

# International Iberian Nanotechnology Laboratory

Your Worldwide Science and Innovation Partner



Director General: Prof. Lars Montelius

# History of INL



2007 ➔



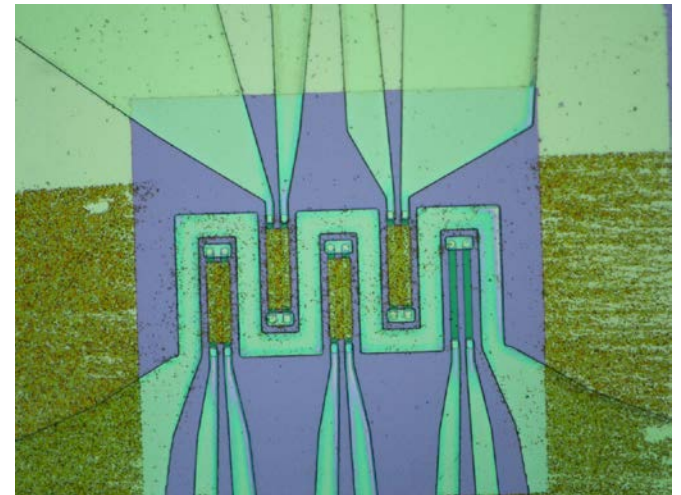
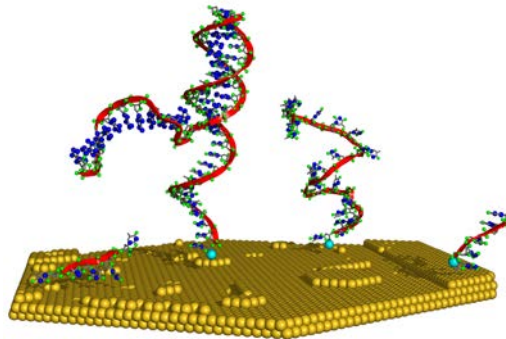
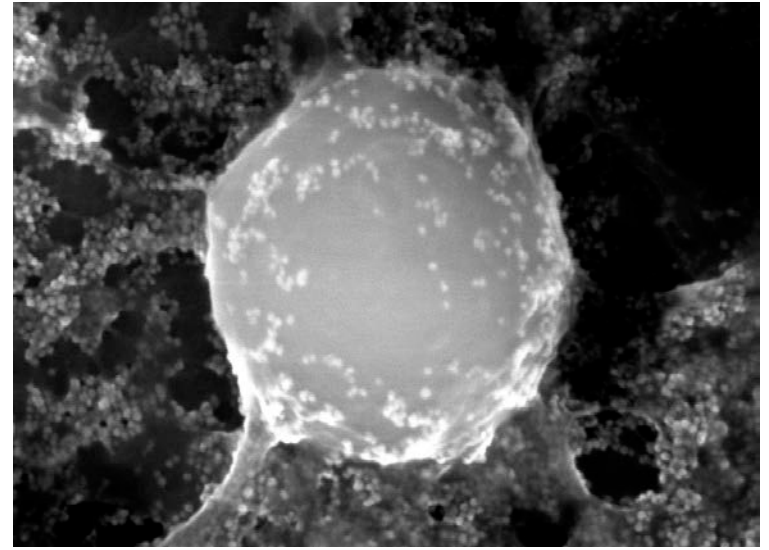
- ◆ Jointly founded by Portugal and Spain
  - Concept in 2005, construction in 2008, personnel in 2011
- ◆ Current statistics in 2016
  - Located in Braga, Portugal
  - About 400 researchers from over 30 countries
  - Main building: 22000 m<sup>2</sup>; laboratories 7500 m<sup>2</sup>; cleanroom 1000 m<sup>2</sup>
  - Modern infrastructure, integrated nano-incubator, social facilities

## ◆ Integrated internally

- Life sciences
- Physical sciences
- Micro- and nano-fabrication
- Engineering and system integration

## ◆ Integrated with partners

- Academic, research, and industrial
- Regional, European, and global





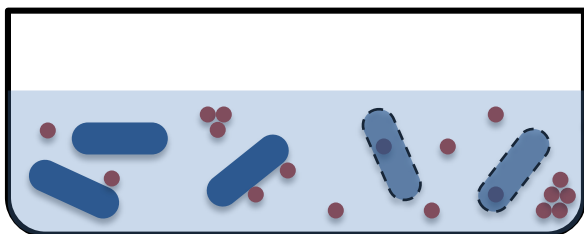
Planktonic cells (in suspension)



Biological analysis

Monitor cell viability, phenotype, proliferation, i.e., **only effects on cells.**

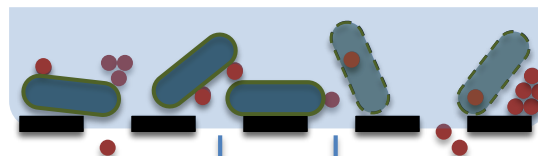
Add nanoparticles and incubate



Physical and chemical characterization

What are properties and interactions in cell+NP systems?

Filter cell/NP mixture

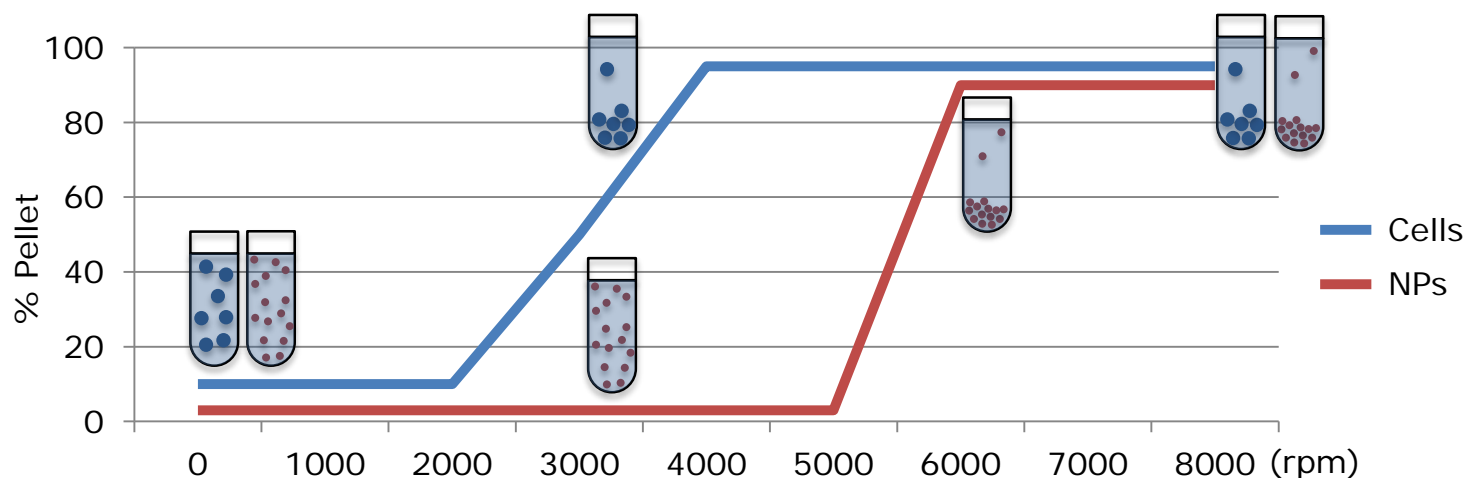


Rinse, fix, dehydrate cells

## Physical chemistry strongly impacts NP–cell bioanalytics

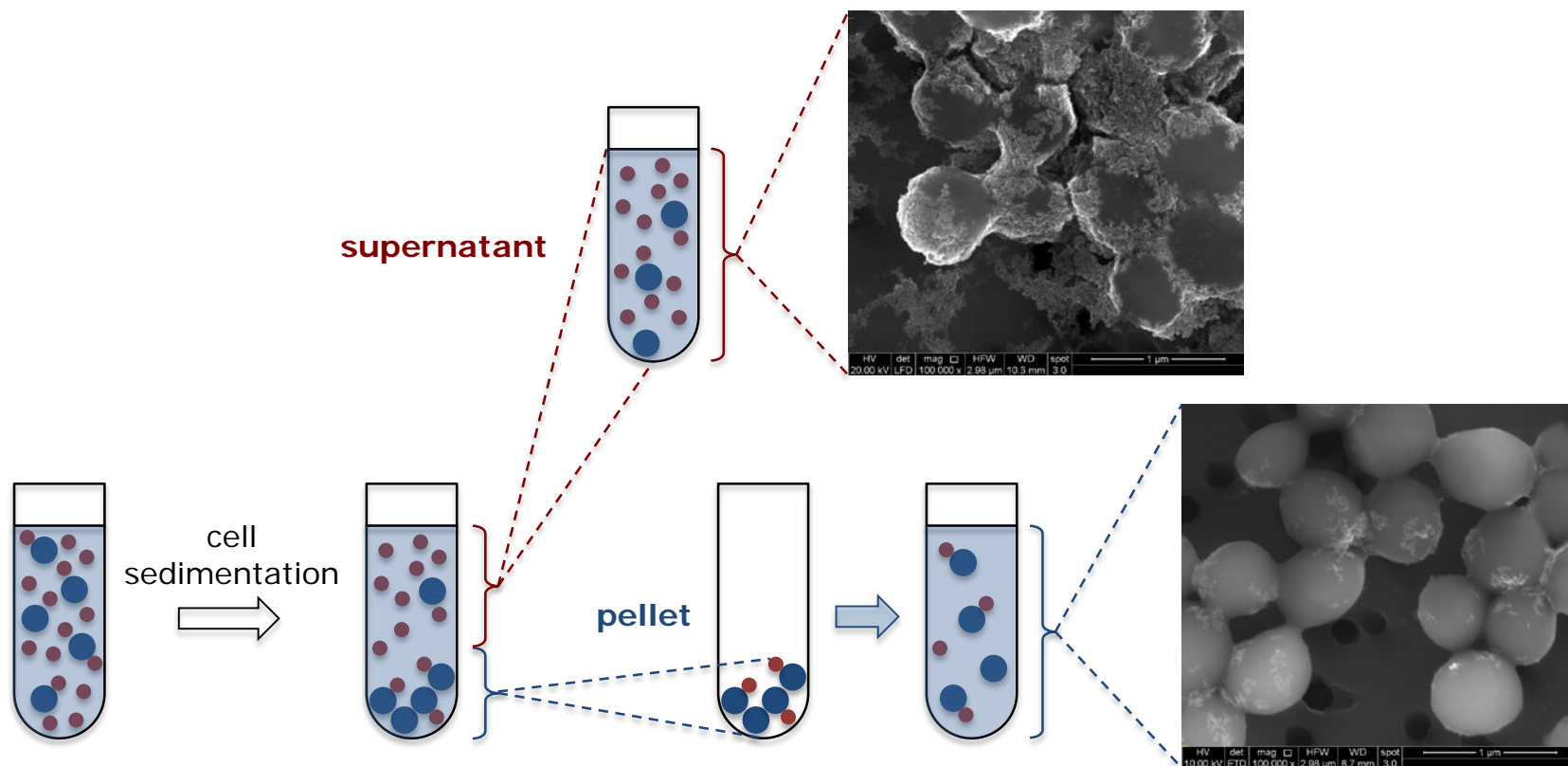
- NP–cell interactions may not be just biological
- Presence of NPs may affect biological assays

# Differential Sedimentation Approach



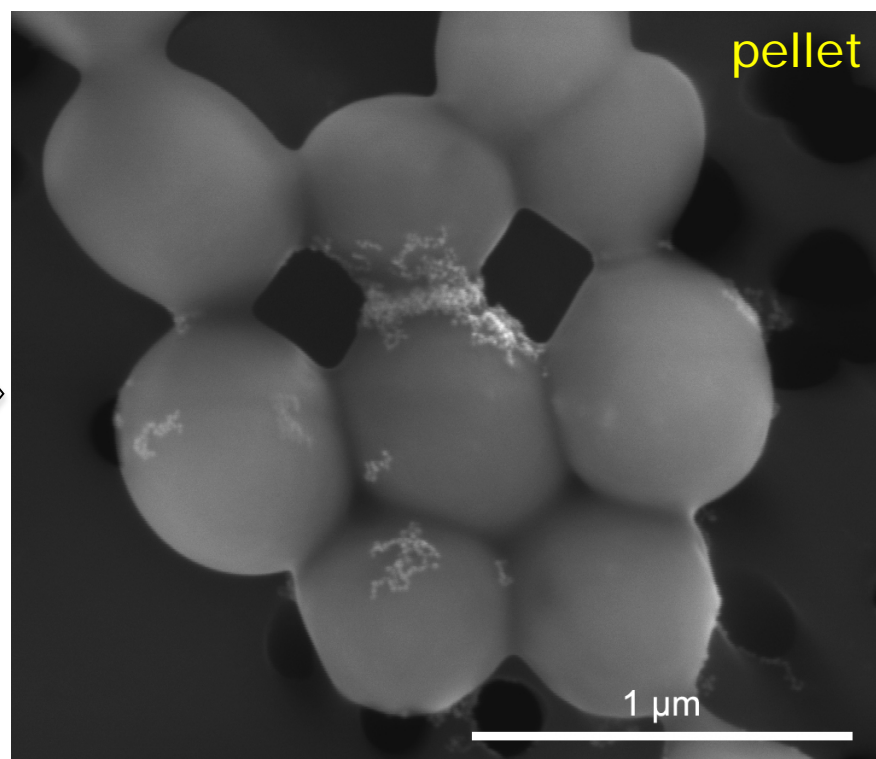
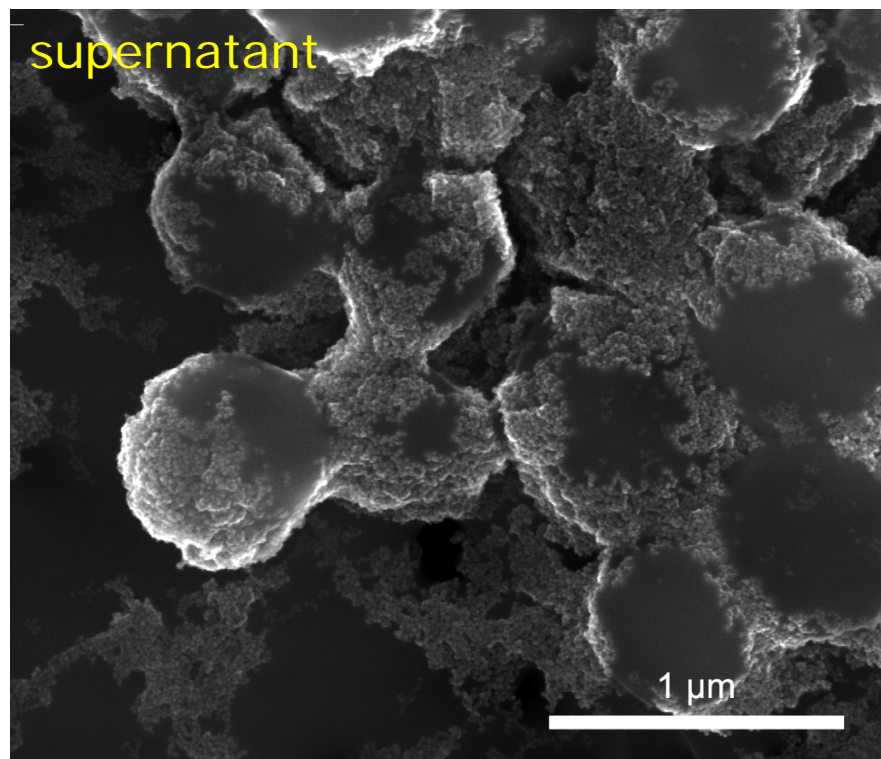
- ◆ Density-based sedimentation may not be sufficient
  - For low NP-loading limit, density changes will be minimal
- ◆ Exploit different physicochemical properties of cells and NPs
  - Solution conditions for different sedimentation thresholds?
    - Viability of cells often limits the range of solution conditions
- ◆ Establish sedimentation thresholds for cells and NPs separately
  - Colloidal stability differences are hard to predict *a priori*

# Differential Sedimentation Scheme



- ◆ Centrifugation just above cell sedimentation threshold
  - Supernatant contains free NPs and some cells
  - Pellet contains cells with any attached/loading NPs for analysis

# Separation of Free NPs & Cells+NPs



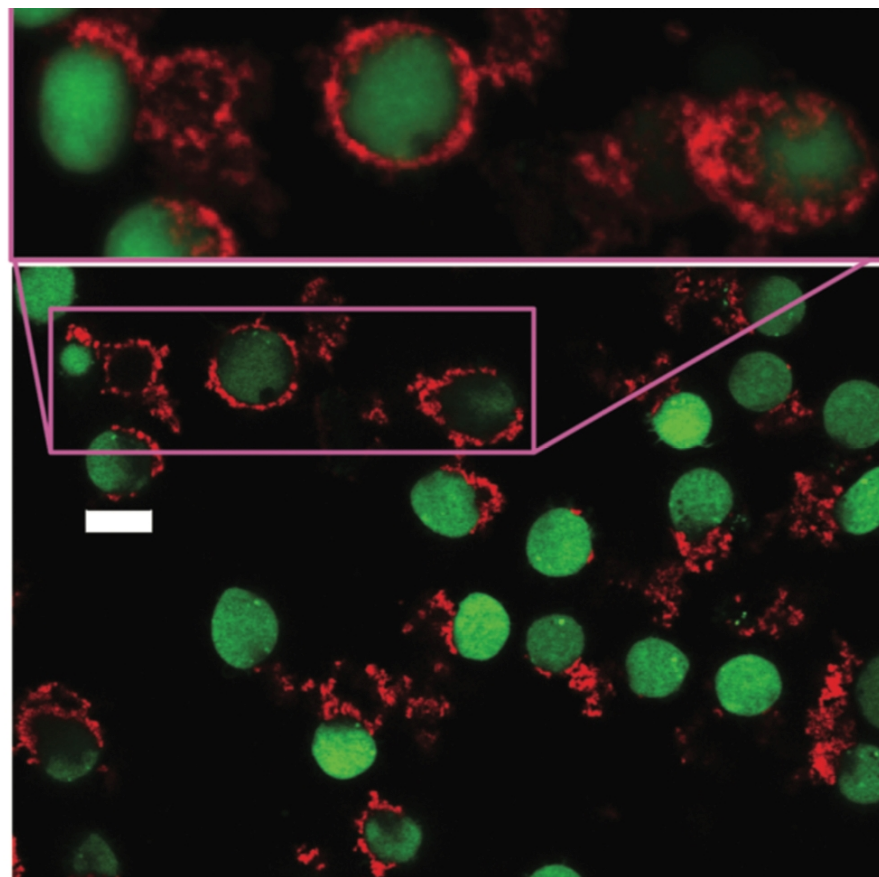
- ◆ Supernatant: some cells, many NPs aggregated during drying
- ◆ Pellet: Only NPs attached to cells *in solution* are observed by SEM
  - No free NPs in the background, different loading of adjacent cells
- ◆ Protocol allows us to preserve evidence of NP–cell interactions

# Details of NP-cell Interactions

## Combining CXCR4-targeted and nontargeted nanoparticles for effective unassisted in vitro magnetic hyperthermia

Vânia Vilas-Boas, Begoña Espiña, Yury V. Kolen'ko, Manuel Bañobre-Lopez, José A. Duarte, Verónica C. Martins, Dmitri Y. Petrovykh, Paulo P. Freitas, and Felix D. Carvalho

- ◆ NPs functionalized to target cancer cells
  - Targeting CXCR4 receptor overexpressed in Jurkat cells
- ◆ Targeting visualized by confocal microscopy
  - The NPs are recognizing the Jurkat cells
  - The high loading of NPs around each cell is primarily due to NP-NP interactions







Contents lists available at [ScienceDirect](https://www.sciencedirect.com)

Journal of the Mechanical Behavior of  
Biomedical Materials

journal homepage: [www.elsevier.com/locate/jmbbm](http://www.elsevier.com/locate/jmbbm)

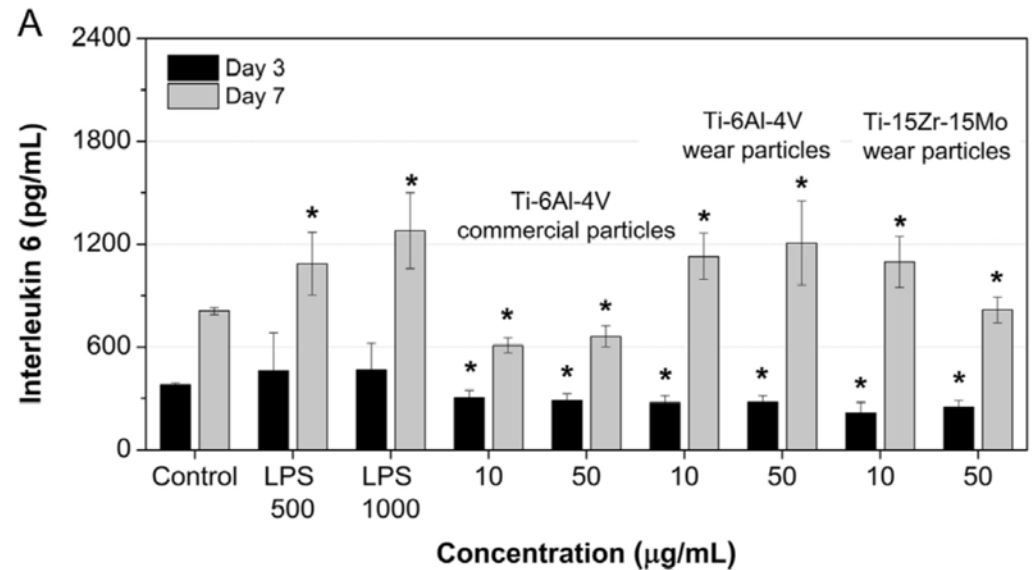


Exposure effects of endotoxin-free titanium-based wear particles to human osteoblasts



Bruna C. Costa<sup>a,\*</sup>, Alexandra C. Alves<sup>b</sup>, Fatih Toptan<sup>b,c</sup>, Ana M. Pinto<sup>b,c</sup>, Liliana Grenho<sup>d,e</sup>,  
Maria H. Fernandes<sup>d,e</sup>, Dmitri Y. Petrovykh<sup>f</sup>, Luís A. Rocha<sup>g</sup>, Paulo N. Lisboa-Filho<sup>g</sup>

- ◆ Metabolic assays can be affected by NP contamination
  - E.g., endotoxin (LPS) in cytokine assays
- ◆ NPs can produce artifacts in assays
  - Particularly in colorimetric ones



## ◆ Expertise

- Nanosafety in the context of nanomedicine, food, environment
- Setting up an ERA Chair on Nanosafety

## ◆ Technology

- Toxicology assays: zebra fish, cell culture, and specialized formats
- Electron and optical microscopy of nanoparticles in cells
- Extensive state-of-the-art nanocharacterization

## ◆ Network and history

- Past and current nanosafety projects: EU, regional, national
- Active interactions with major projects on nanoparticle characterization in nanomedicine and metrology contexts
- Membership in organizations involved in nanosafety: ETPN, NIA

## ◆ Questions?

- Dmitri Petrovykh: [dmitri.petrovykh@inl.int](mailto:dmitri.petrovykh@inl.int)