Aspectos metrológicos na avaliação da toxicidade de nanopartículas

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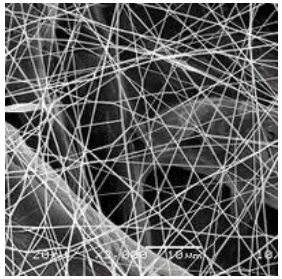
- O que é nanotoxicologia?
- Risco
- Perigo
- Exposição
- Mecanismos da nanotoxicidade
- Estado da arte – desafios
- O Inmetro e a nanotoxicidade

O que é a nanotoxicologia?

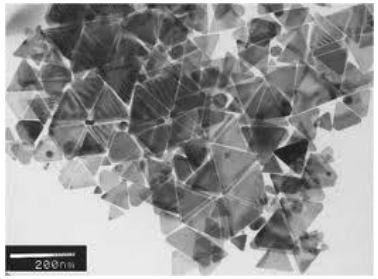
POR QUE DEVEMOS NOS PREOCUPAR?

O que são nanomateriais?

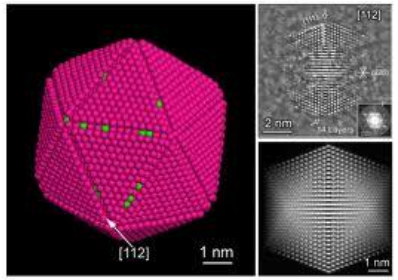
ISO/TS 27687:2008



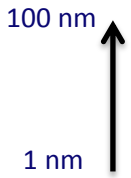
Nanofibra de NTC

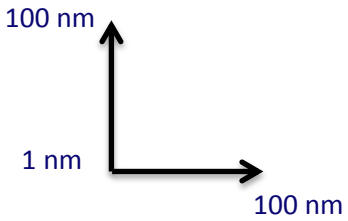


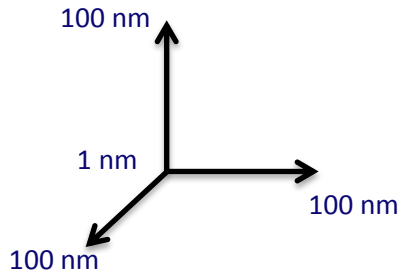
Nanoplaca de Ag



Nanopartícula Fe/Pt

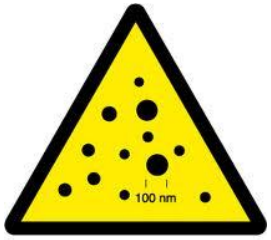






Teoria da toxicologia

Risco = Perigo + Exposição



O perigo das nanomateriais

- **Os nanomateriais são mais tóxicos que seus homólogos não-nano?**
- **Como eles afetam as células?**
  - **Efeito**
    - **Agudo**
    - **Crônico**
- **Eles se transformam em formas mais tóxicas?**

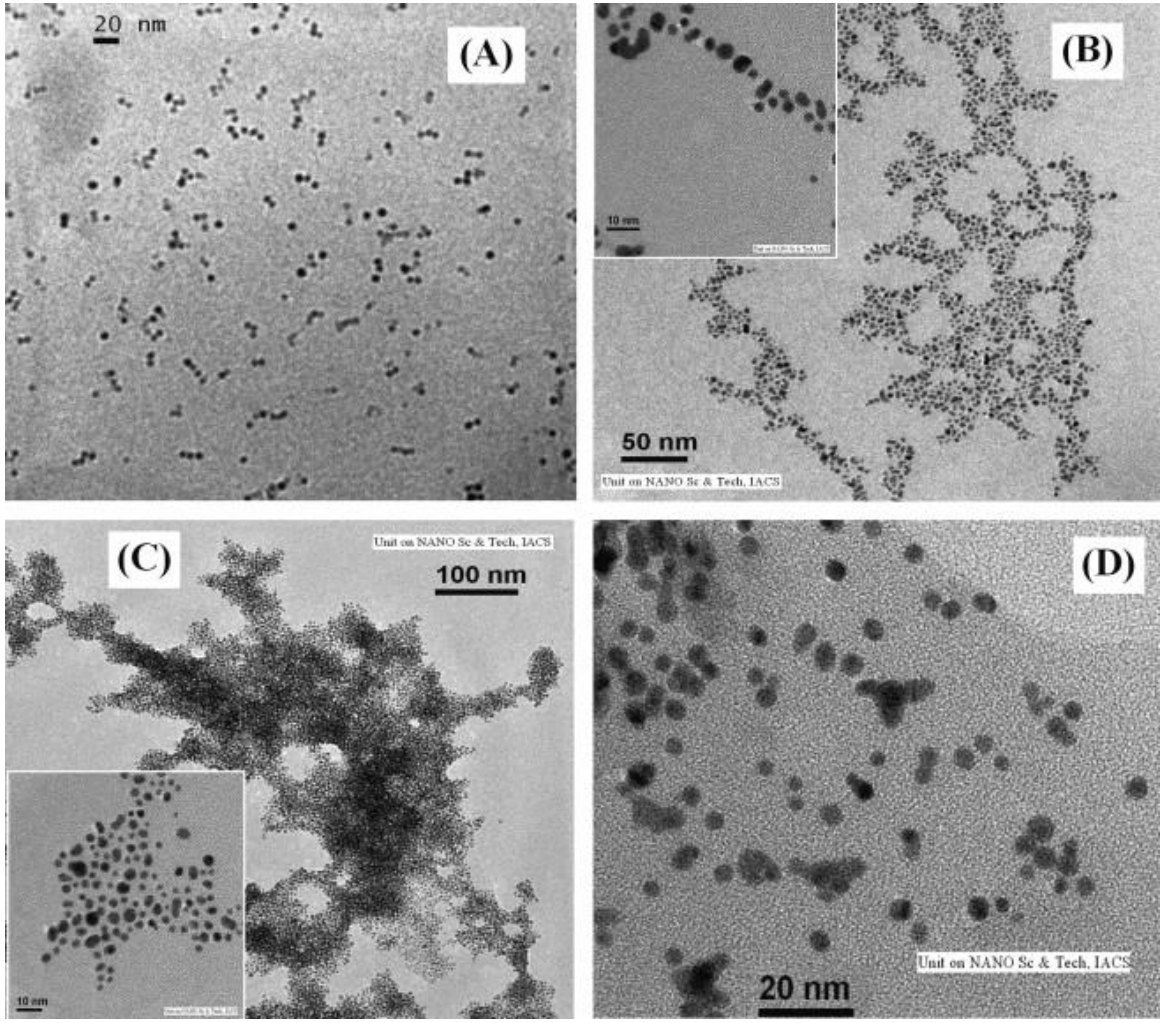
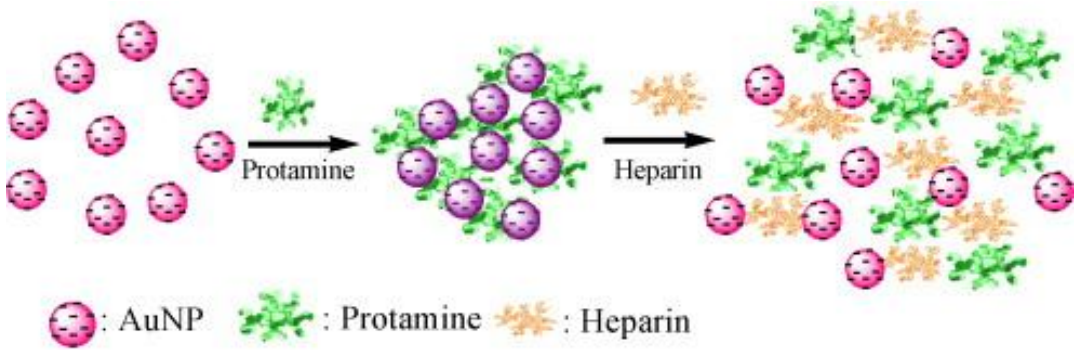


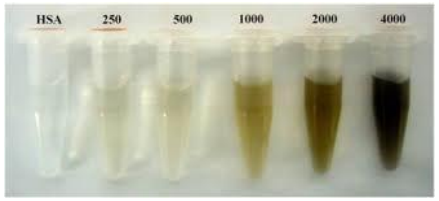
Fig. 4. Representative TEM images demonstrating the protamine-induced aggregation of AuNPs and the subsequent de-aggregation by heparin. (A) Citrate-stabilized native AuNPs. (B and C) Aggregated AuNPs ([protamine], B: 0.7 µg/ml; C: 1.6 µg/ml). (D) De-aggregated AuNPs ([heparin]: 10.2 µg/ml). Inset shows the enlarged view of the 2D aggregates.

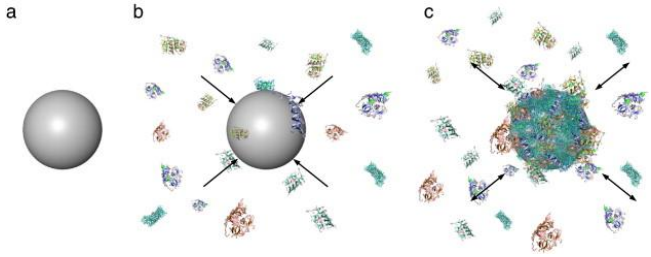
Jena & Raj, 2008, http://dx.doi.org.ezproxy.med.nyu.edu/10.1016/j.bios.2007.11.0



Jena & Raj, 2008, http://dx.doi.org.ezproxy.med.nyu.edu/10.1016/j.bios.2007.11.014,

Exposição a nanomateriais: desafio metrológico





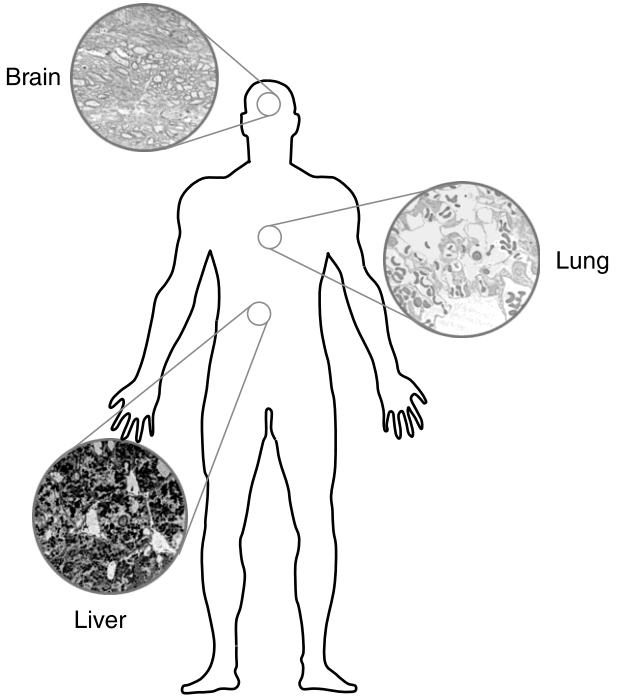
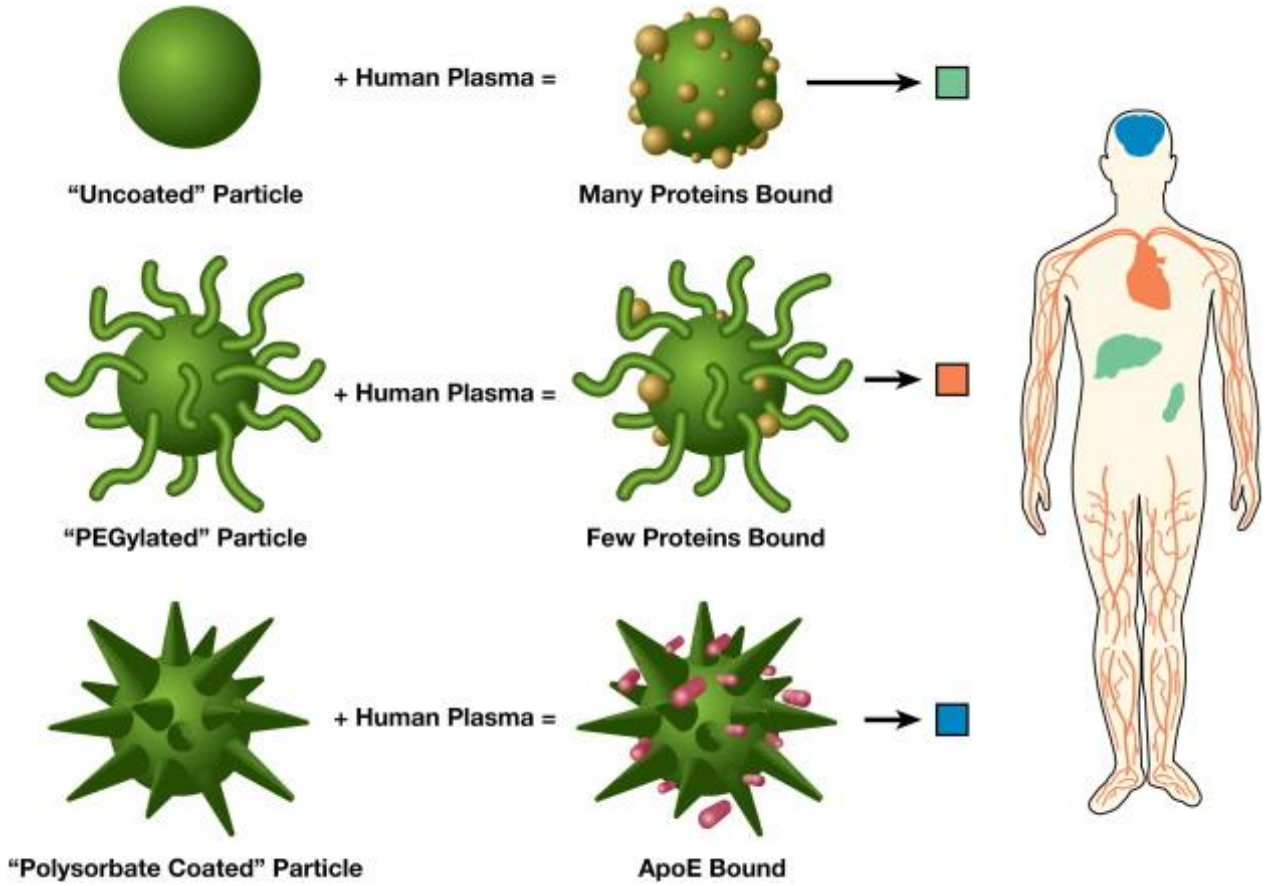


Fig. 3. Nanoparticle entry route into the body via the lung, particle accumulation in the liver and the most vulnerable site: the brain.

Biodistribuição de nanomateriais em função do revestimento



Alvos intracelulares dos nanomateriais

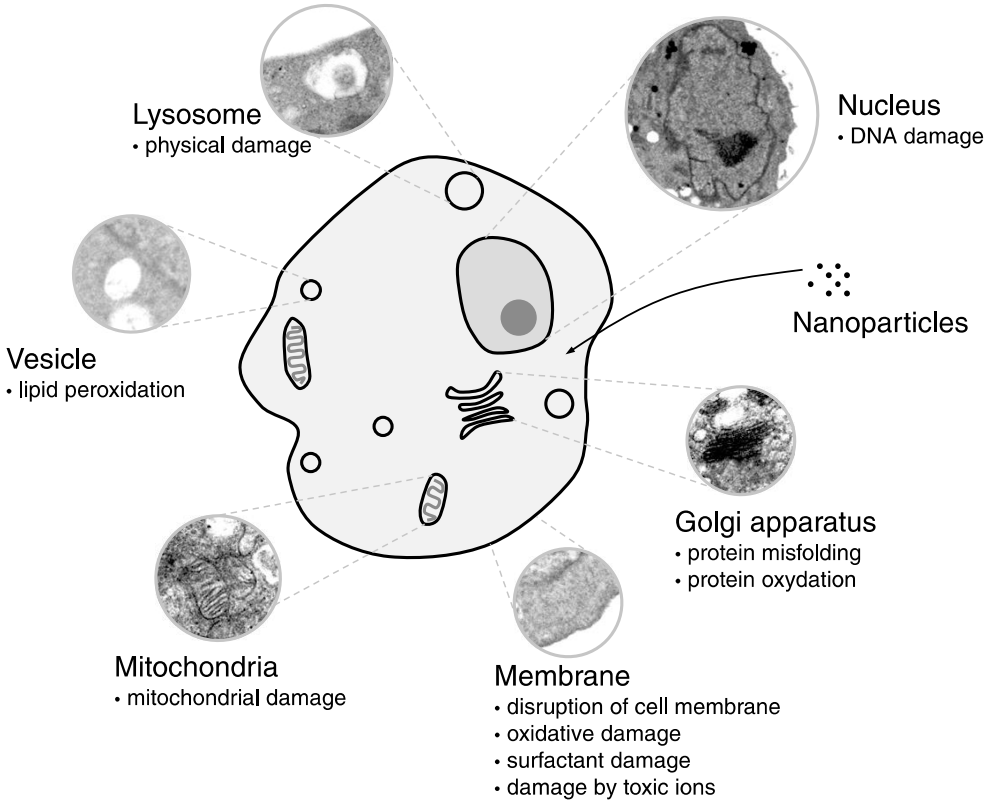


Fig. 2. Nanoparticle interaction with cells: intracellular targets and nanotoxicological mechanisms.

Vias de administração dos nanomateriais

Toxicological considerations of clinically applicable nanoparticles

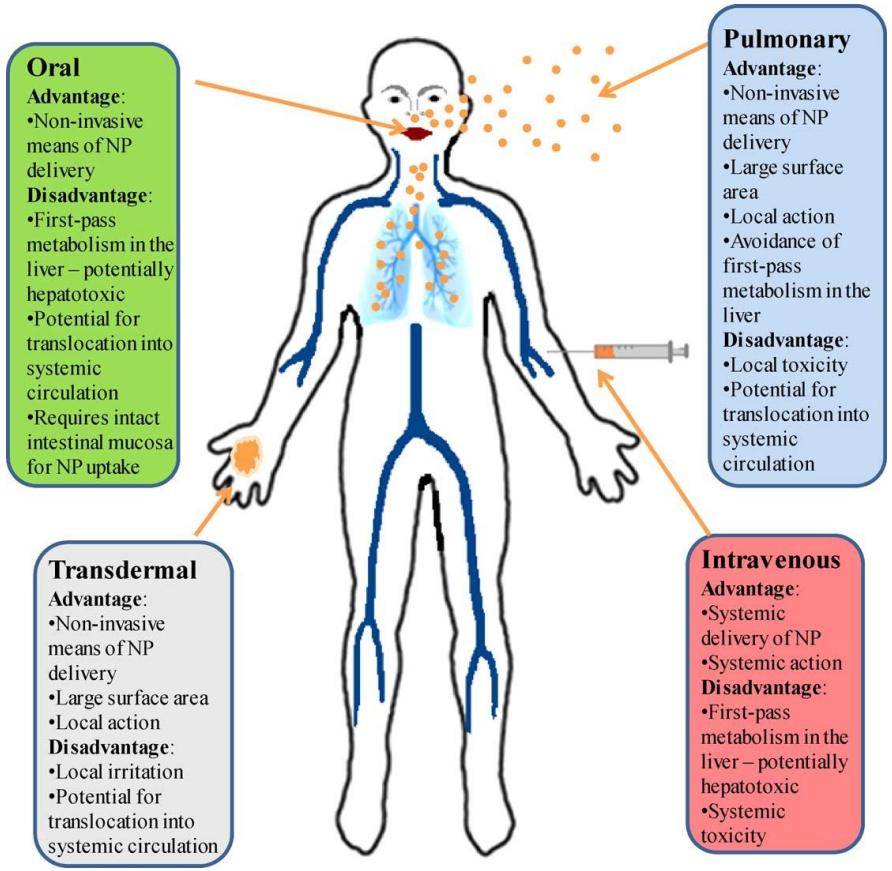


Figure 1 Routes of administration of nanoparticles and their advantages and disadvantages.

Nanomateriais biologicamente úteis

Toxicological considerations of clinically applicable nanoparticles

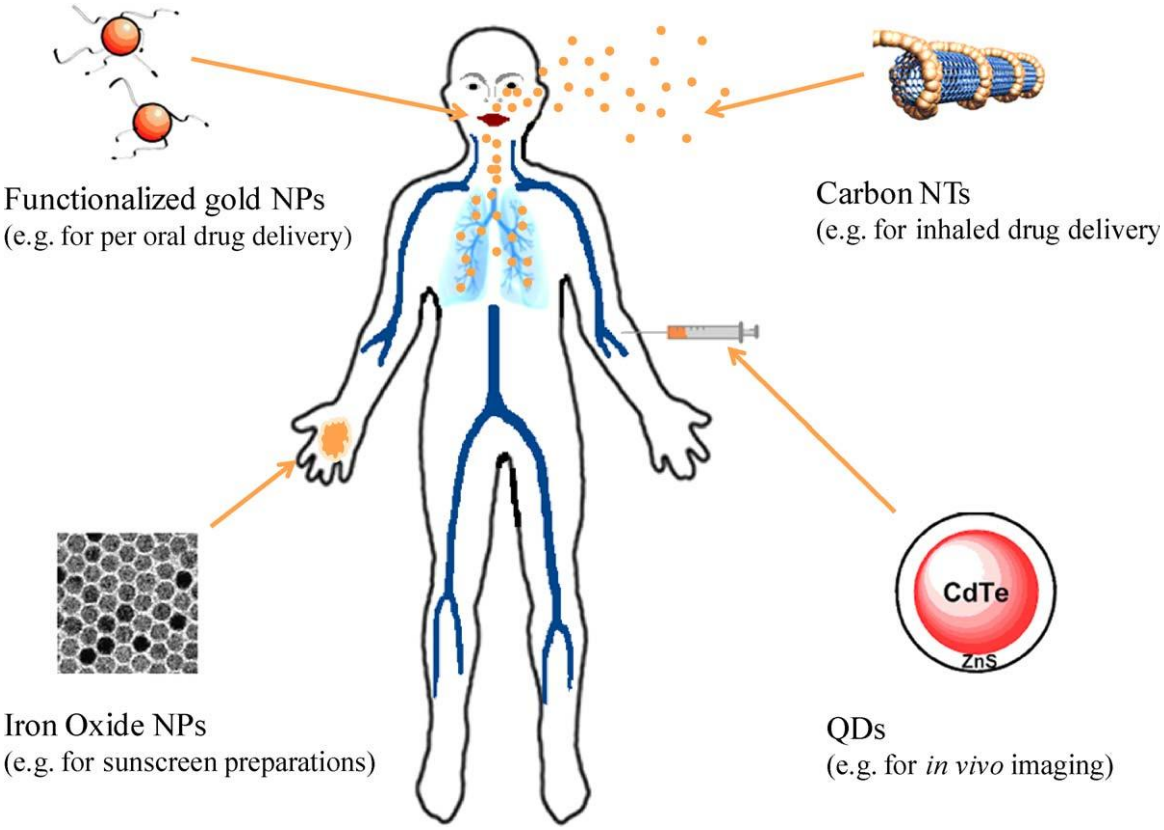
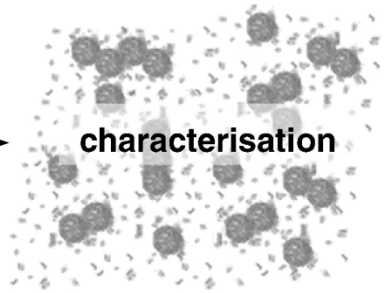


Figure 2 Selection of biologically useful nanoparticles [30].

Nanoparticle characterisation, pathways and toxicological impact



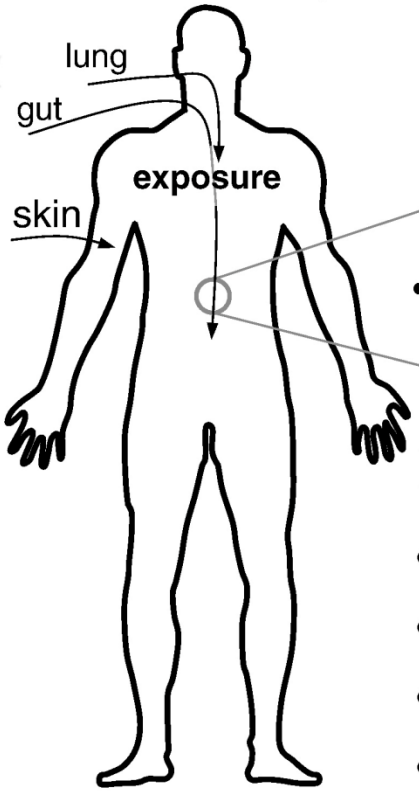
Nanoparticles:

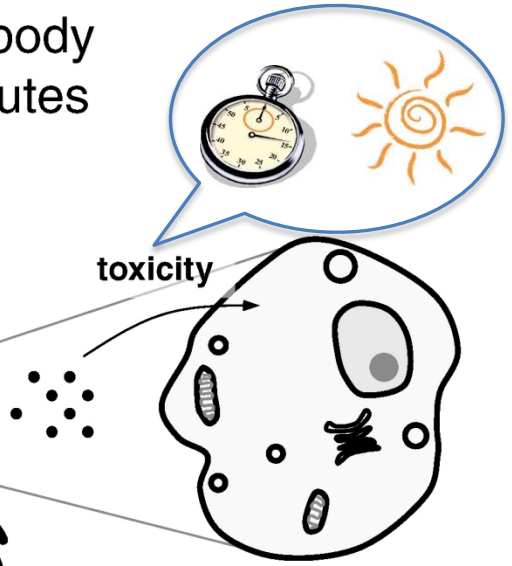
- industry
- research
- medicine

Nanoparticles in the environment:

- alteration of surface
- protein corona
- agglomeration

Nanoparticles in the body and possible entry routes



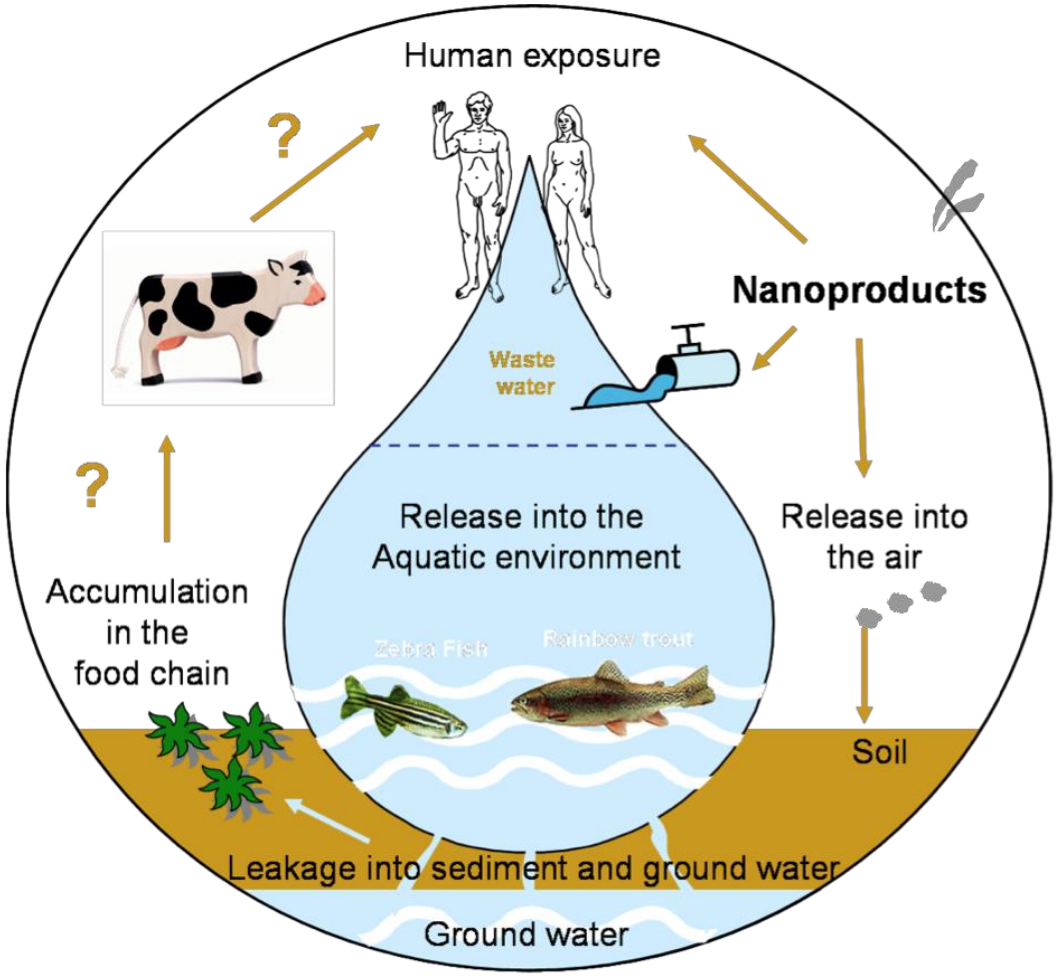


Nanoparticles in cells:

- production of reactive oxygen species
- protein misfolding
- membrane damage
- mitochondrial damage
- DNA damage

Yildirimer et al., 2001, doi:10.1016/j.nantod.2011.10.001

Exposição ambiental de nanomateriais



Mecanismos de nanotoxicidade

Estado da arte - desafios

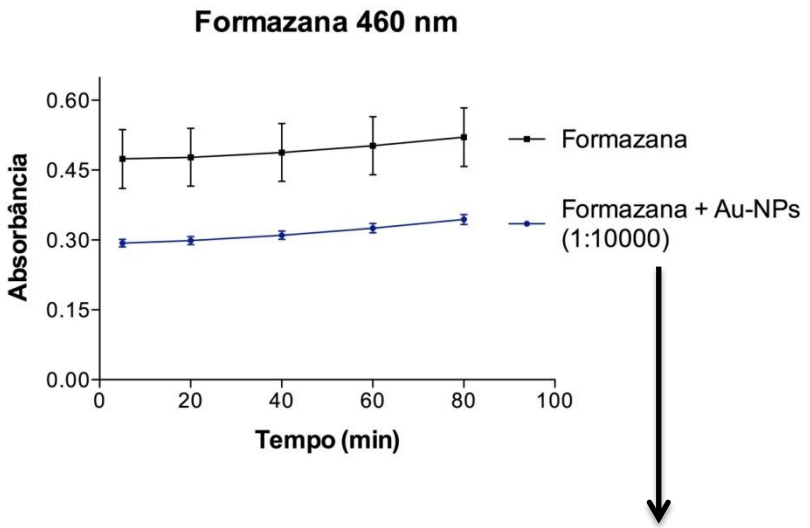
- **Estresse oxidativo**
  - **Grande área de superfície das NMs**
    - **Radicais livres // ROS**
    - **Oxidação de macromoléculas (P/L/AN)**
      - **Estrutura e função comprometida**
    - **Regulação pró-inflamatória / estado de defesa celular**

Estado da arte - desafios

- **Fluxo de cálcio**
  - **Consequencia de ROS**
    - **Ativação descontrolada de fatores de transcrição**
    - **Regulação pró-inflamatória**

Estado da arte - desafios

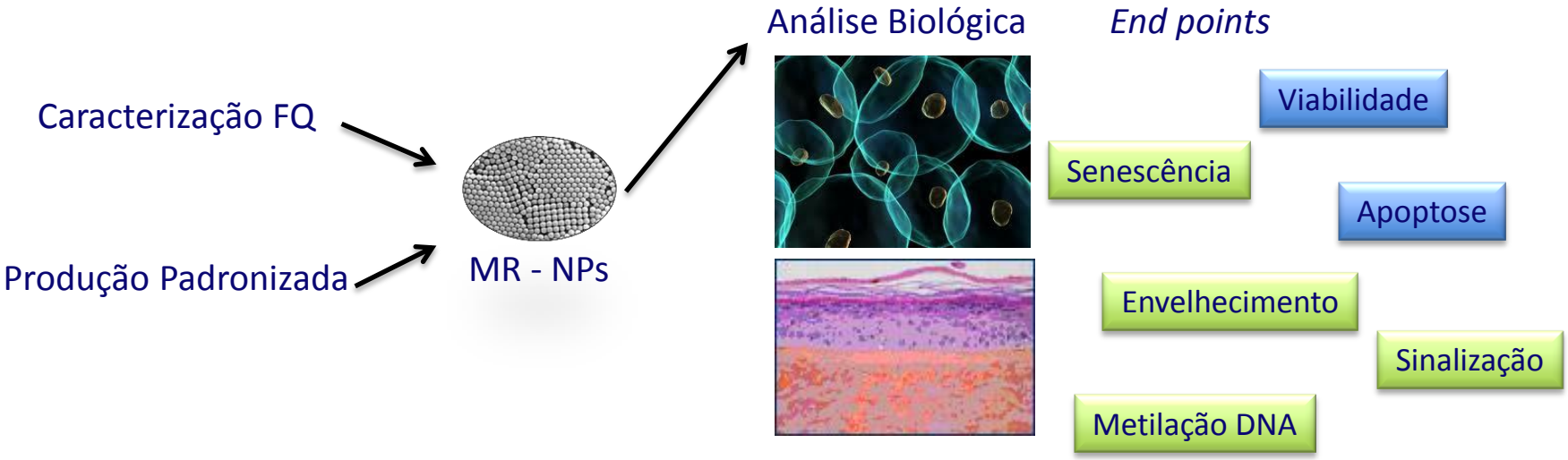
- **Viabilidade celular**
  - **Adequabilidade dos métodos atuais**
    - **M/XTT, Alamar blue**
    - **NRU**
    - **CV**
  - **Novos biomarcadores**
  - **Ensaios de toxicidade capazes de acessar o risco**
  - **Sistemas biomiméticos**
  - **Comparabilidade de ensaios de toxicidade**



~ 10.000 AuNPs / células

O Inmetro e a nanotoxicidade

- **Coordena a Rede Nanotox**
- **Coordena a Rede Nacional de Métodos Alternativos**
- **Participa do Nanovalid (FP7 – CE)**



Modelos in vitro

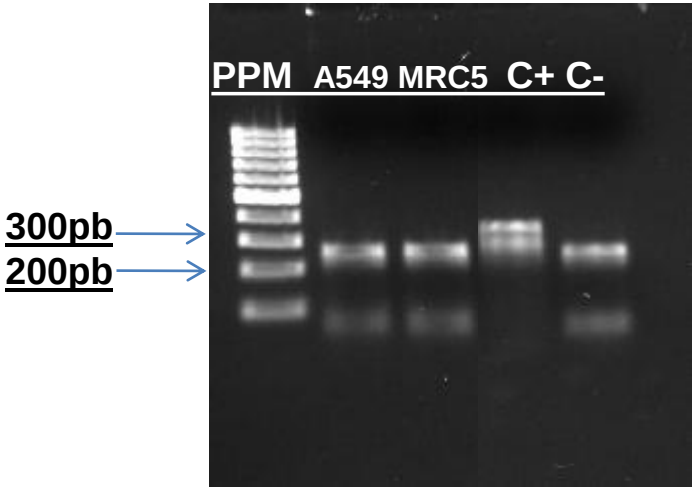
- **Células**
  - **Pulmonar**
    - **MRC5**
    - **A549**
  - **HUVEC**
    - **Endotélial**
    - **Estromal**
  - **Gástrica**
    - **Caco**
    - **Fibroblasto**
  - **Pele**
    - **Queratinócito**
    - **Fibroblasto**

- **Tecidos equivalentes**
  - **Pulmão**
  - **Vaso**
  - **Gastrico**
  - **Pele**

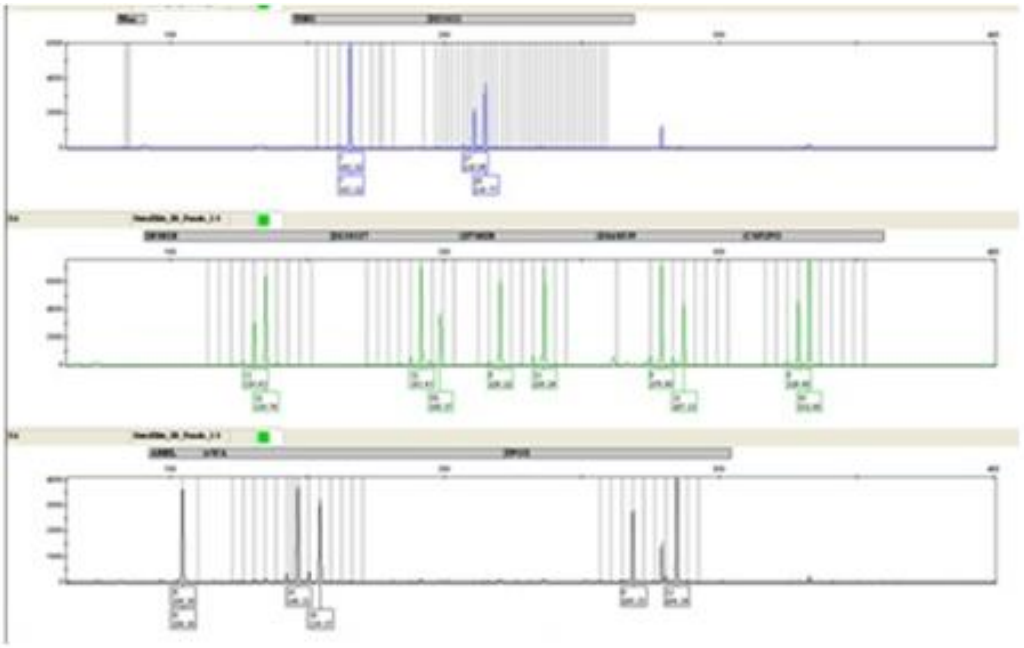
BCRJ

Pureza e autenticidade

Detecção de micoplasma (PCR)

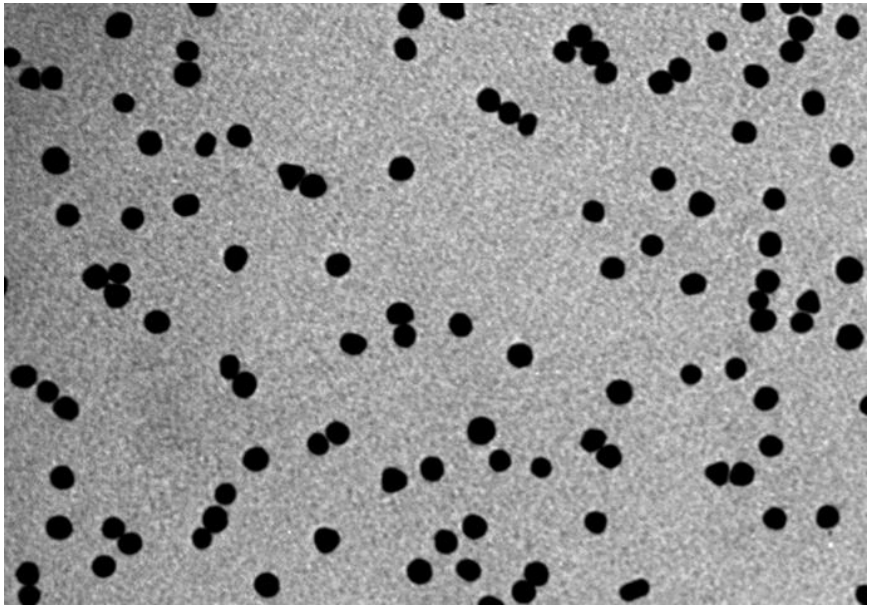


STR para todas as linhagens humanas

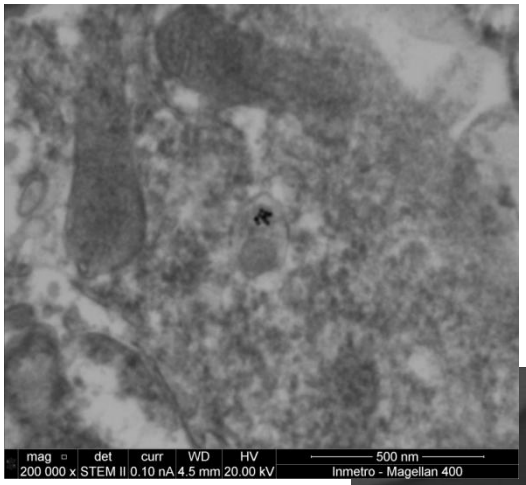


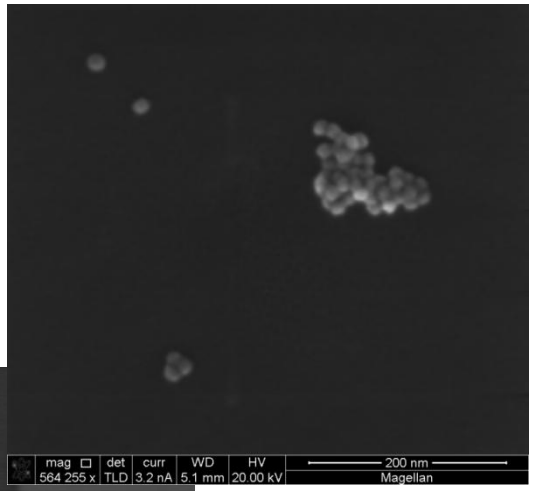
Au-NPs

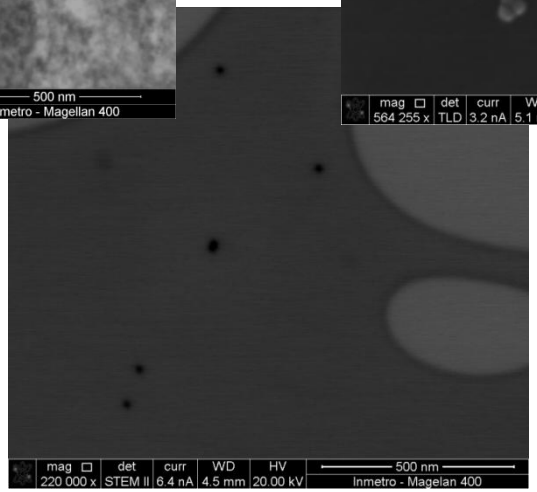
Produção e caracterização Dipro/Dimci



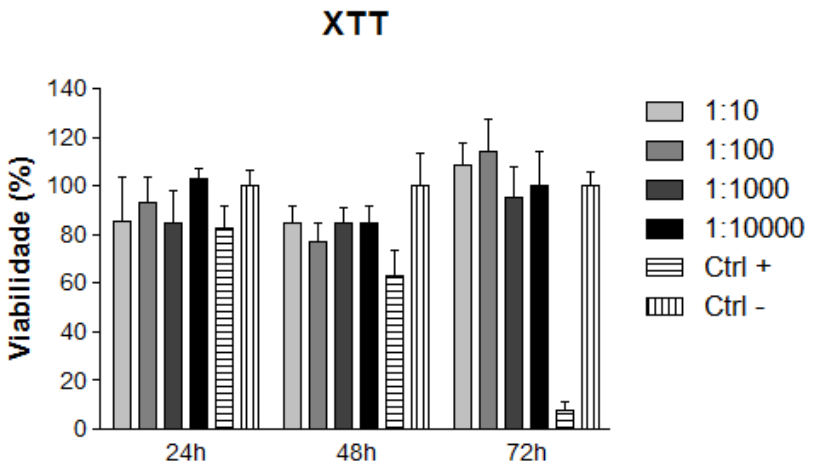
Interação célula - NP

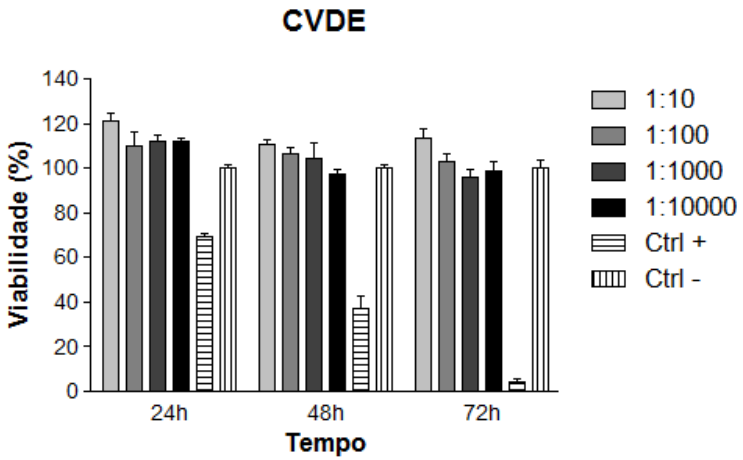


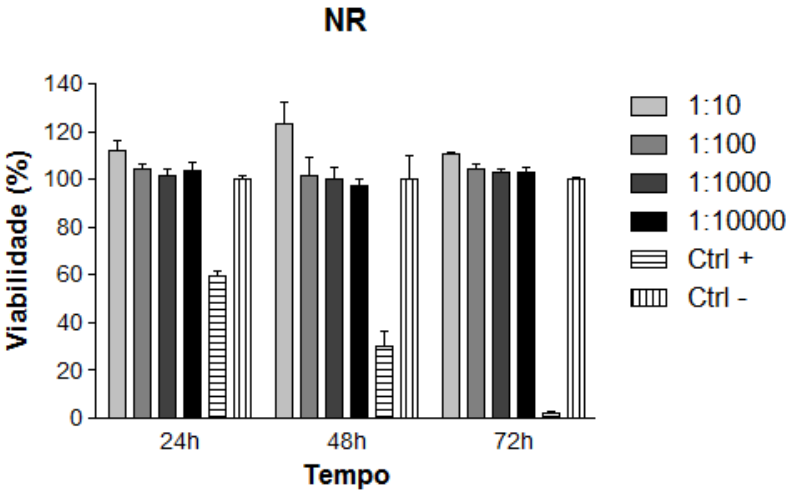




Ensaio de citotoxicidade

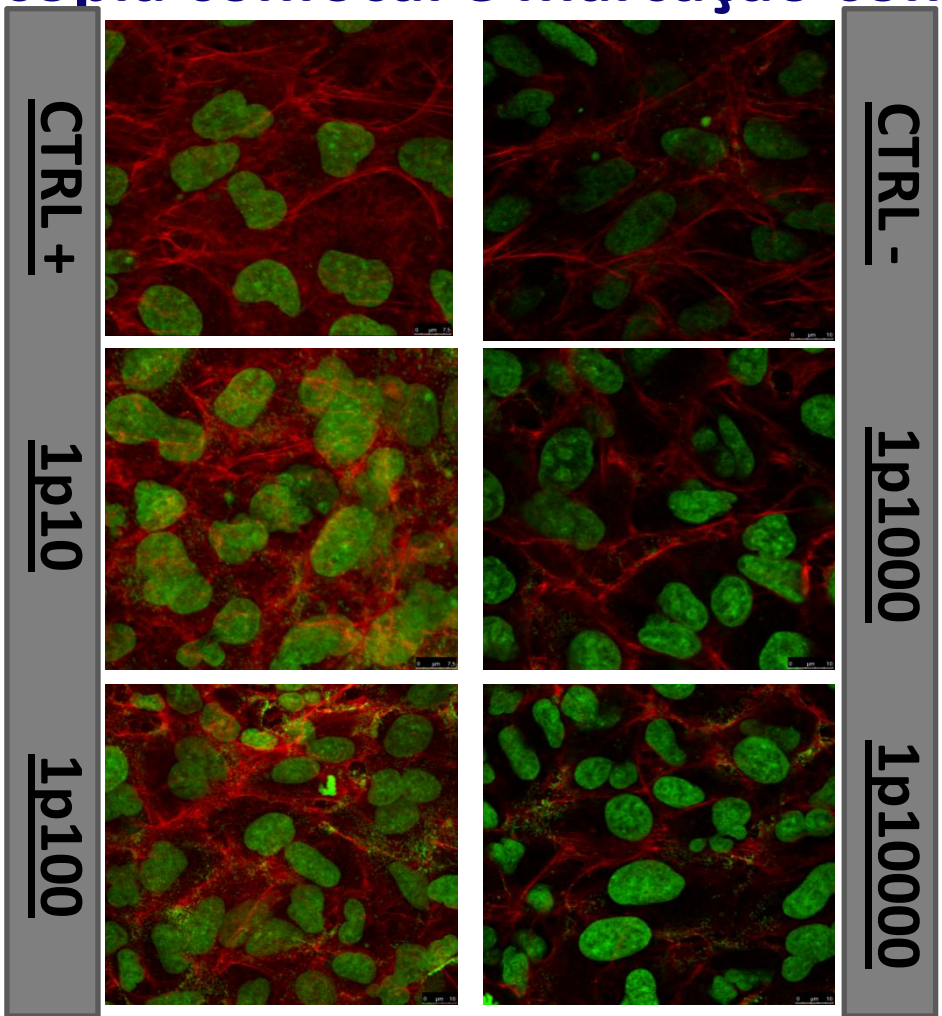






Análise do citoesqueleto

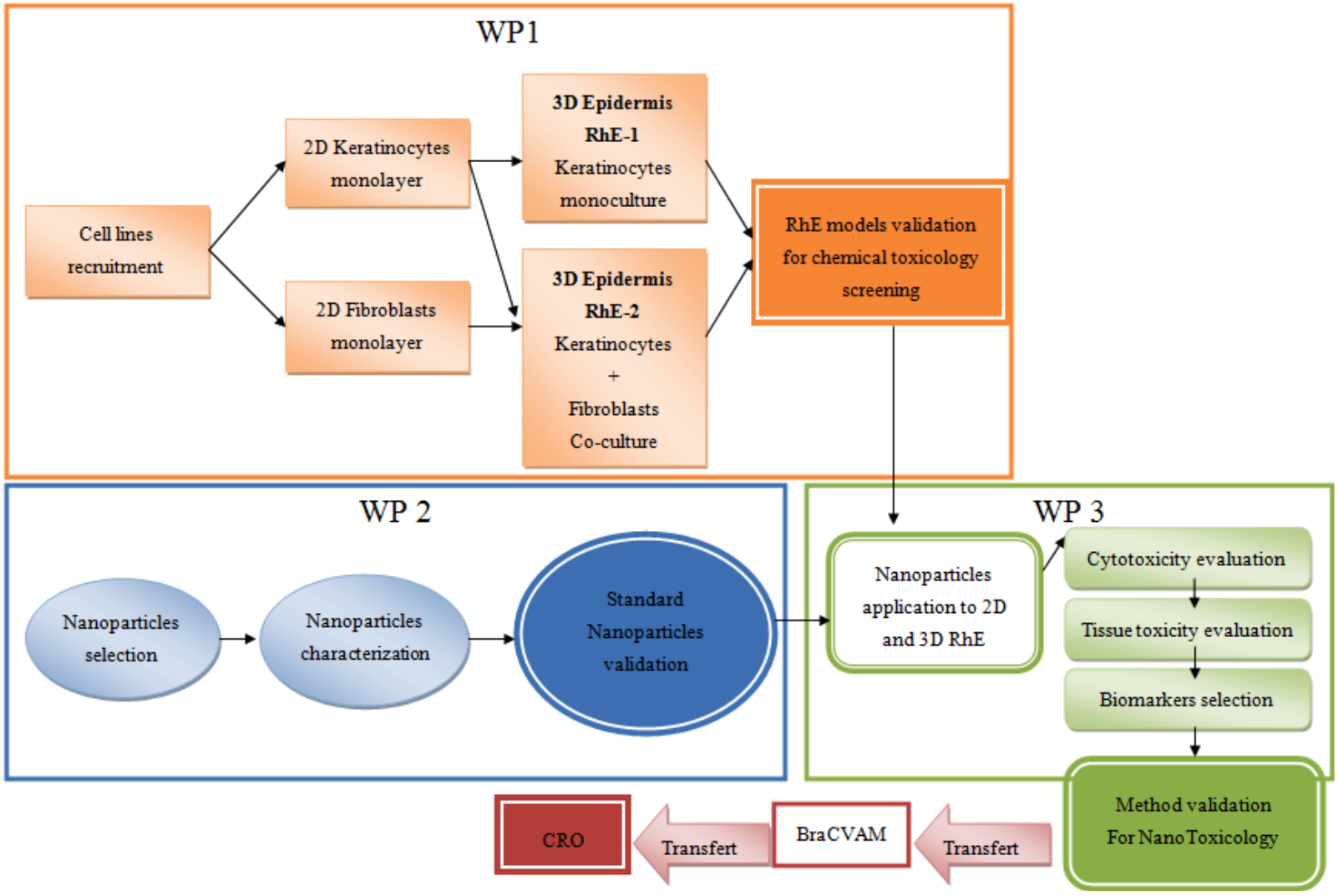
(microscopia confocal e marcação com faloidina)



Nanotoxicidade e Métodos Alternativos (MA)

- **Análise da adequação dos MA validados ao ensaio de toxicidade de NPs (*guides* da OECD)**
  - **Fototoxicidade (GD OECD 432)**
  - **Irritação cutânea (GD OECD 439)**
- **Desenvolvimento de tecido equivalente de pele (Pascal Sommer e Leslie Laquieze, IBPC/CNRs/Lion/Fr)**
- **BraCVAM - RENAMA**

Tecido equivalente de pele



Obrigado

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