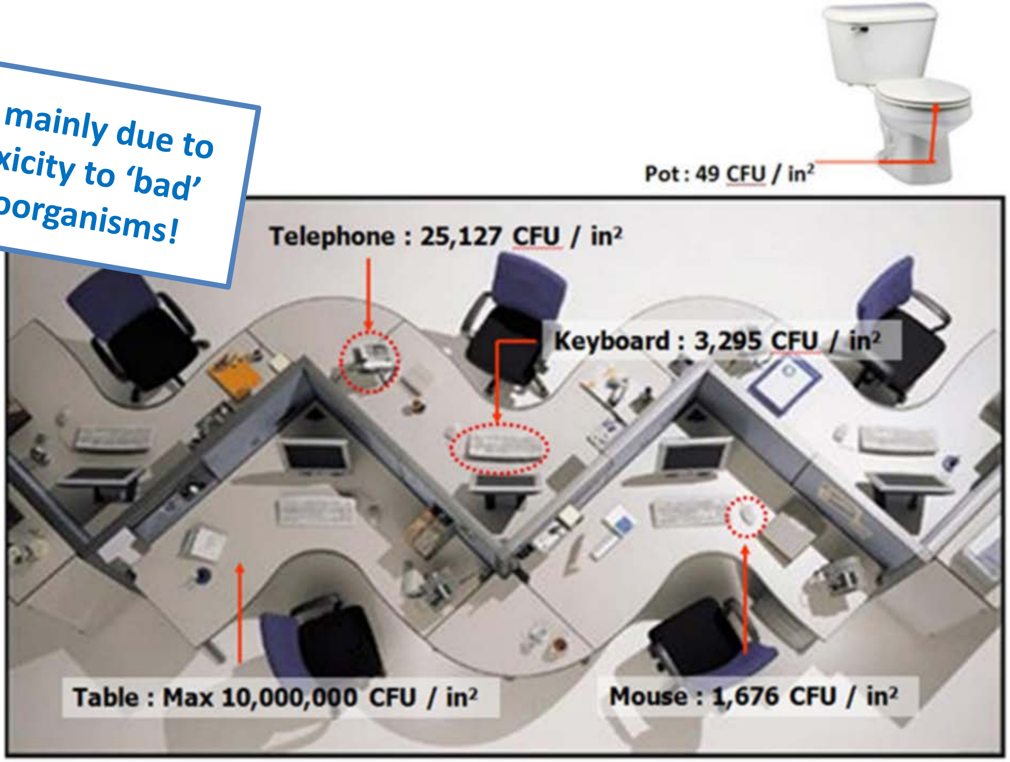
Benefit
+443 products contain AgNPs

Used mainly due to its toxicity to 'bad' microorganisms!

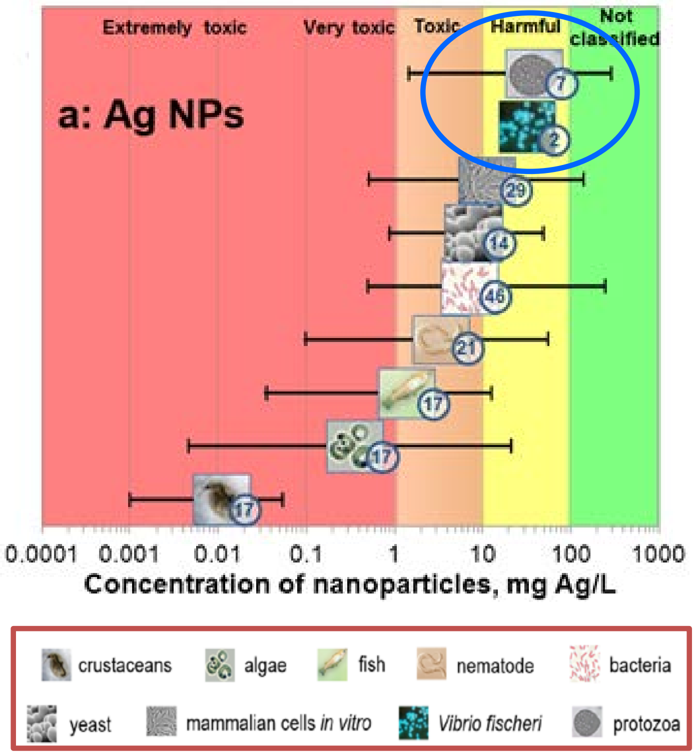
Benefits of silver compared to antibiotics



http://www.hirshfields.com/designresource/flooring/silverknight.html



The downside



Nanosilver is more toxic to non-target organisms than to bacteria

Hazard ranking

L(E)C50 or MIC	EU classification
> 100 mg/L	Not harmful/not classified
10-100 mg/L	Harmful
1-10 mg/L	Toxic
< 1mg/L	Very toxic

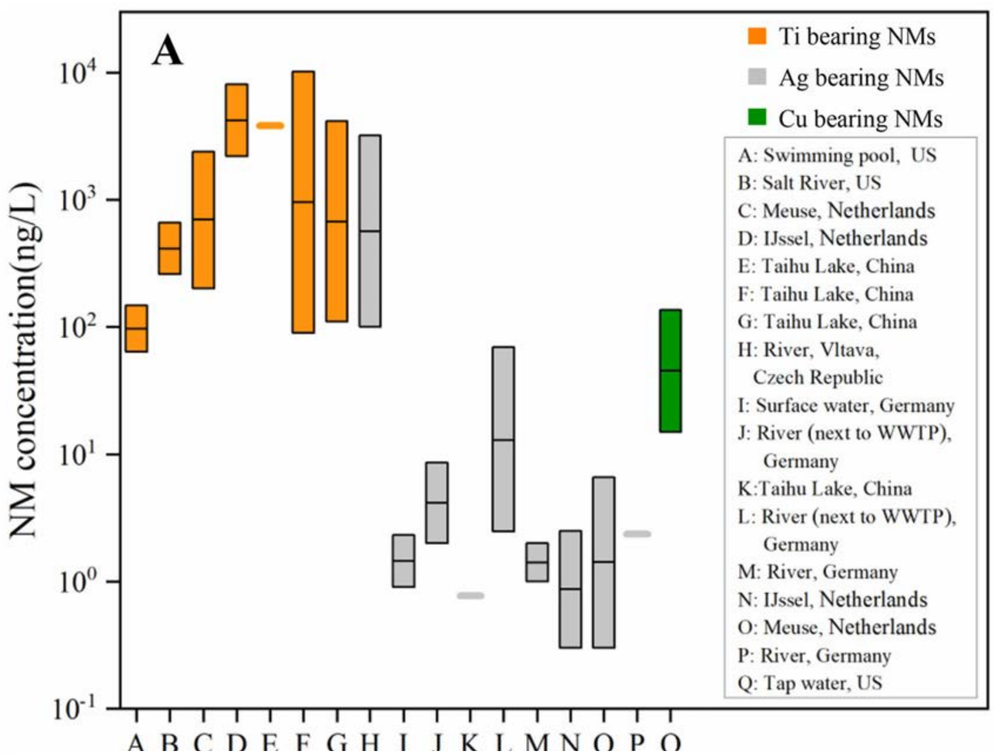
Median *L(E)C50 and MIC ± minimum and maximum are shown*

Bondarenko et al. Archives of toxicology, 1181 (2013)

How to benefit by the nanotechnology, while minimizing and avoiding possible risks?

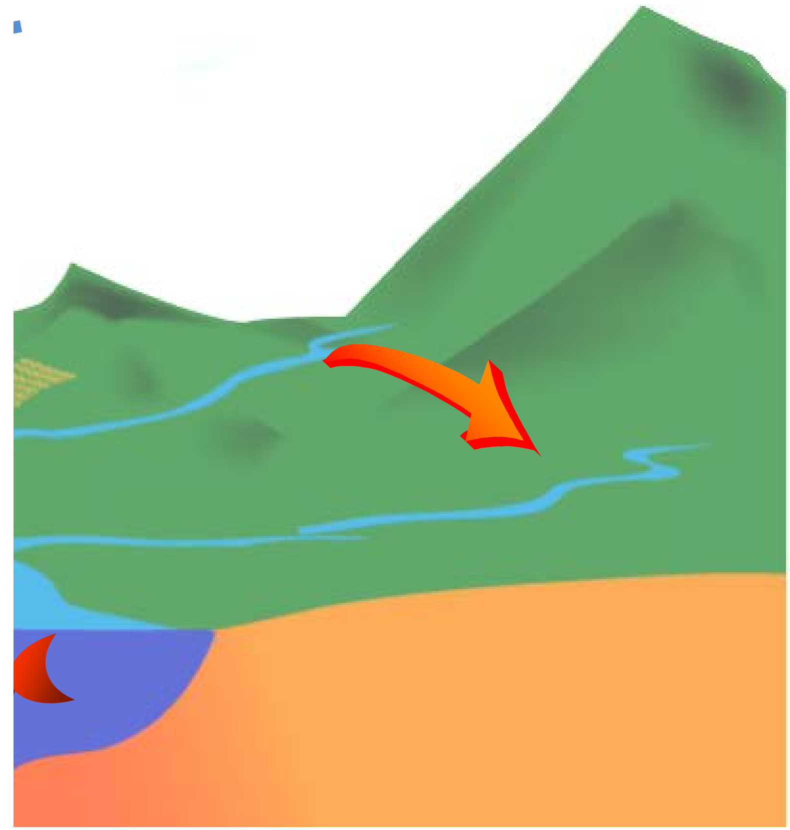
Do ENPs represent environmental risk?

- **Measured AgNPs concentrations 0.3 to 3200 ng/L in surface waters**



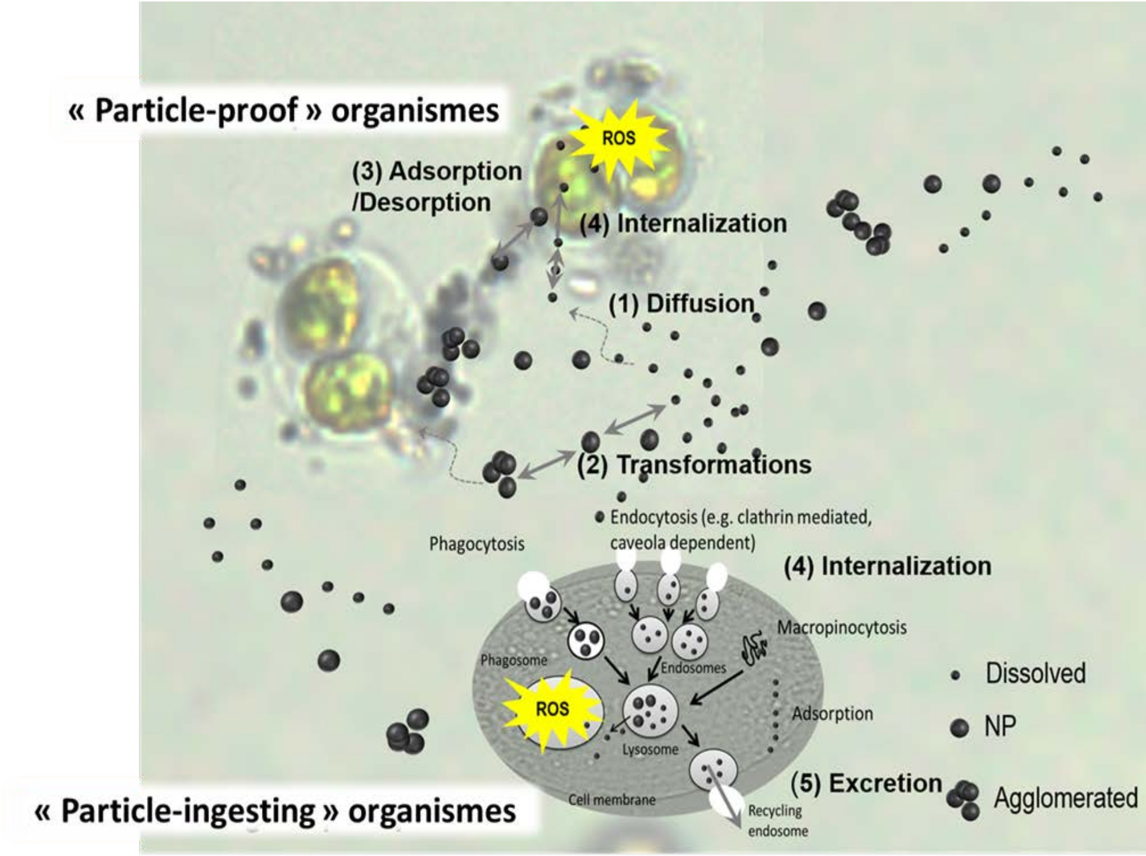
J. Zhao et al. Crit. Rev. Environ. Sci. Technol., 51 (2021), 1443

- **Effects (PNEC)**



Understanding of the interactions of ENPs with environmental and living systems is a key for enabling an appropriate risk assessment

ENPs - aquatic microorganisms interactions



Slaveykova et al. E J Prot 55, 39 (2016)

- **How do ENPs affect aquatic microorganisms?**
- **How do aquatic microorganisms alter ENPs fate?**

Experimental design

Nanomaterials:

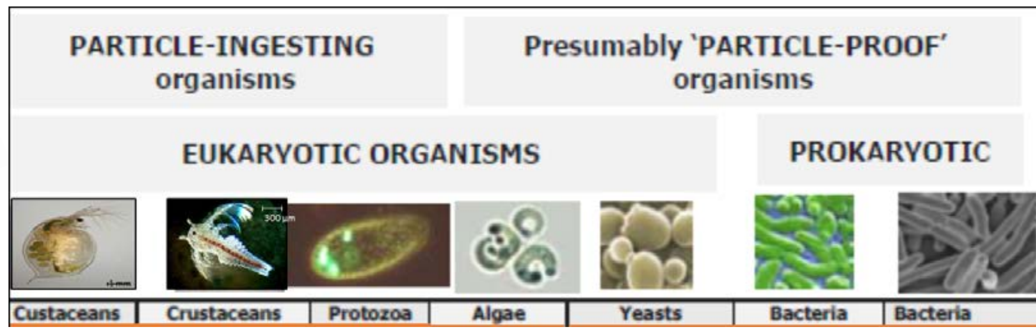
Amidine Latex **Carboxyl Latex**

Pt, Au, Ag TiO_2, CuO, Co_3O_4, Mn_2O_3 ZVI, Fe_xO_y

Organisms: at various levels of the food-web and different feeding pattern

Chlamydomonas reinhardtii

Ochromonas malhamensis



Dissolution: *GIME, A4F-UV-ICP-MS, Ultrafiltration*
Aggregation: *FCS, A4F-UV-ICP-MS*
Abiotic ROS
Potential for shading: *Attenuation of the different light spectrum components by spectrophotometry*
Association to biota: *FCS, FCM, Imaging, spICPMS*
Internalization and excretion: *FCM, Imaging, ICP-MS*

Bioassays: Growth inhibition, oxidative stress, membrane damage *FCM*; photosynthetic yield;

Metabolomics: Metabolome responce /quantification of metabolite abundance LC MS/MS

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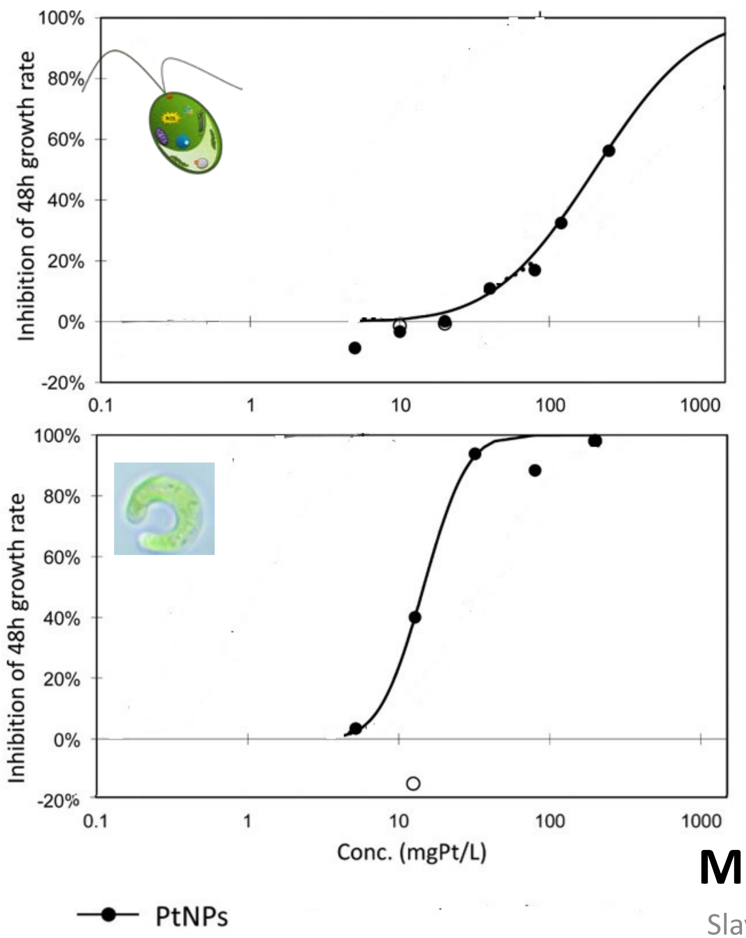
PtNPs effect on green algae

- **Concentration–response courbes for PtNPs based on the total Pt concentration**

48h- growth inhibition



2 nm starch-coated Pt NPs
Hydrodynamic size of 10 nm
Z-potential (48h): −28 ± 0.3 mV
Contain dissolved Pt about 3% of the total Pt content (1-48h, ultracentrifugation)

- Decreased growth rates of both *P. subcapitata* and *C. reinhardtii*
- *P. subcapitata* is more sensitive to PtNPs than *C reinhardtii*
- PtNPs would be classified as "harmful" to algae in accordance with the EU regulation

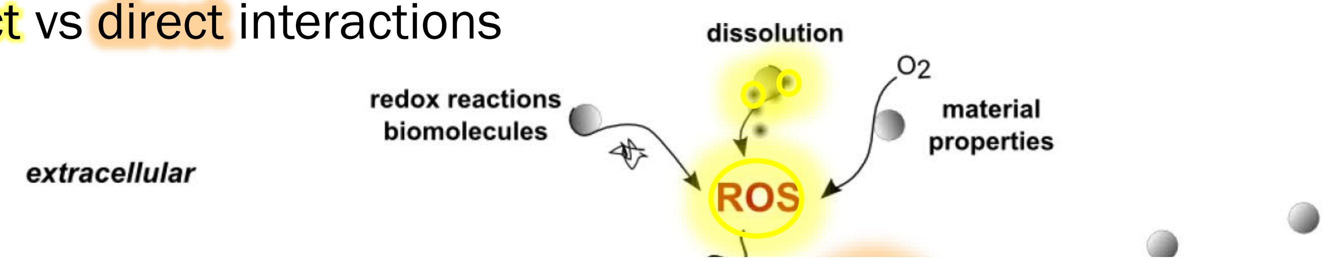
L(E)C50 or MIC	EU classification
> 100 mg/L	Not harmful/not classified
10-100 mg/L	Harmful
1-10 mg/L	Toxic
< 1mg/L	Very toxic

Multiple mechanisms behind the observed effects

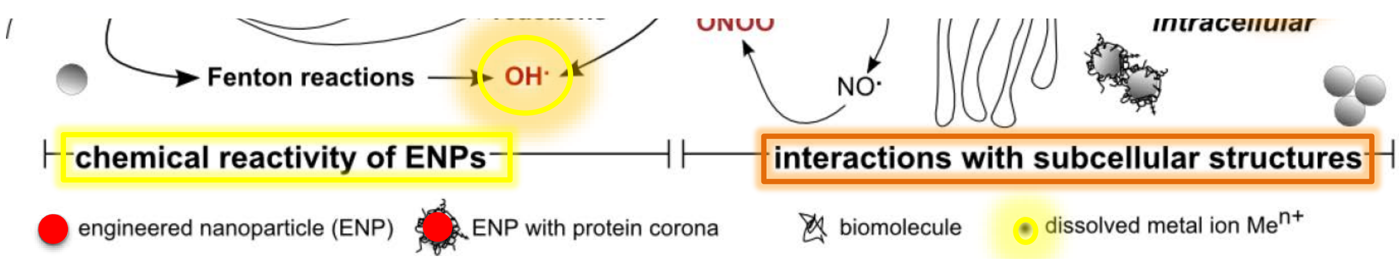
Sorensen et al. Env. Sci Technol.50: 10653 (2016)

Effect is a result of a myriad of interactions

indirect vs direct interactions



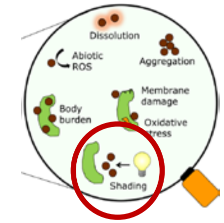
1. **Contribution of shading**
2. **Dissolved Pt**
3. **Cellular ROS generation**
4. **Pt accumulation by algae**



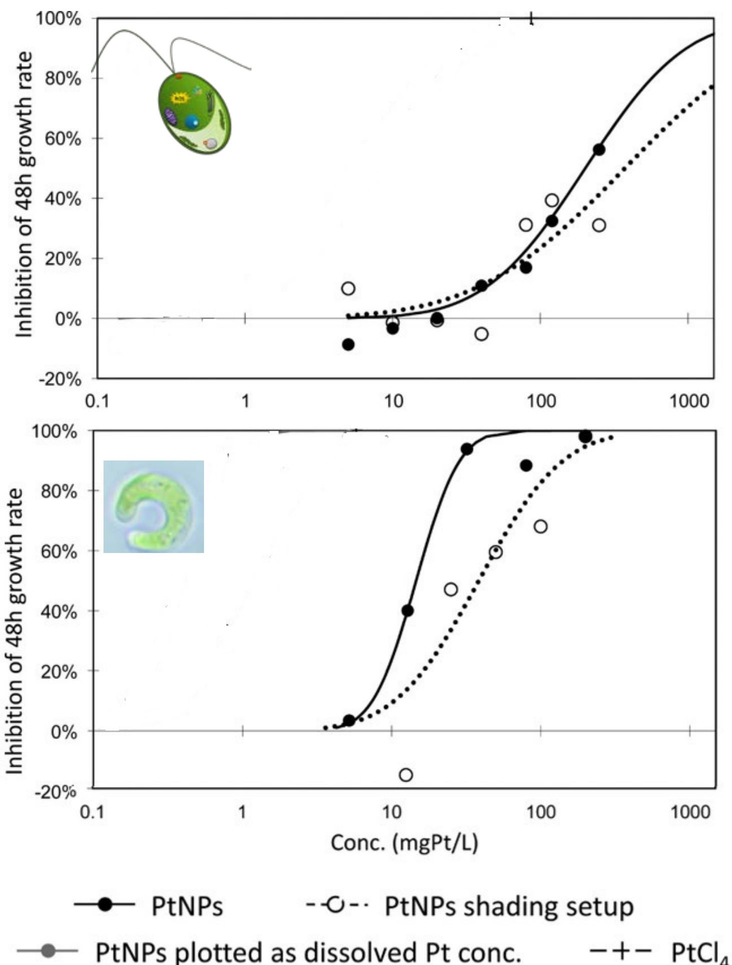
Von Moos and Slaveykova, Nanotoxicology 2014

Need to address to avoid potential artefacts and misinterpretations in nanoecotoxicology testing

1. Contribution of shading effect



48h- growth inhibition

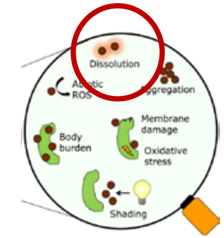


PtNPs limited the available light for algal growth by shading, thus inhibiting growth physically rather than by a toxic action of the PtNPs

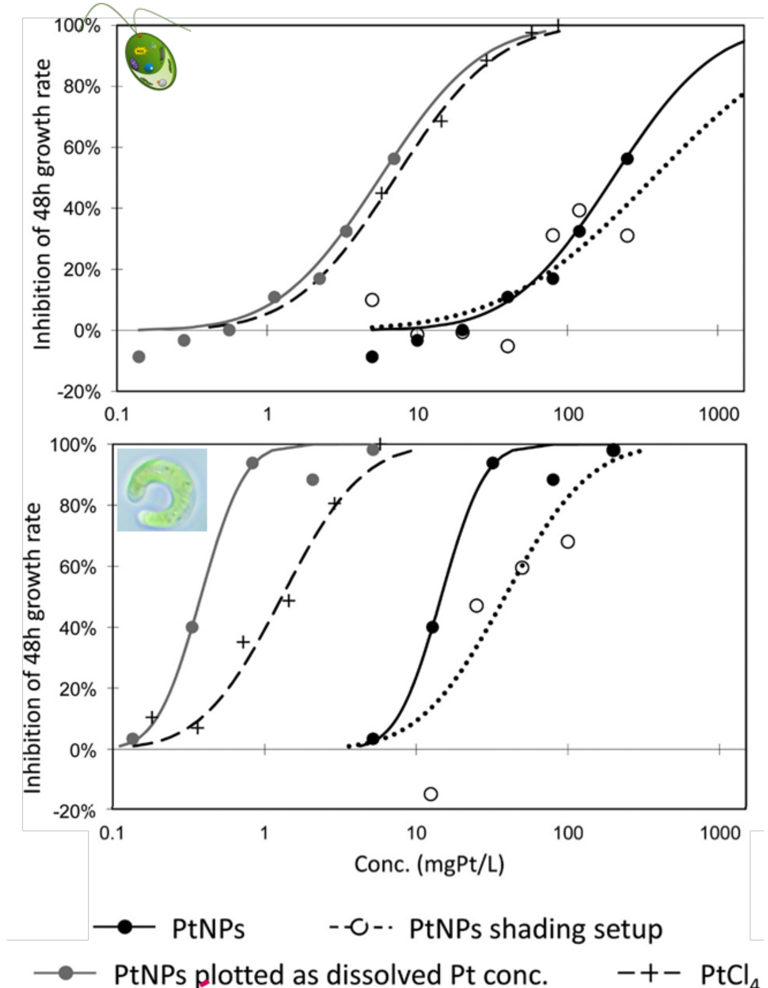
- physical shading from PtNPs lowered the algal growth rates
- but also indicate that PtNPs inhibit algal growth rates by other means than shading, possibly by direct toxic effects

Sorensen et al. Env. Sci Technol.50: 10653 (2016)

2. Contribution of dissolved Pt



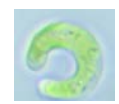
- **Dose–response curves for PtNPs based on the dissolved Pt concentration**



C. reinhardtii

- PtNPs data aligned with the $PtCl_4$ data
  - → suggesting that the PtNP toxicity may be caused by the dissolved Pt.

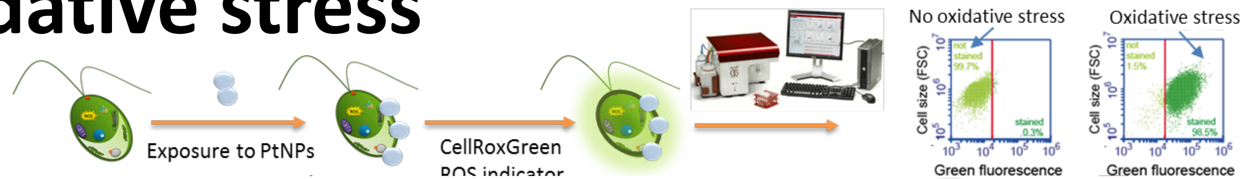
P. subcapitata

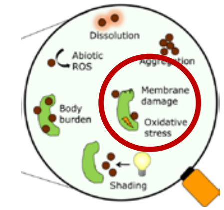


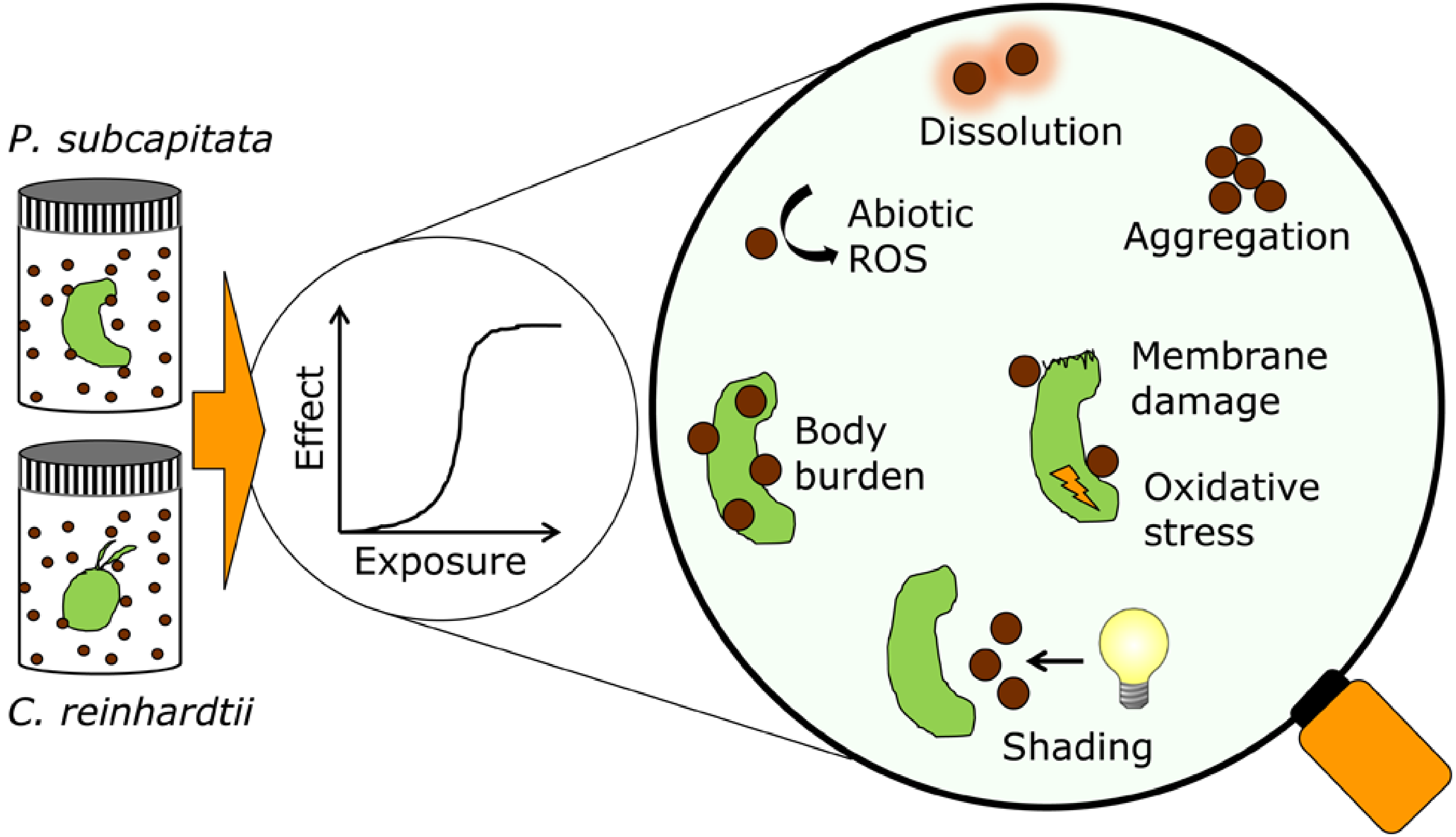
- PtNPs data based on dissolved Pt showed greater inhibition than $PtCl_4$
  - → suggesting a possible NP-specific effect

Sorensen et al. Env. Sci Technol.50: 10653 (2016)

3. Oxidative stress



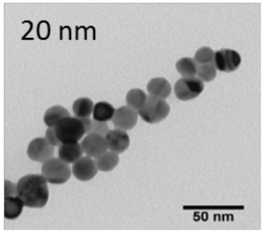




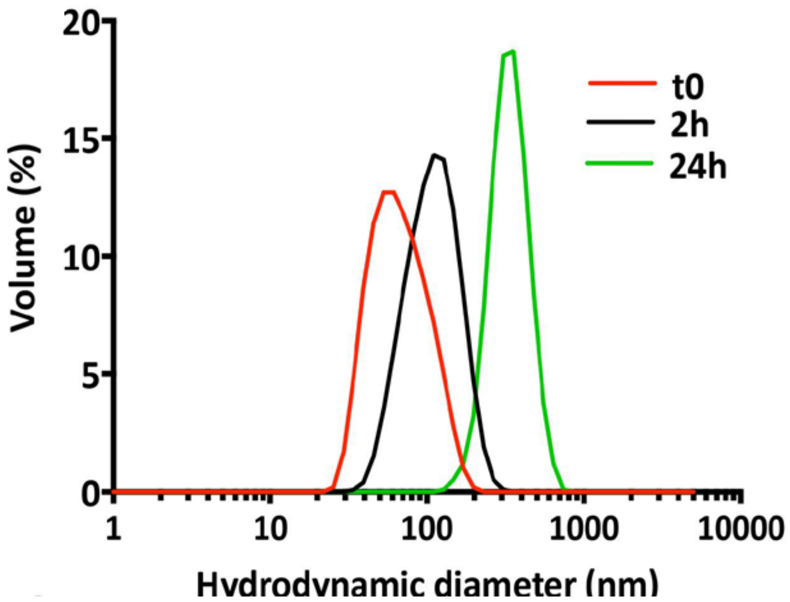
- Pt-NPs induced high oxidative stress, but recovery at 48h
- Different sensitivity of algae to Pt-NPs
- Pt ions contribute to the effect to *C. reinhardtii*, but not to the effect to *P. subcapitata*

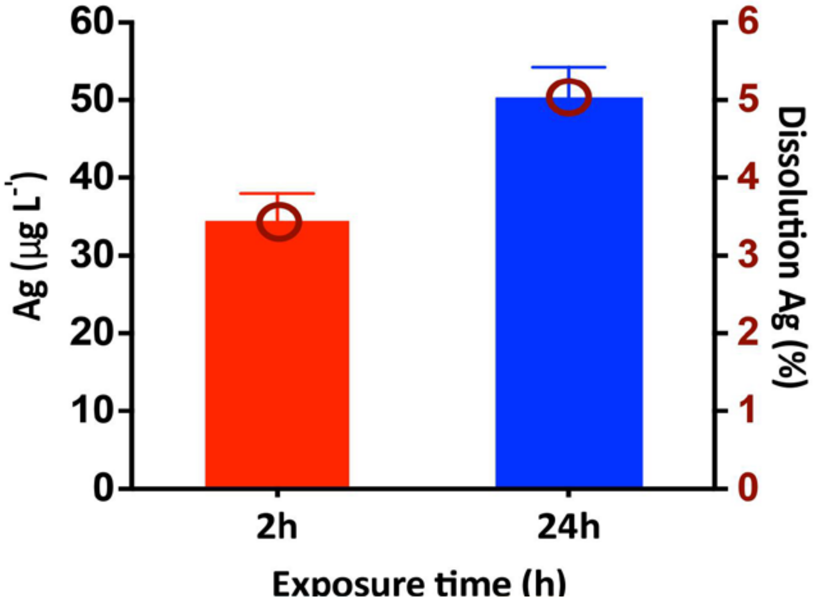
Sorensen et al. Env. Sci Technol.50: 10653 (2016)

AgNPs and golden-brown alga *Ochromonas malhamensis*



- 20nm citric coated AgNPs
- Z-potential (24h): −23 ± 4.3 mV
- Hydrodynamic size of 60nm in synthetic lake water, agglomeration
- Dissolved Ag 4-5% of the total Ag content (ultracentrifugation)



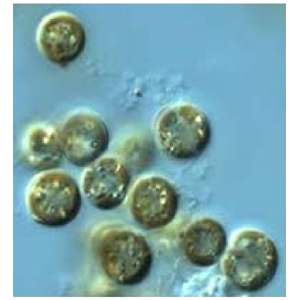


Liu et al. Sci Rep. 10, 20563 (2020)

AgNPs are toxic to golden-brown alga *Ochromonas malhamensis*

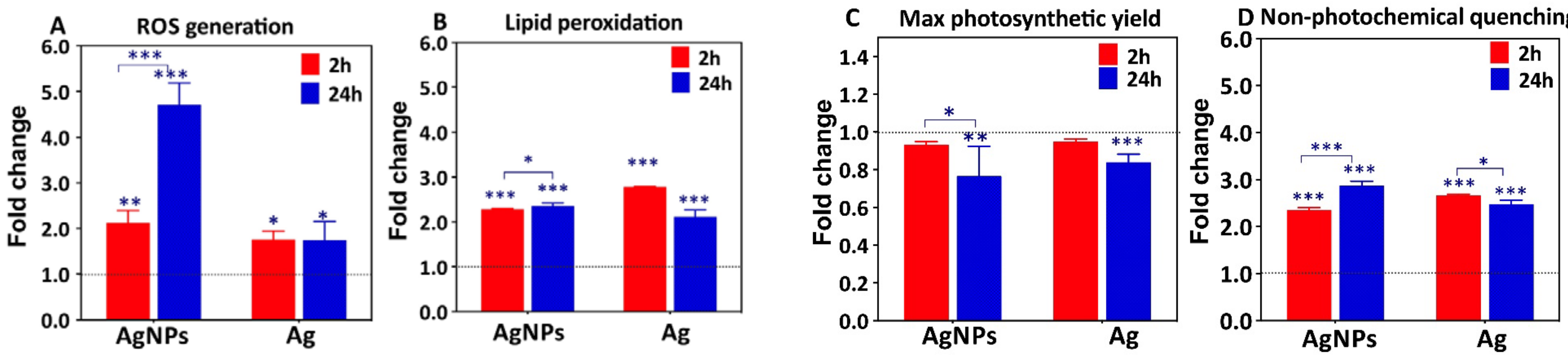
EC 50 AgNPs
8 mg/L

L(E)C50 or MIC	EU classification
> 100 mg/L	Not harmful/not classified
10-100 mg/L	Harmful
1-10 mg/L	Toxic
< 1mg/L	Very toxic



bigelow.org

1 mg/L AgNPs; 40 μg/L Ag^+

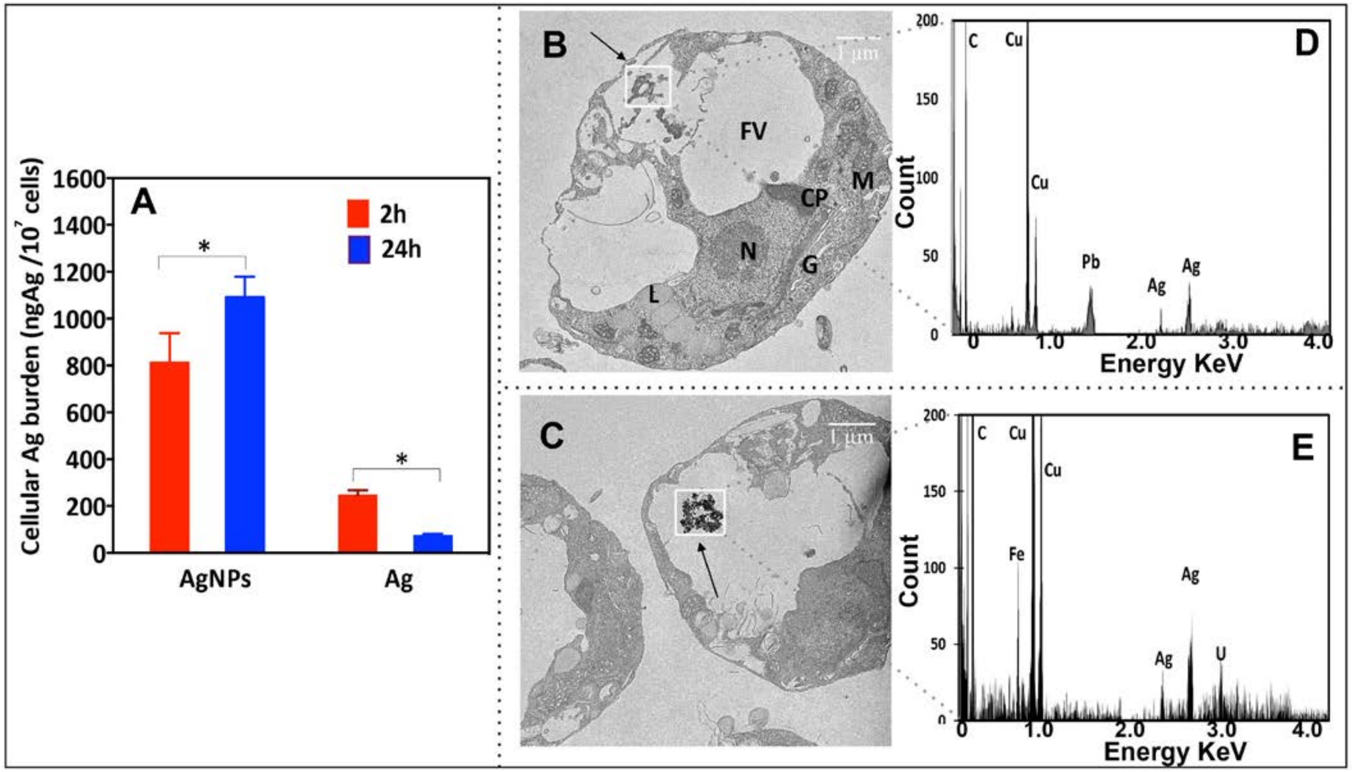


- Excessive cellular ROS generation
- Membrane damage; Photosynthetic yield decrease

Liu et al. Sci Rep. 10, 20563 (2020)

AgNPs are ingested by golden-brown alga *Ochromonas malhamensis*

TEM imaging and Energy-dispersive X-ray spectroscopy of *O. malhamensis* exposed to 1mg/L AgNPs, 2h and 24h



2h

2h

- Single AgNPs of 20nm size are localized in the cellular vacuole

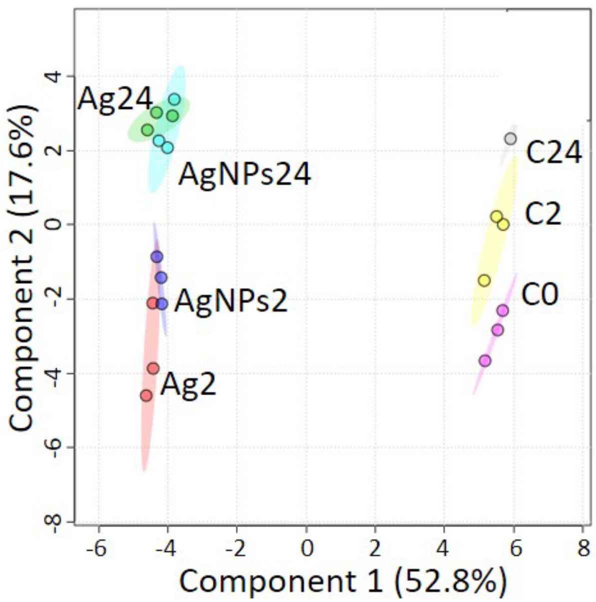
Liu et al. Sci Rep. 10, 20563 (2020)

AgNPs dysregulate algal metabolism

2h exposure to 1 mg/AgNPs and 40µg/L Ag^+

Targeted metabolomics LC MS/MS

Partial Least Squares - Discriminant Analysis



90 metabolites quantified
Antioxidants, Amines, Nucleobase/side/tide, amino, organic and fatty acids, Sugar/sugar alcohols

- Good separation between Ag-treatments and control
- AgNPs and dissolved Ag have distinct effect at 2h on the metabolic pathways, but not at 24h

Liu et al. Sci Rep. 10, 20563 (2020)

AgNPs dysregulate algal metabolism

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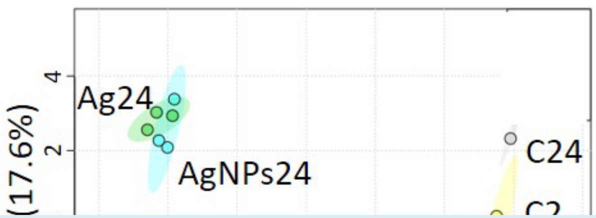
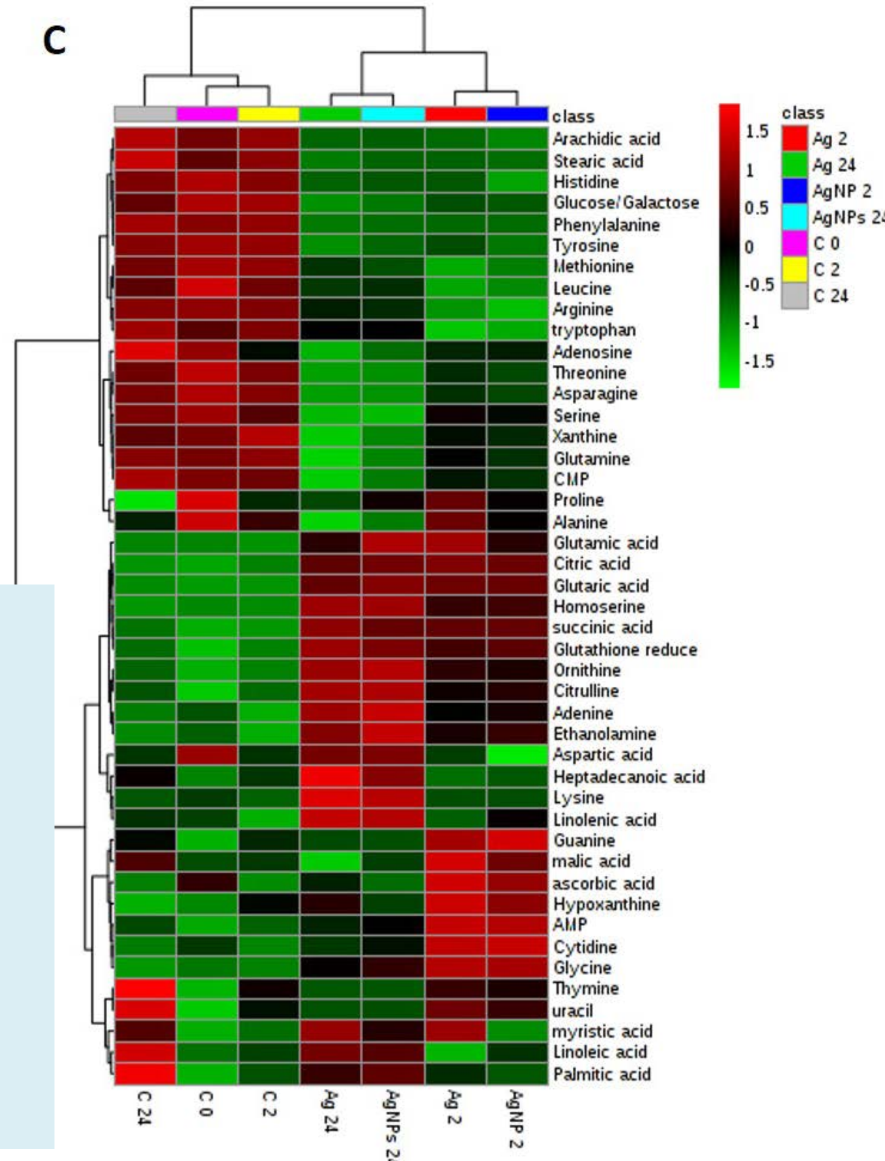
Targeted metabolomics LC MS/MS

Partial Least Squares - Discriminant Analysis



- Overlap of differentially impacted metabolites in AgNPs and Ag^+
  → Significant fraction of AgNPs effects originate from released Ag^+

- Some changes were specific to Ag NPs treatment

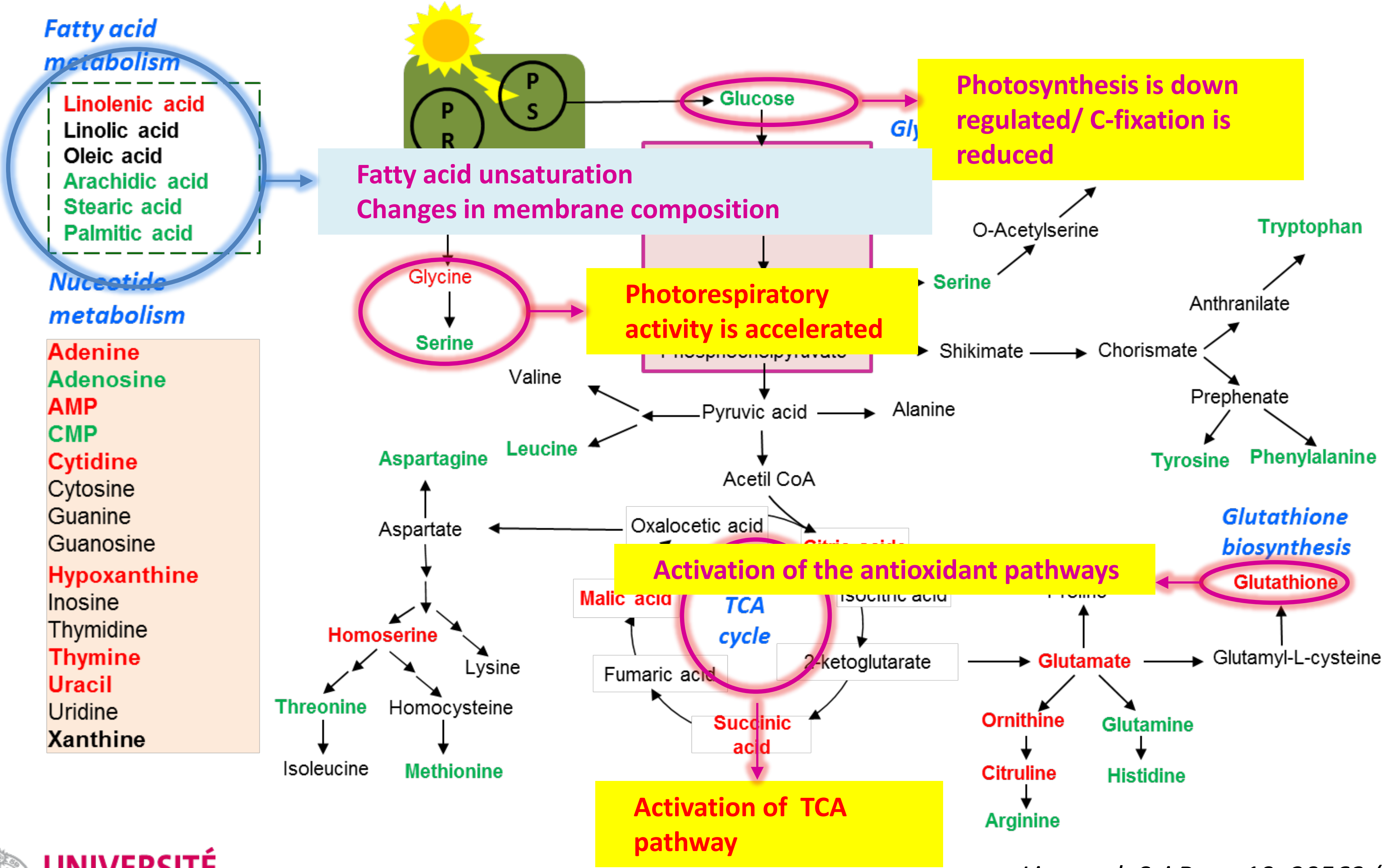


Liu et al. Sci Rep. 10, 20563 (2021)

Overview on AgNPs - induced metabolic pathway alteration

Upregulated Metabolites

Downregulated Metabolites



Liu et al. Sci Rep. 10, 20563 (2021)

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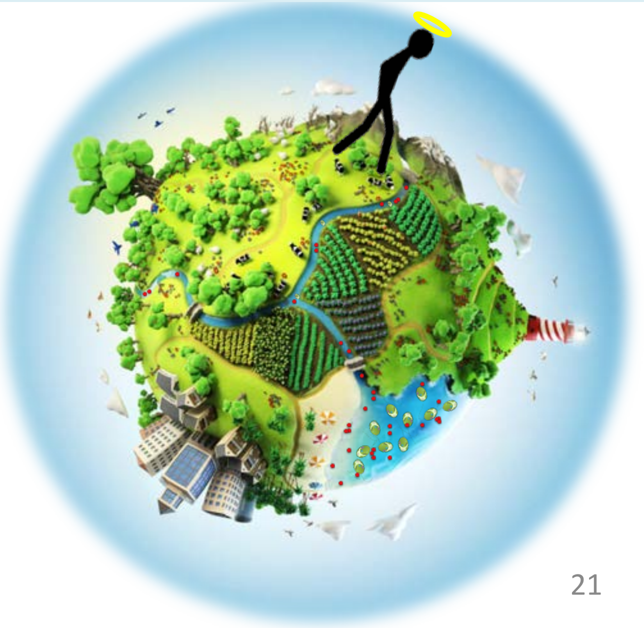
Take-home message

- NPs-organism interactions have multiple dimensions and the NPs-induced effects are a result of a myriad of direct and "indirect" interactions
- Knowledge is necessary to guide the informed decision about the possible environmental consequences of the nanotechnology and selection of the materials in environmental applications

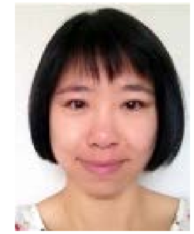
Outlook

The role of aquatic microorganisms in ENPs fate and transformations

Environmental implications of nano- and microplastics



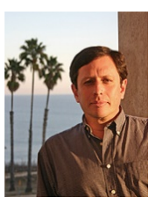
Thank you for your attention!

Swiss National
Science Foundation

Opportunités et risques des nanomatériaux
Programme national de recherche PNR 64

UCSB

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