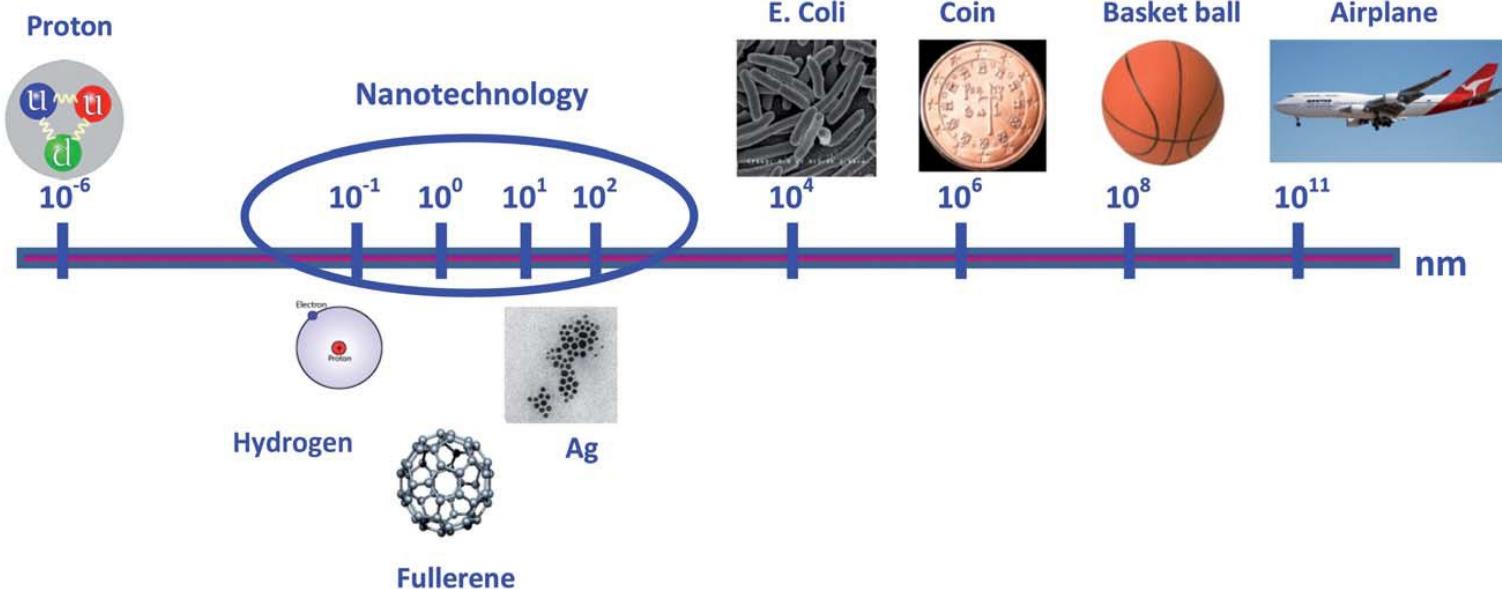


## “BUENAS PRÁCTICAS PREVENTIVAS EN EL TRABAJO CON NANOMATERIALES. EVALUACIÓN, MEDICIÓN Y PROTECCIÓN COLECTIVA”

# *RETOS Y DESARROLLO DE HERRAMIENTAS ANALÍTICAS PARA LA CARACTERIZACIÓN DE NANOMATERIALES EN ESTUDIOS TOXICOLÓGICOS*

**Rosa Carmen Rodríguez Martín-Doimeadios**

Catedrática Química Analítica  
Departamento Química Analítica y Tecnología de los Alimentos  
Facultad Ciencias Ambientales y Bioquímica  
UCLM



**Nanomaterials (NMs)** should consist for 50 % or more particles having a size in the range  
**1 – 100 nm\***



# SEARCH THE NANODATABASE

Your inventory for products that contain Nanomaterials

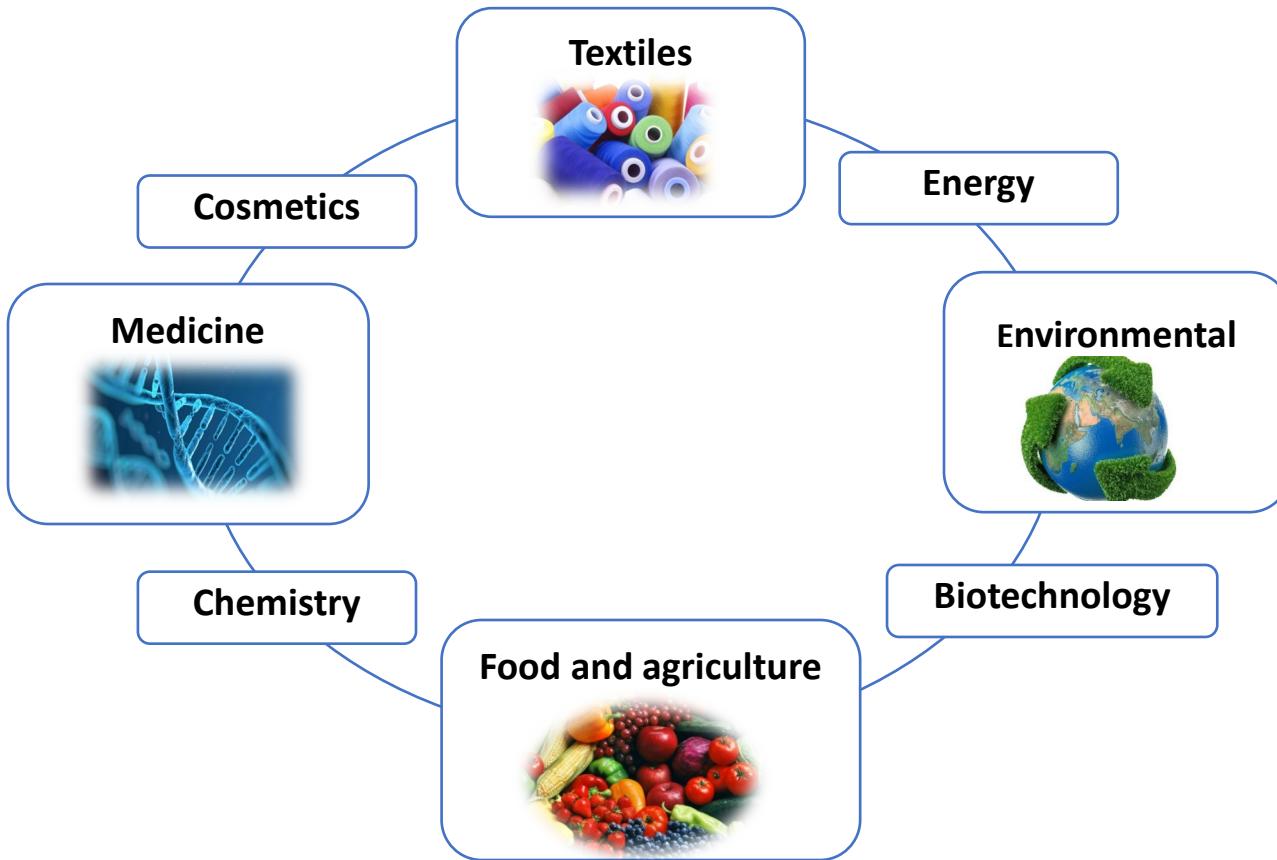
Search for Product Name, Nano Material, Manufacturer..



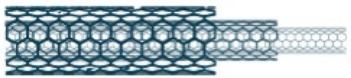
There are currently

5 , 3 6 7

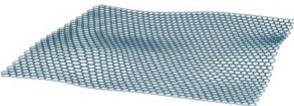
products in our database



# Types of nanomaterials



Carbon nanotubes



Graphene



Core-Shell nanoparticles



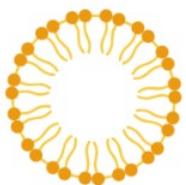
Metal oxide nanoparticles



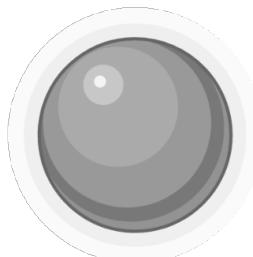
Quantum Dots



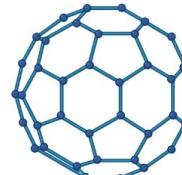
Polymeric nanoparticles



Liposomes



Metallic nanoparticles



Fullerene



Dendrimers

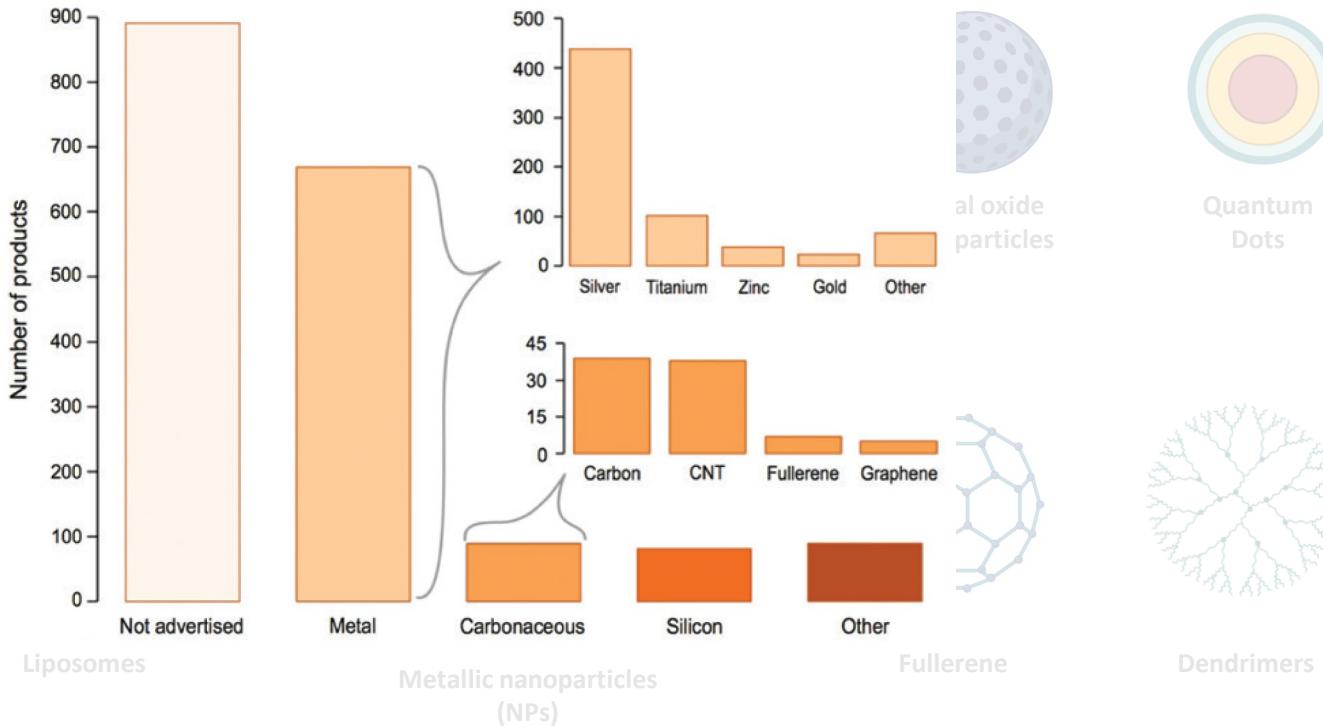
# Types of nanomaterials



Carbon nanotubes



Polymeric nanoparticles





## Toxicological risks on human health induced by NP



*Inhalation*

Asthma  
Bronchitis

*Ingestion*

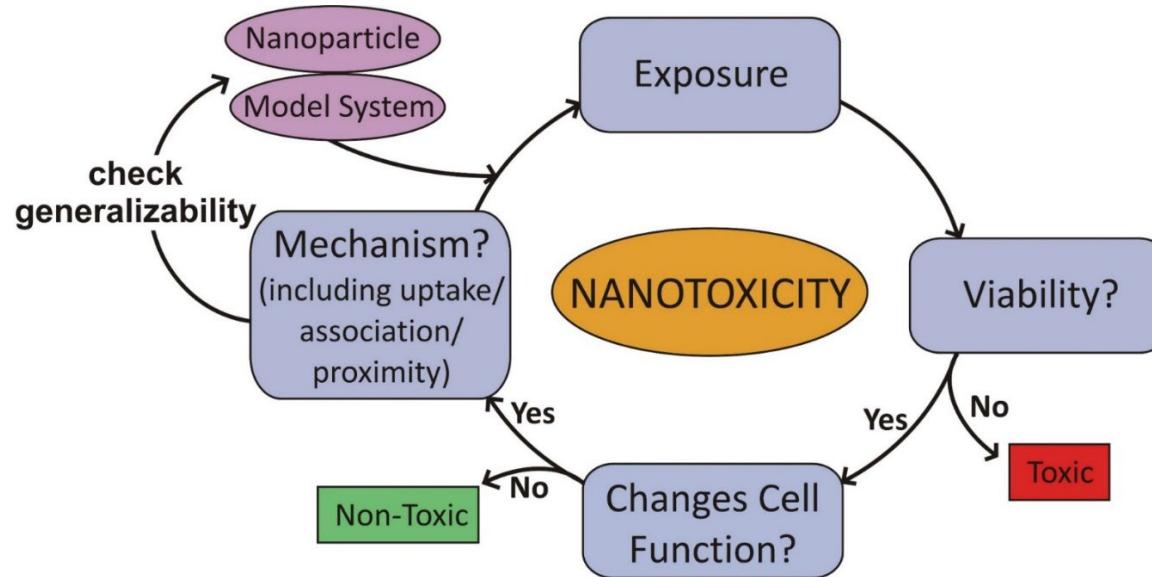
Crohn's disease  
Colon cancer

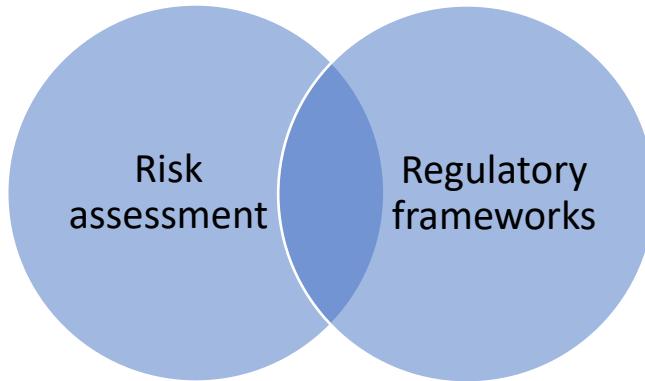
*Dermal*

Auto-immune diseases  
Dermatitis

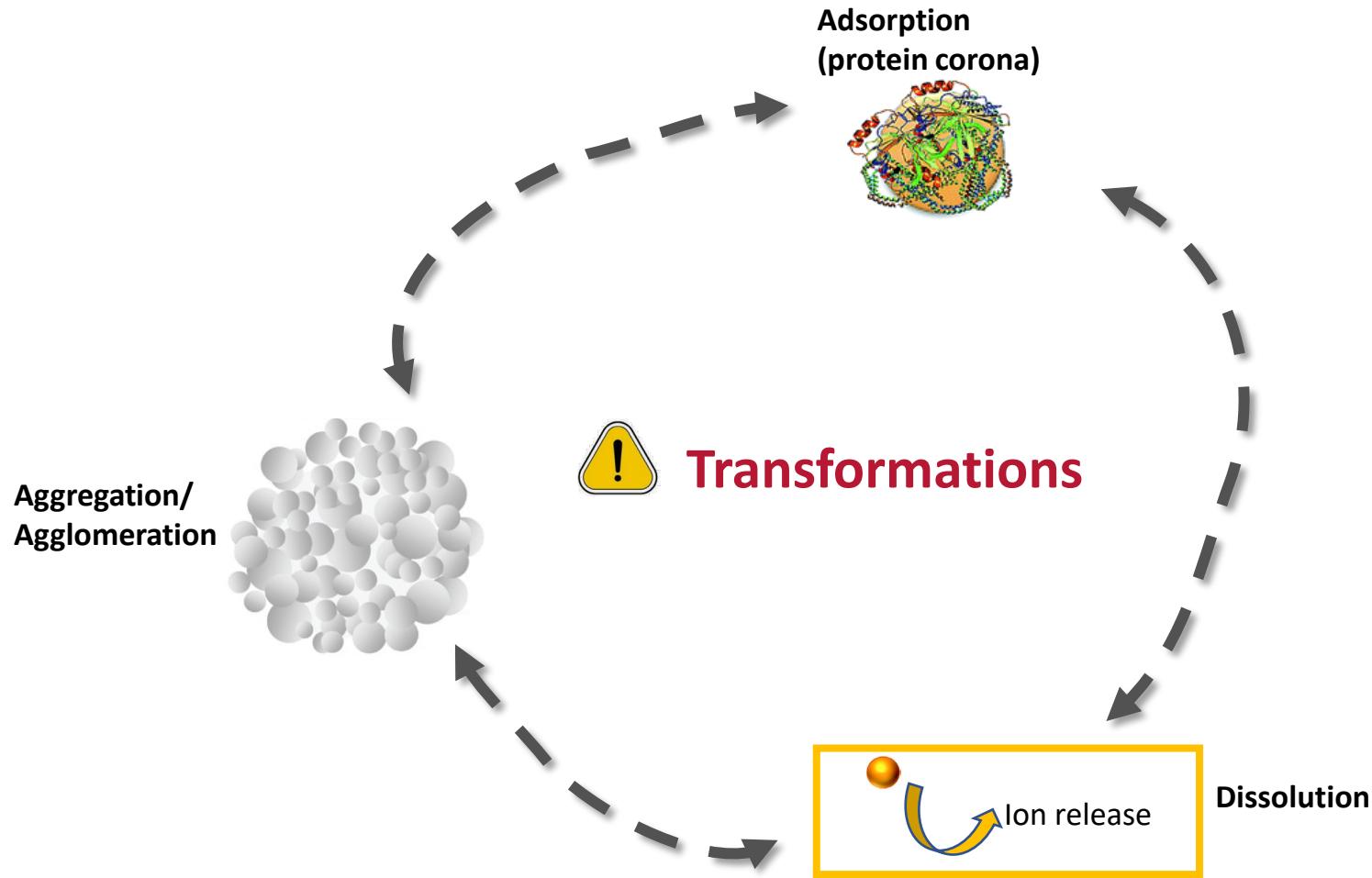
*Cell internalization*

Nucleus  
Membrane

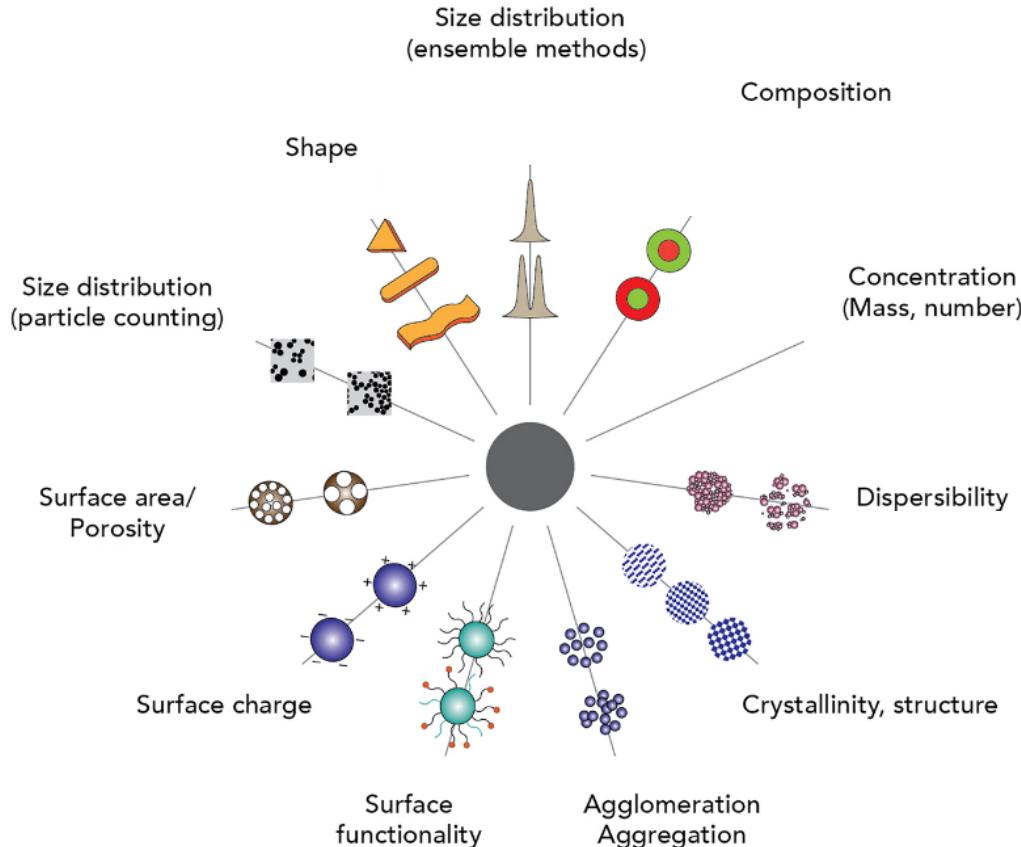




*Valid and comparable analytical methods are required to support risk assessment for regulation*

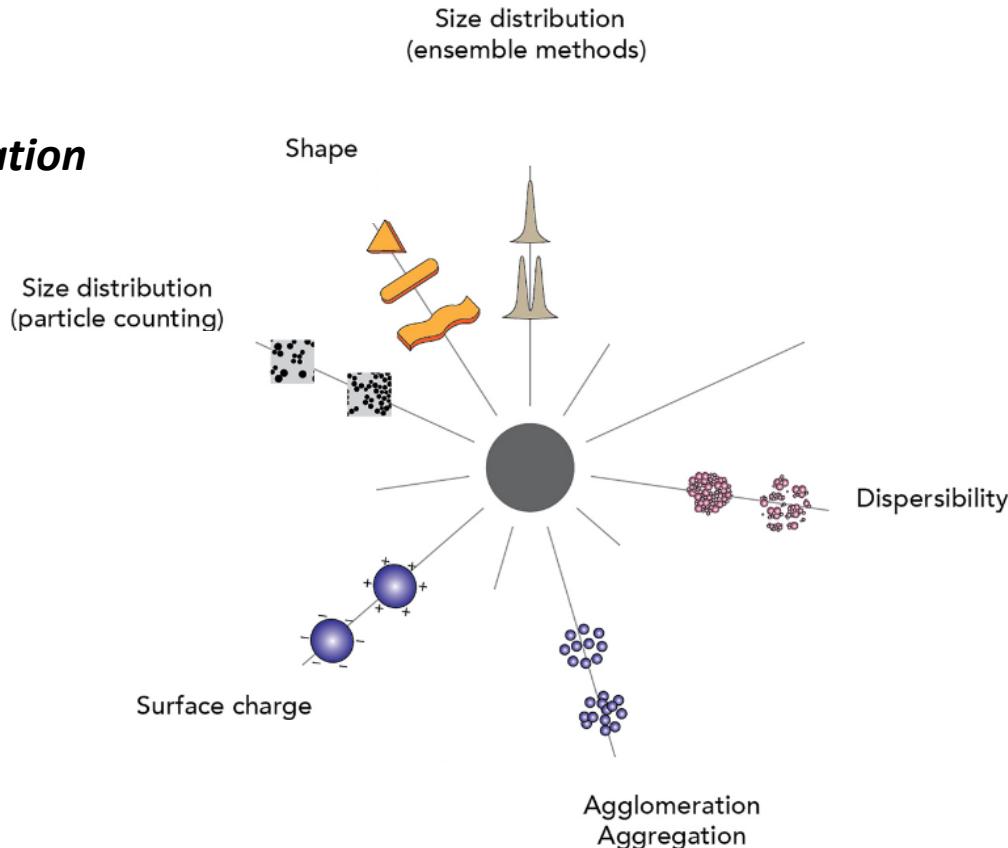


# Characterization



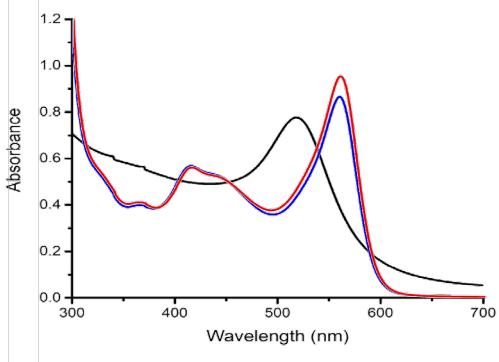
# Characterization

## Physical information

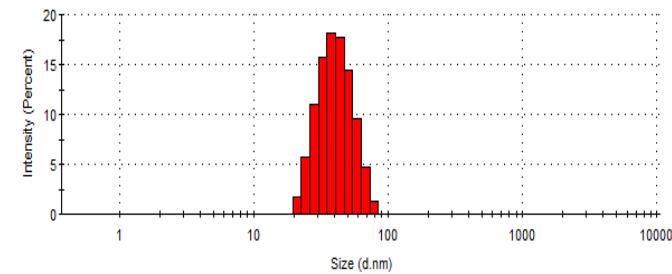


# Classical techniques

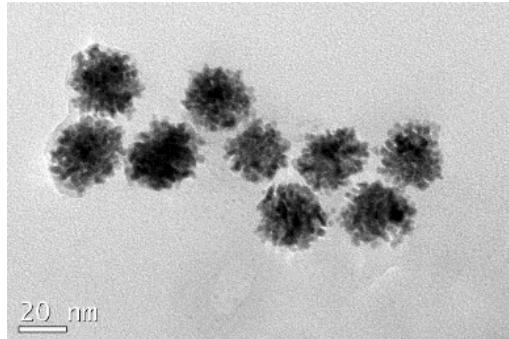
## Ultraviolet-visible spectroscopy (UV-vis)



## Dynamic light scattering (DLS)



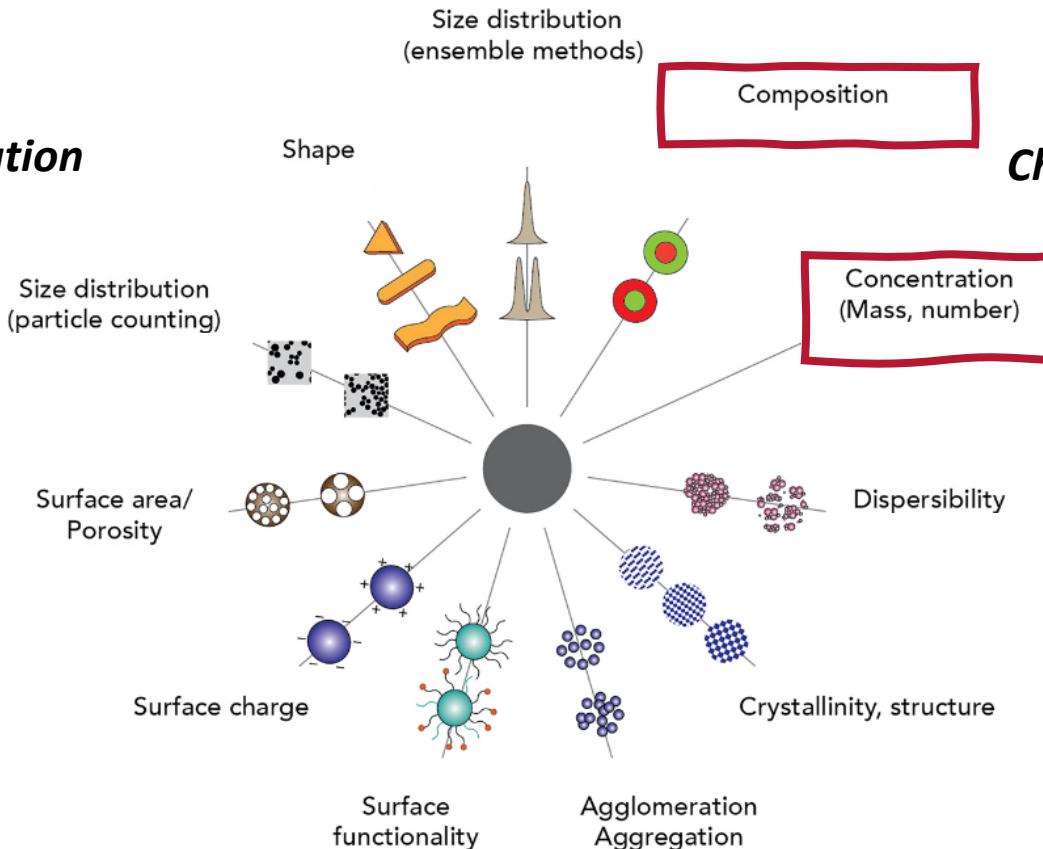
## Electron microscopy techniques (EM)



# Characterization

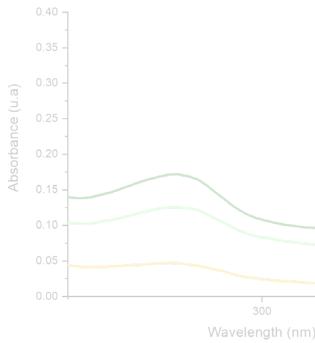
*Physical information*

*Chemical information*

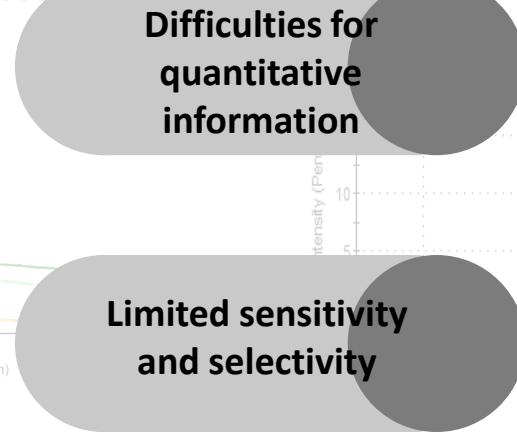


# Classical techniques

Ultraviolet-visible spectroscopy (UV-vis)



Dynamic light scattering (DLS)



Electron microscopy techniques (EM)



**Difficulties for quantitative information**

**Limited sensitivity and selectivity**

**Risk of important alterations due to sample preparation**



↑ *Number of NMs*

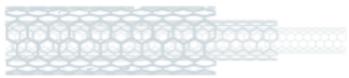
↑ *Diversity of NMs*

*Transformations*

*Characterization/Quantification  
(chemical/physical parameters)*

*Need for appropriate/validated analytical  
methods/techniques*

# Types of nanomaterials



Carbon nanotubes



Graphene



Core-Shell nanoparticles



Metal oxide  
nanoparticles



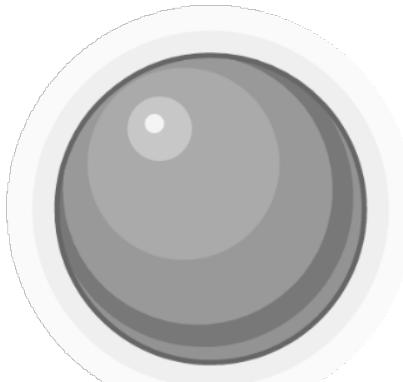
Quantum  
Dots



Polymeric  
nanoparticles



Liposomes



**Metallic nanoparticles  
(NPs)**

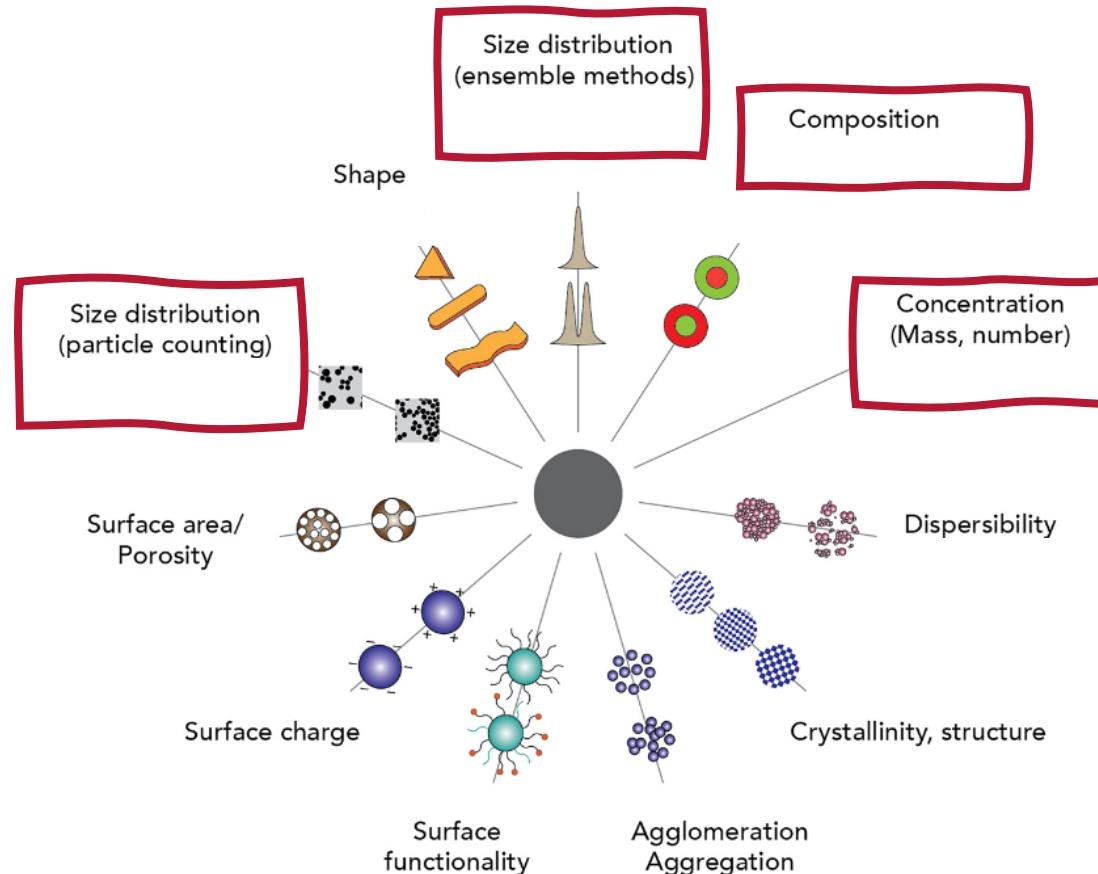


Fullerene

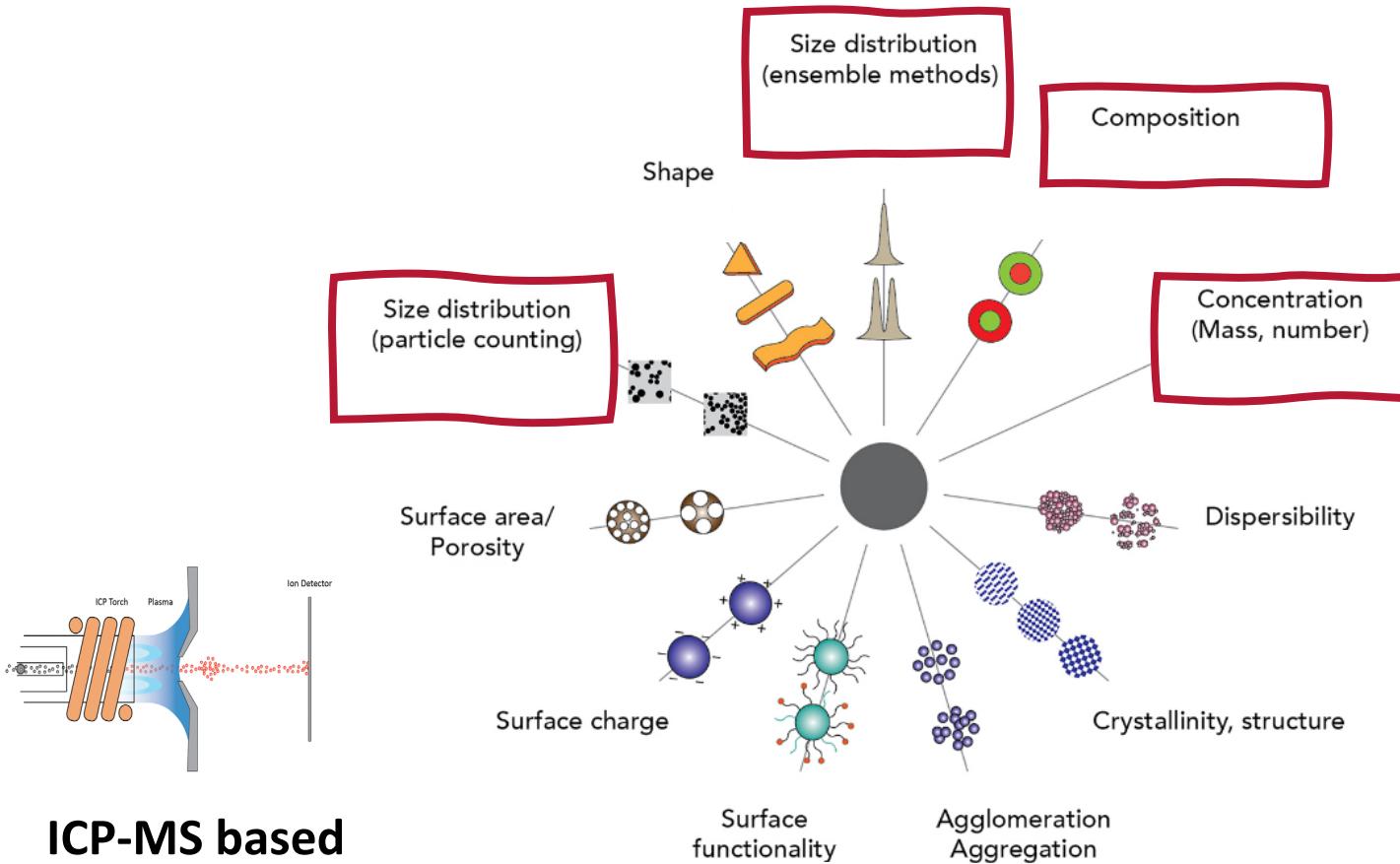


Dendrimers

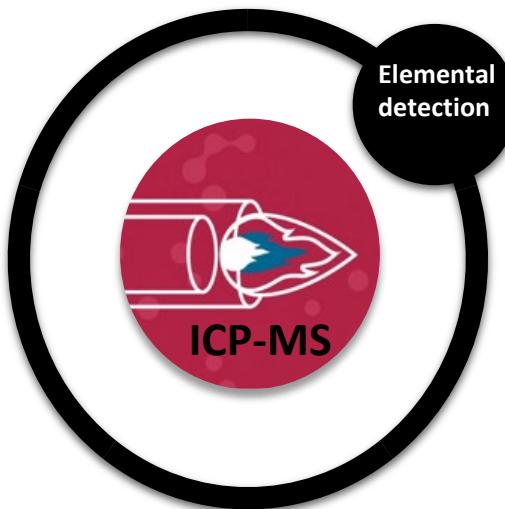
# Characterization



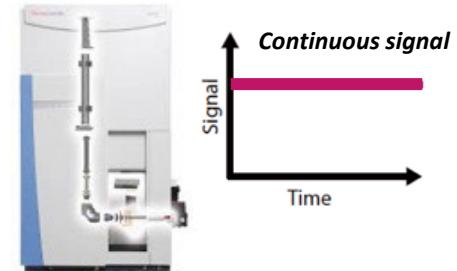
# Characterization



**ICP-MS based  
techniques**



*Conventional*



**Analytical information**

*Elemental identification*

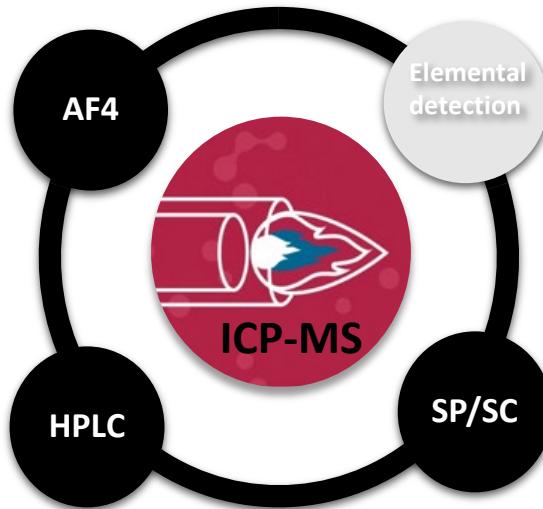
*Total mass concentration*



*Asymmetric flow  
field-flow  
fractionation*

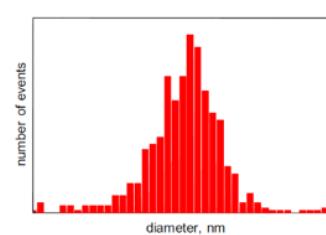


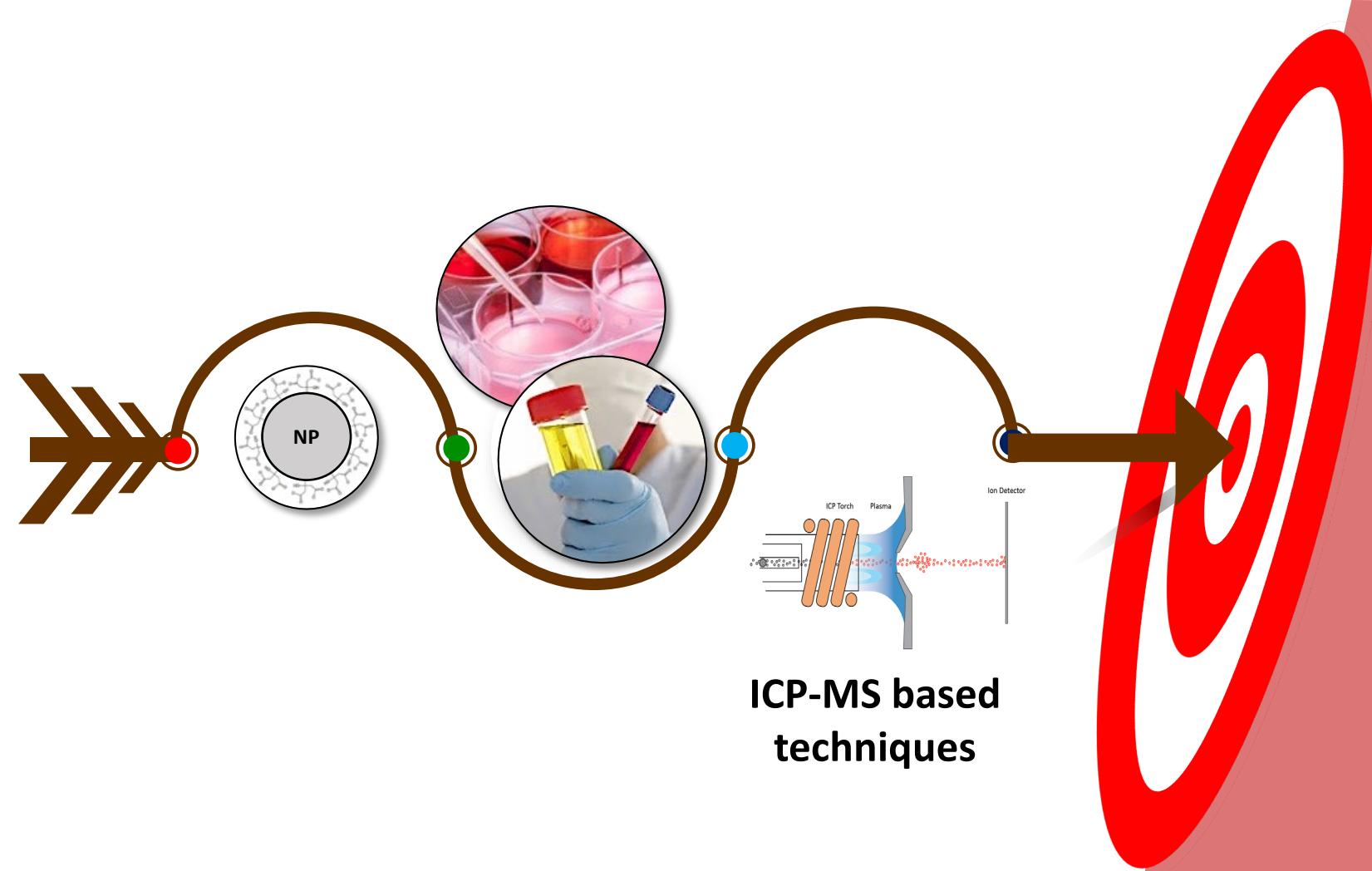
*High performance  
liquid chromatography*

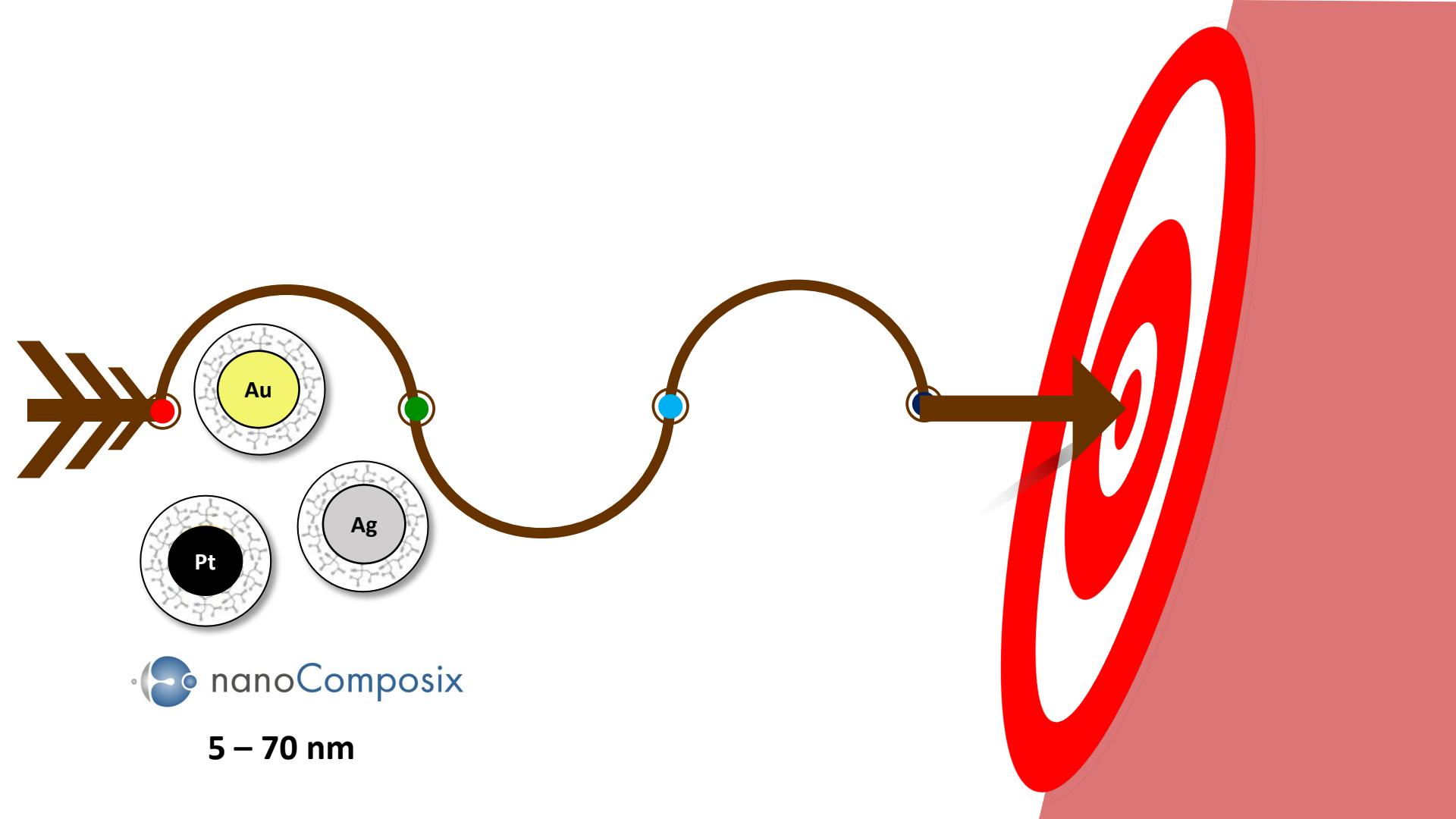


*Single Particle / Cell*

*Conventional*







nanoComposix

5 – 70 nm



[www.nanoComposix.com](http://www.nanoComposix.com)

## SAFETY DATA SHEET

Preparation Date: 10/11/2010  
Revision Date: 02/10/2021

---

### SECTION 1: Identification

---

## NanoXact™ Gold Nanoparticles (AUCN)

**Manufacturer:** nanoComposix, Inc.  
4878 Ronson CT STE K  
San Diego, CA 92111-1806

**NANOCOMPOSIX CUSTOMER SERVICE:** (858) 565-4227  
CHEMTREC (EMERGENCY ONLY): (800) 424-9300  
POISON CENTER: (800) 562-8236

**Relevant Identified Uses:** Laboratory chemicals, Manufacture substances

---

### SECTION 2: Hazards Identification

---

#### EMERGENCY OVERVIEW:

**OSHA Hazards:** No known OSHA hazards. Not a dangerous substance according to GHS.

**Hazards not otherwise classified (HNOC) or not covered by GHS - None**

## **SECTION 8: Exposure Controls and Personal Protection**

---

**CONTROL PARAMETERS:** Contains no substances with occupational exposure limit values.

**EXPOSURE CONTROLS:**

**APPROPRIATE ENGINEERING CONTROLS:** General industrial hygiene practice.

**PERSONAL PROTECTIVE EQUIPMENT:**

**EYE PROTECTION:** Use equipment for eye protection tested and approved under appropriate government standards such as NIOSH (US) or EN 166(EU).

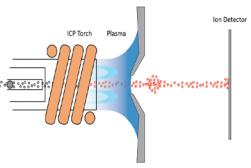
**SKIN PROTECTION:** Wear protective clothing and gloves.

**VENTILATION:** None.

**RESPIRATOR:** Respiratory protection not required. For nuisance exposures use type OV/AG (US) or type ABEK (EU EN 14387) respirator cartridges. Use respirators and components tested and approved under appropriate government standards such as NIOSH (US) or CEN (EU).

**ADDITIONAL PROTECTION:** No additional protection required.

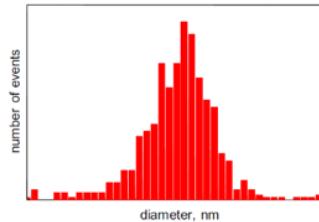
---



ICP-MS based  
techniques



## Single particle (SP-ICP-MS)

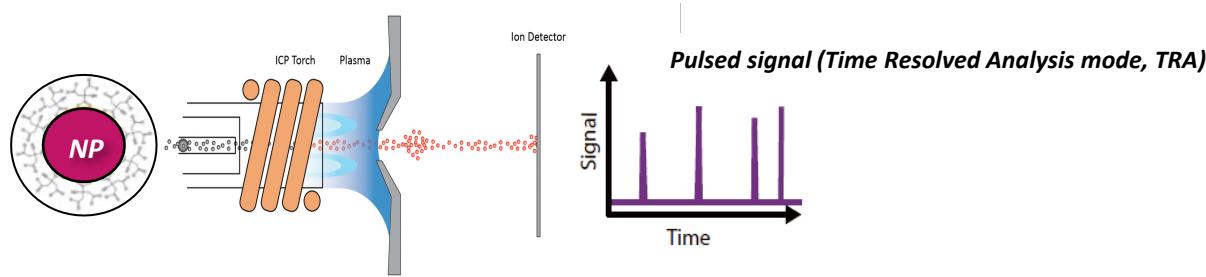


## Hyphenated techniques (AF4/HPLC-ICP-MS)



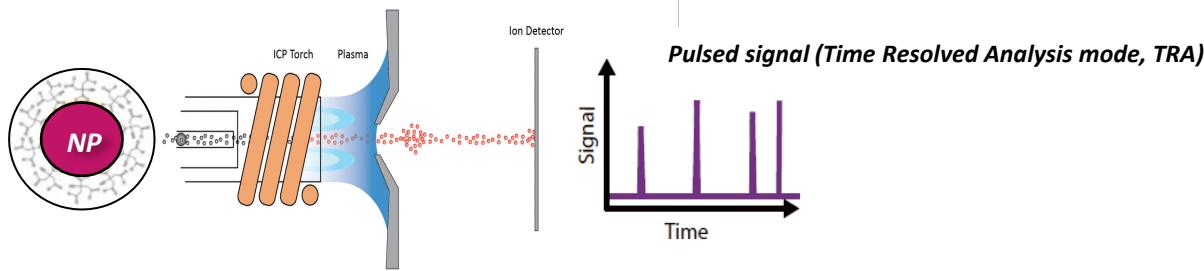
# Single Particle-ICP-MS

## Sizing and counting



# Single Particle-ICP-MS

## *Sizing and counting*



## Analytical information

*NP identification*

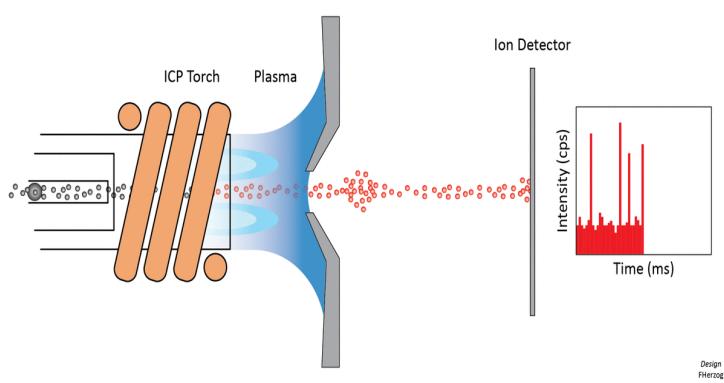
*NP core size*

*Size distribution*

*Particle number concentration*

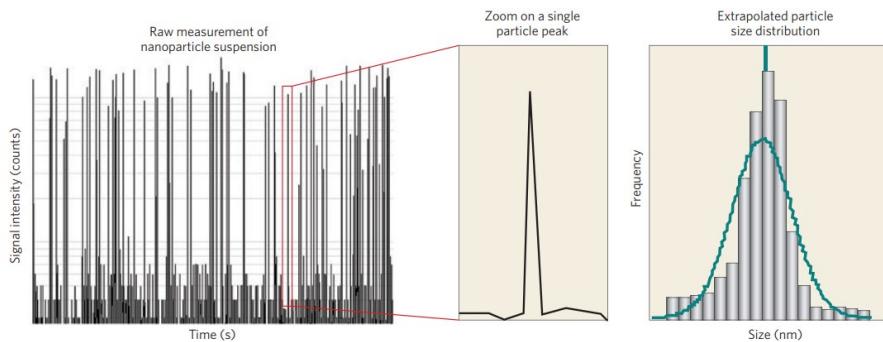
*Particle mass-based concentration*

*Mass concentration of ionic forms*



## Data acquisition

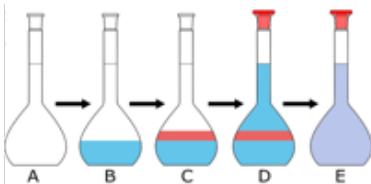
- Concentration
- Nebulization efficiency
- Dwell time
- Acquisition time
- ...



## Data treatment

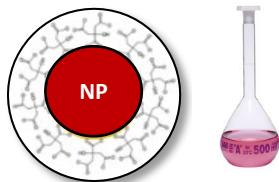
- Mathematical tools

# Single Particle-ICP-MS

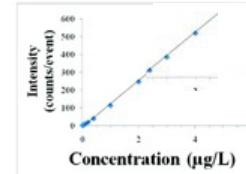


Calibration Curve in Milli-Q water  
(0.05-5  $\mu\text{g L}^{-1}$ )

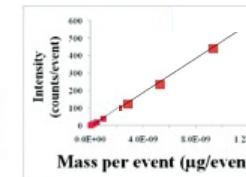
- Transport efficiency ( $\eta_n$ )**
- Sample flow rate ( $q_{liq}$ )**
- Dwell time ( $t_{dt}$ )**
- ICP-MS response factor**



Corresponding  
Dissolved Metal Standards

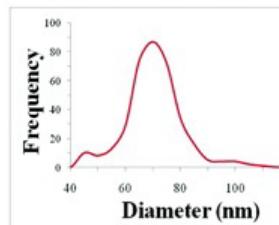


$$W = [\eta_n * q_{liq} * t_{dt} * C] \downarrow$$

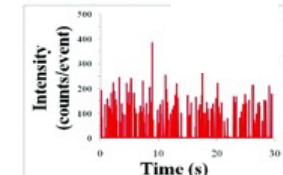


$$d = \sqrt[3]{\frac{6 * m_p}{\rho * \pi}} \downarrow$$

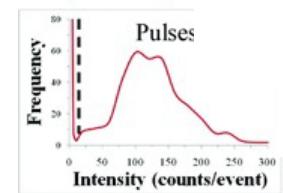
Particle size distribution



Unknown  
Nanoparticle Sample



Background  
↓



$$N_p = \frac{f(I_p)}{q_{liq} * \eta_n} \downarrow$$

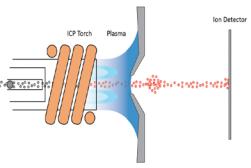
Particle number  
concentration

$$N_p \text{ (particles/volume)}$$

# SP-ICP-MS: Data treatment



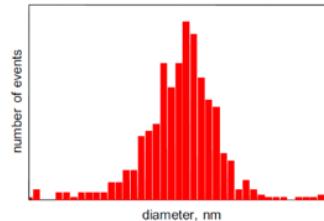
Not standardized



ICP-MS based  
techniques



## Single particle (SP-ICP-MS)

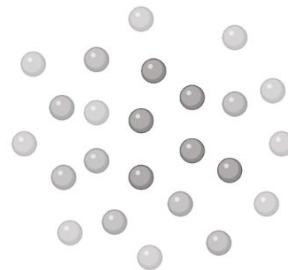


**Complejo Hospitalario de Toledo**



## Hyphenated techniques (AF4/HPLC-ICP-MS)





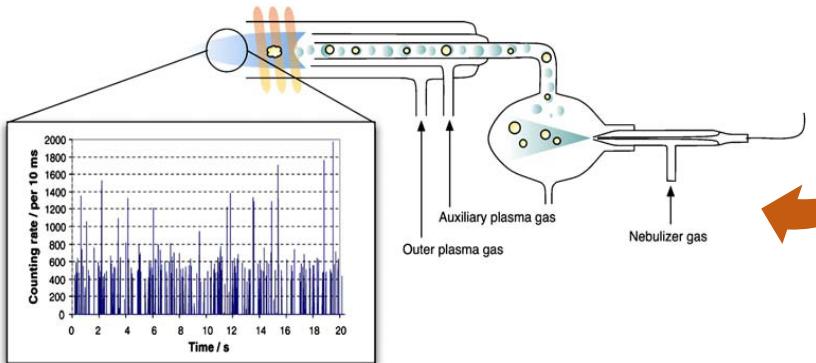
30 & 50 nm PtNPs

Dilution  
and  
Vortex agitation  
(1,200 rpm 30 s)

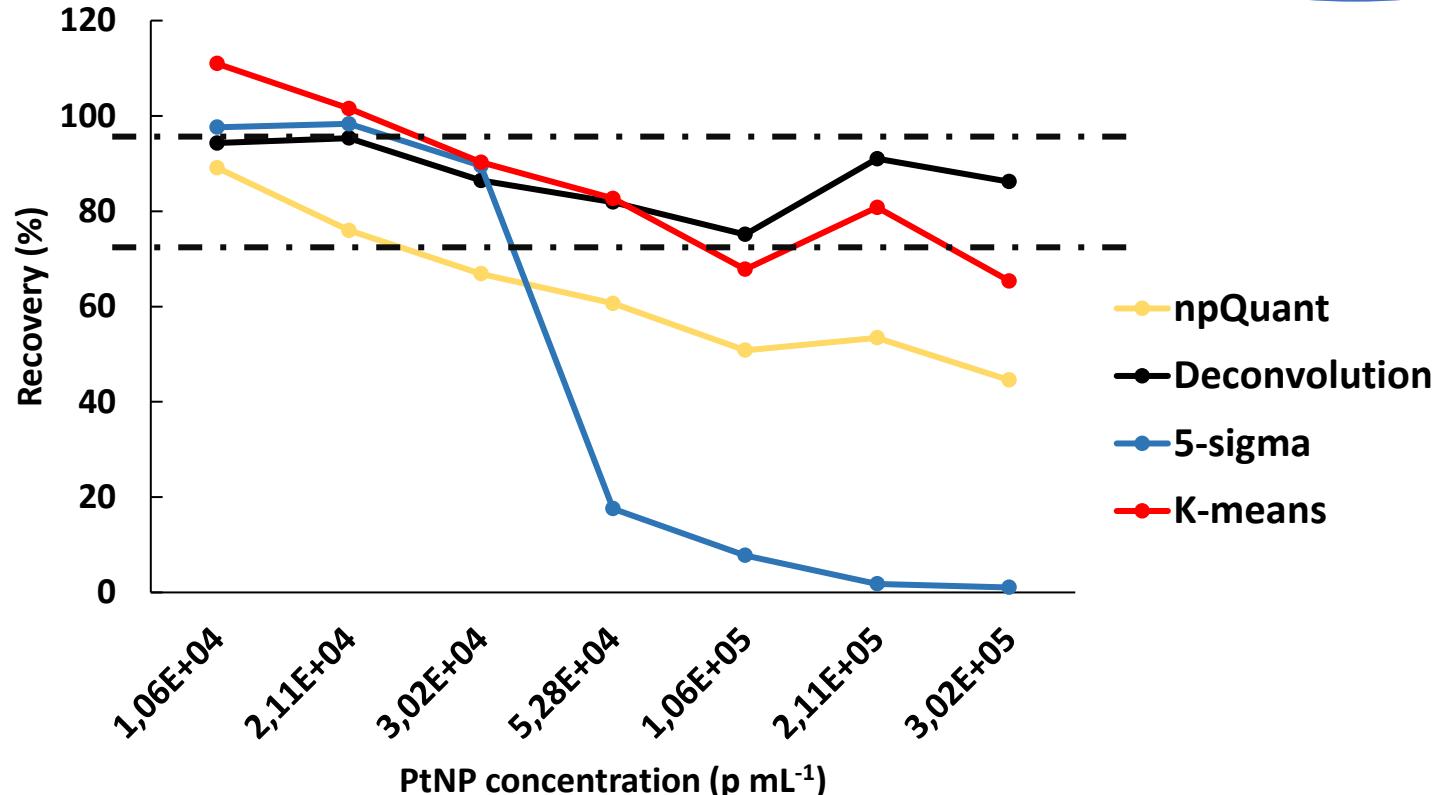
PtNP concentration:  
 $1.1 \times 10^4 - 3.1 \times 10^5 \text{ p mL}^{-1}$

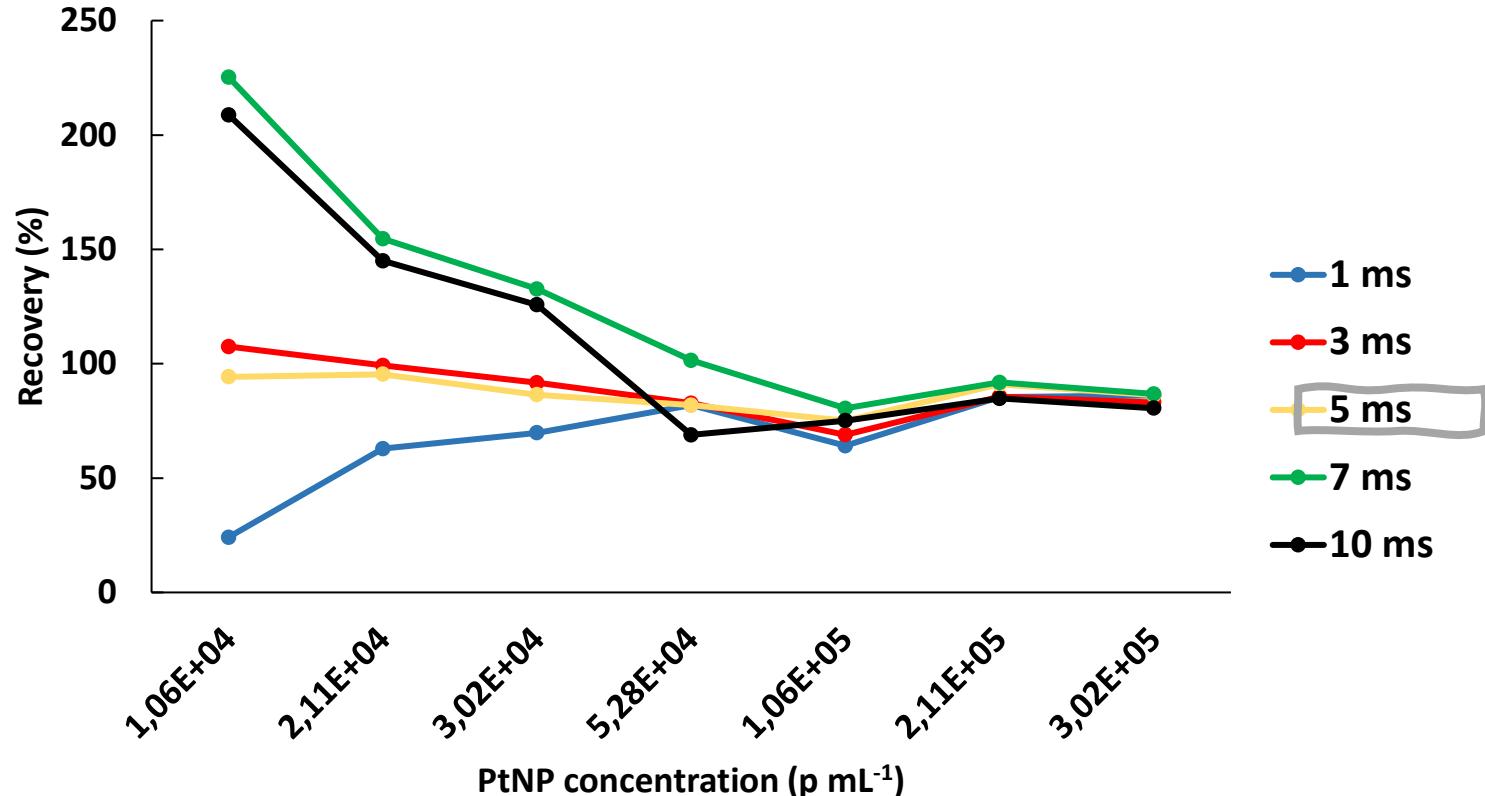
Initial data points:  
**30,000**

Dwell time:  
**5 ms**

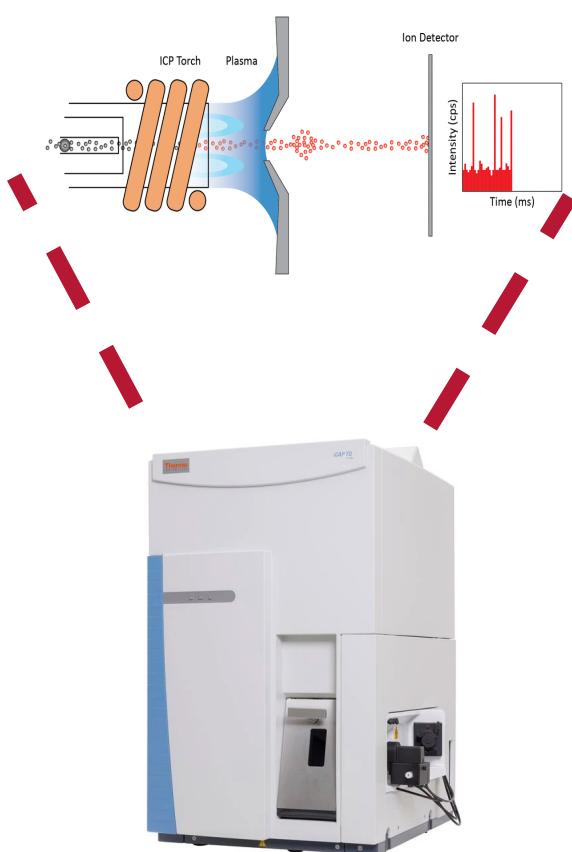


**SP-ICP-MS analysis**





# Optimum conditions



iCAP-TQ-ICP-MS ThermoFisher Scientific

ICP-MS settings	
RF-power	1,500 W
Nebulizer	Meinhard
Spray chamber	Cyclonic
Gas flows (L min <sup>-1</sup> )	
Plasma gas	14
Nebulizer gas	1.0
Auxiliary gas	0.8
Data acquisition	Time Resolved Analysis
Mode	SQ-KED
He flow rate (mL min <sup>-1</sup> )	4.9
Ion monitoring	<sup>195</sup> Pt, <sup>197</sup> Au
Analyte mass (uma)	194.96
Density (g cm <sup>-1</sup> )	21.45
Transport efficiency (%)	9 -12
Sample flow rate (mL min <sup>-1</sup> )	0.32 - 0.40
Dwell time (ms)	5
Data points	40,000

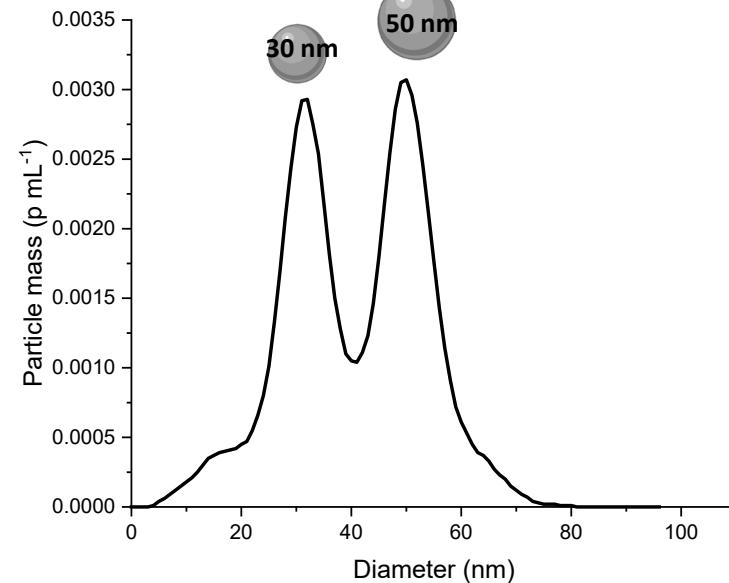
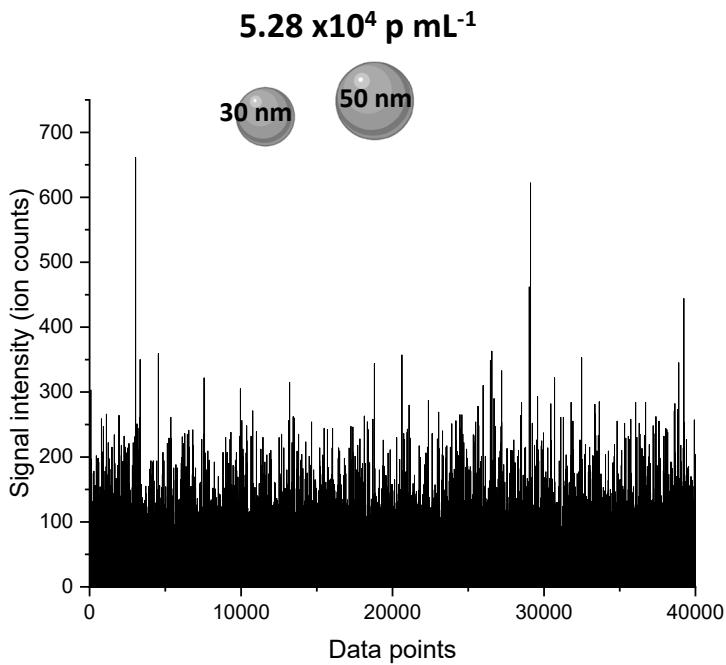
## Data processing: Deconvolution

# Analytical performance

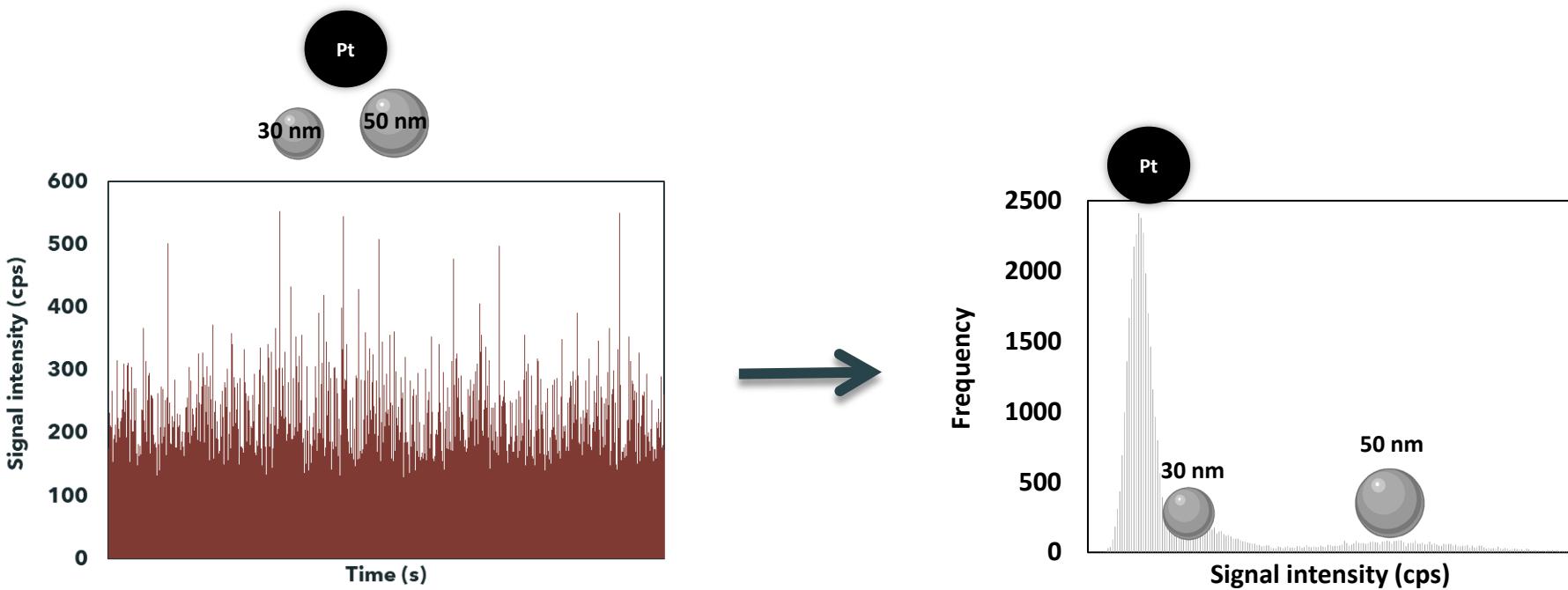
	Particle concentration	Size
Trueness (%)	$94 \pm 8$	$100 \pm 7$
RSD <sub>intra</sub> (%)	1.8	0.7
RSD <sub>inter</sub> (%)	1.9	0.9
LOD*	$0.029 \text{ ng L}^{-1}$	13.78 nm

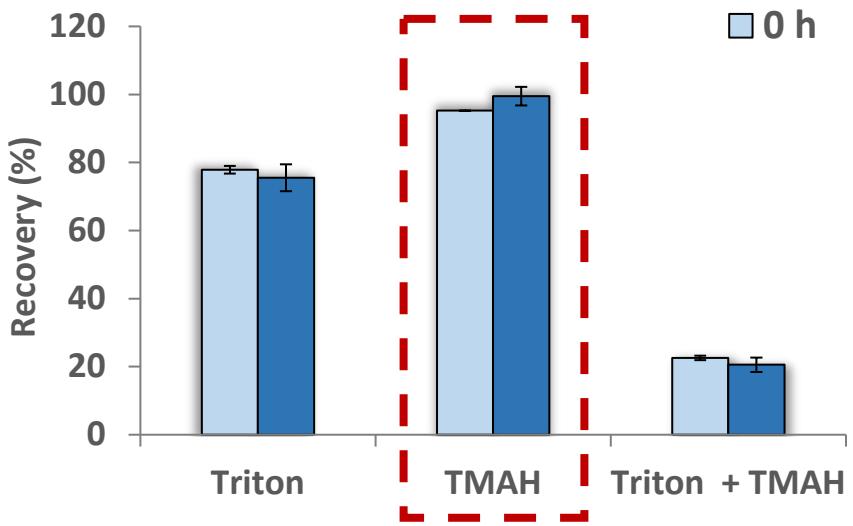
\*Determined using the calculation tool developed by Laborda *et al.*, Spectrochim. Acta Part B, 2020

# Discrimination capabilities



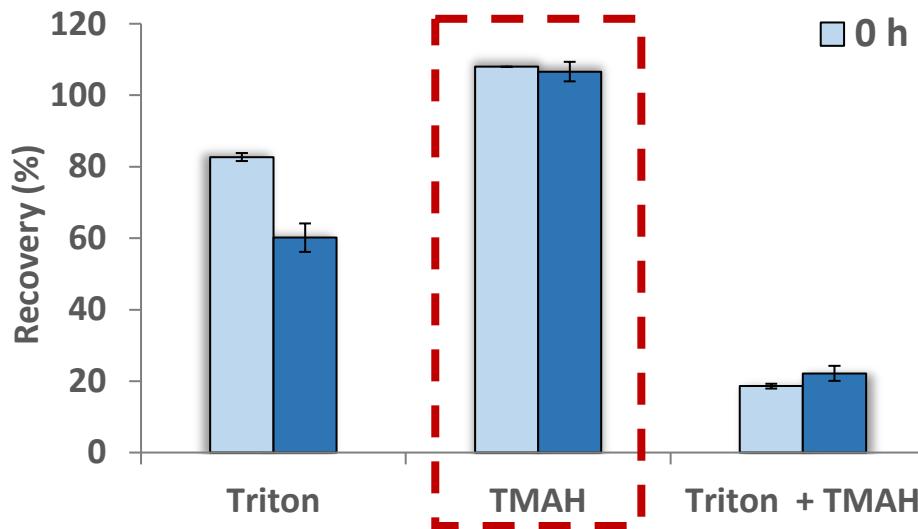
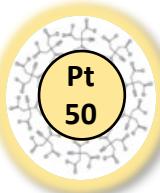
# Discrimination capabilities





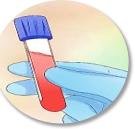
TMAH: Tetramethylammonium hydroxide

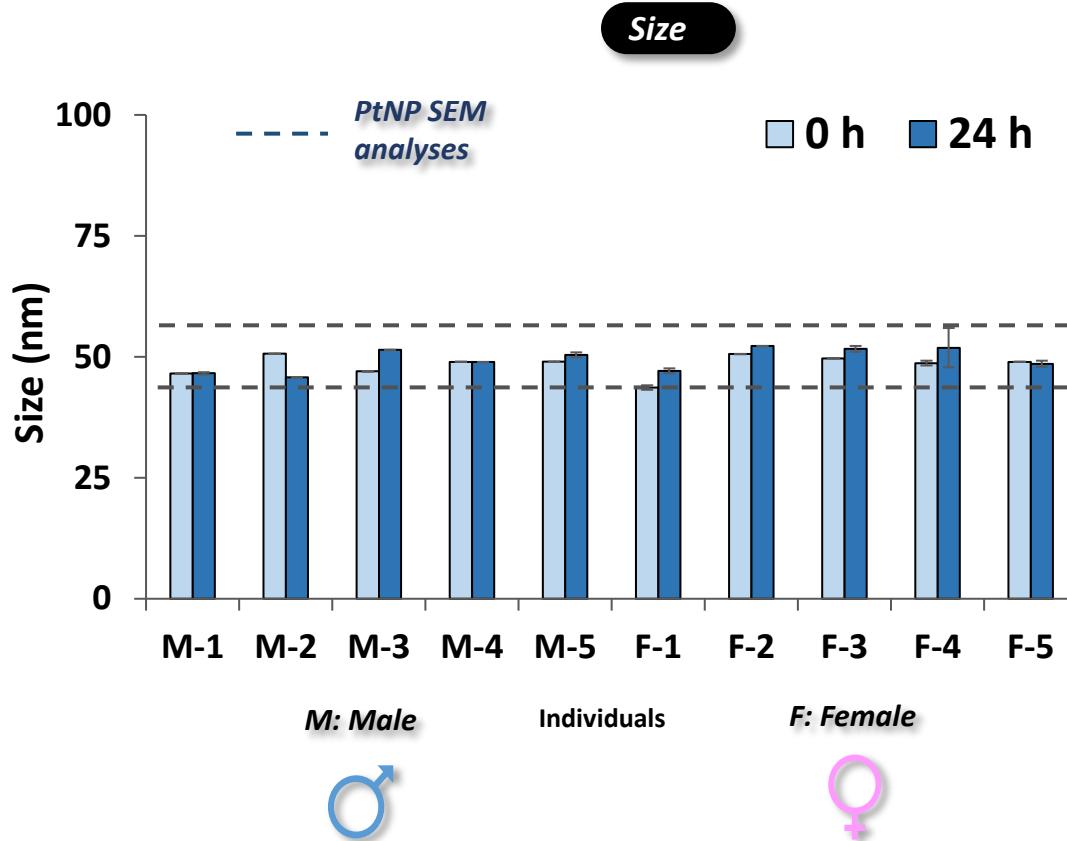
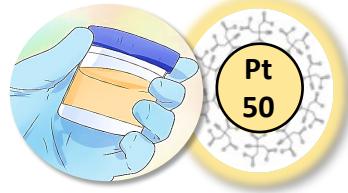
*Particle mass concentration*

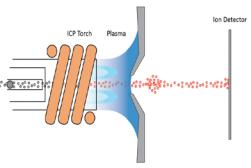


## Recoveries (%) at 0 and 24 h incubation time

*Particle mass concentration*

TMAH CONCENTRATION	1 %		2.5 %		5 %	
	0 h	24 h	0 h	24 h	0 h	24 h
 Pt 50	95.7 ± 0.6	99.3 ± 0.4	77 ± 1	72.9 ± 0.6	94 ± 1	100 ± 6
	101 ± 2	103 ± 2	105 ± 1	94 ± 1	87.3 ± 0.1	99 ± 2
 Pt 50	93.3 ± 0.3	101 ± 2	76 ± 2	78 ± 9	82.4 ± 0.3	107 ± 1
	99 ± 4	100 ± 2	68 ± 4	97 ± 4	92 ± 2	86.9 ± 0.3

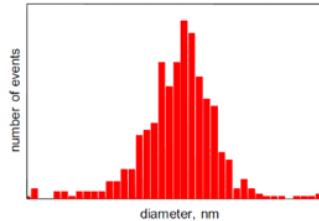




ICP-MS based  
techniques



## Single particle (SP-ICP-MS)



## Hyphenated techniques (AF4/HPLC-ICP-MS)

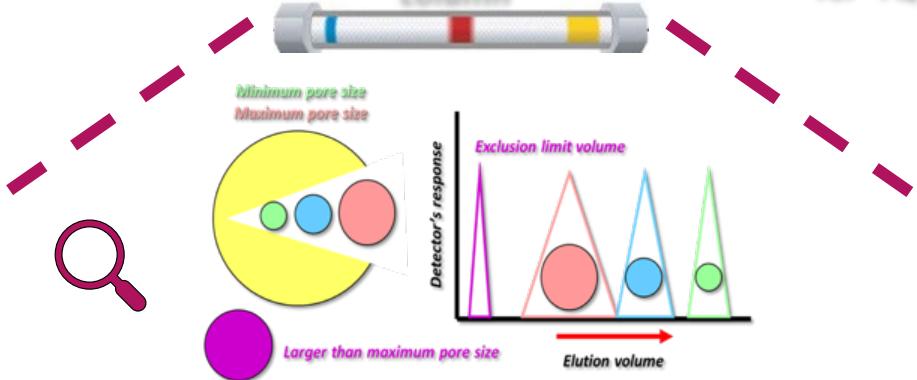


# HPLC-ICP-MS



*Reverse-phase liquid chromatography (RP-HPLC)*

# HPLC-ICP-MS



*Reverse-phase liquid chromatography (RP-HPLC)*

## Analytical information

Characterization    **Size (hydrodynamic diameter)**

*Size distribution*

Quantification    **Mass concentration**

Speciation    **NP + Dissolved species**

# HPLC-ICP-MS

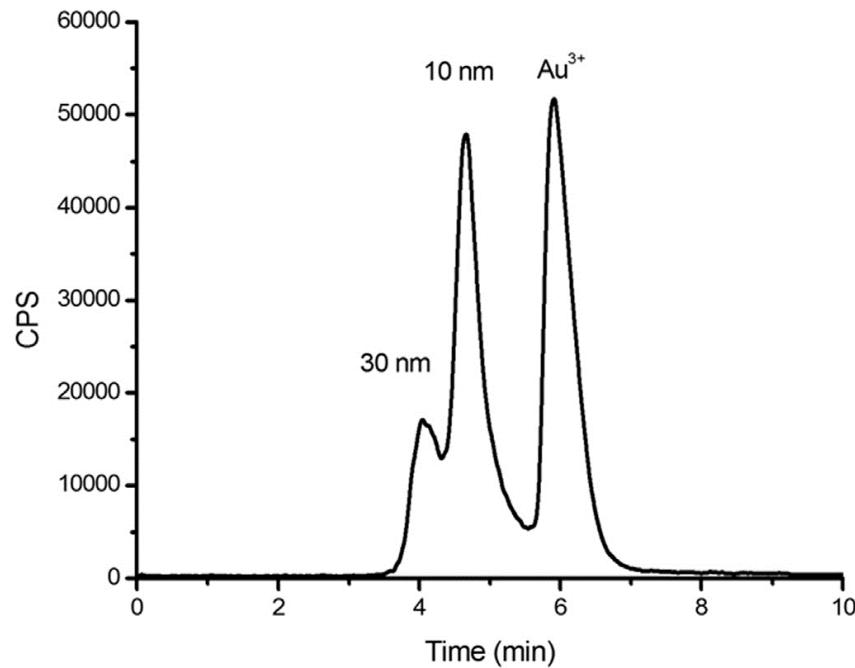


Optimized separation conditions

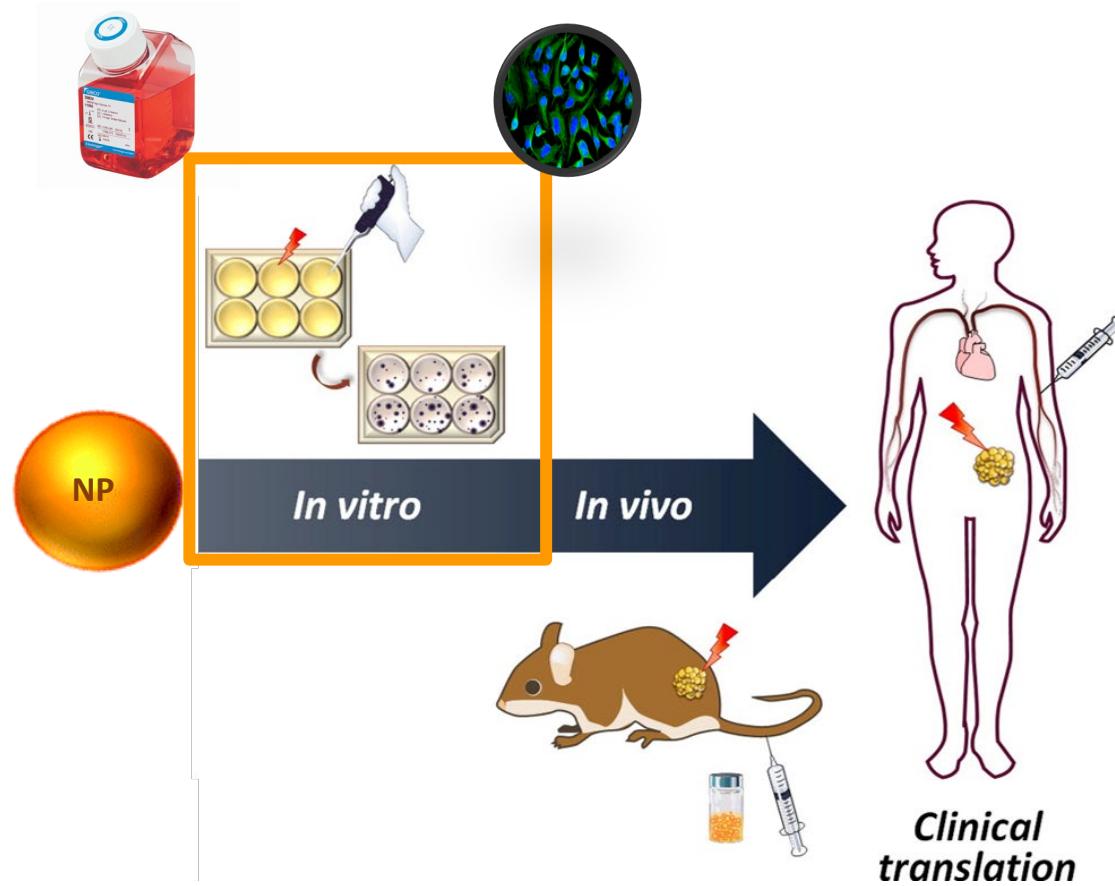
RP-LC	
<b>Mobile phase</b>	<b>10 mM SDS, 1 mM NaH<sub>2</sub>PO<sub>4</sub>, 1 mM Na<sub>2</sub>HPO<sub>4</sub>, pH = 7.3</b>
<b>Column</b>	Nucleosil 7 µm particle size, <b>C18, 1000 Å pore size</b> , 250x4.6 mm
<b>Injection volumen (µL)</b>	200
<b>Flow rate (mL min<sup>-1</sup>)</b>	0.5



# HPLC-ICP-MS



10 nm (0.034 nM) and 30 nm (0.003 nM) AuNPs, and dissolved Au<sup>3+</sup> (0.25 μM)



# DMEM (Dulbecco's Modified Eagle Medium)

## Inorganic Salts (g/liter)

CaCl <sub>2</sub> (anhydrous)	0.20000
Fe(NO <sub>3</sub> ) <sub>3</sub> ·9H <sub>2</sub> O	0.00010
MgSO <sub>4</sub> (anhydrous)	0.09770
KCl	0.40000
NaHCO <sub>3</sub>	1.50000
NaCl	6.40000
NaH <sub>2</sub> PO <sub>4</sub> ·H <sub>2</sub> O	0.12500

## Amino Acids (g/liter)

L-Arginine-HCl	0.08400
L-Cystine·2HCl	0.06260
L-Glutamine	0.58400
Glycine	0.03000
L-Histidine-HCl·H <sub>2</sub> O	0.04200
L-Isoleucine	0.10500
L-Leucine	0.10500
L-Lysine-HCl	0.14600
L-Methionine	0.03000
L-Phenylalanine	0.06600
L-Serine	0.04200
L-Threonine	0.09500
L-Tryptophan	0.01600
L-Tyrosine·2Na·2H <sub>2</sub> O	0.10379
L-Valine	0.09400

## Vitamins (g/liter)

Choline Chloride	0.00400
Folic Acid	0.00400
myo-Inositol	0.00720
Nicotinamide	0.00400
D-Pantothenic Acid (hemicalcium)	0.00400
Pyridoxine-HCl	0.00400
Riboflavin	0.00040
Thiamine-HCl	0.00400

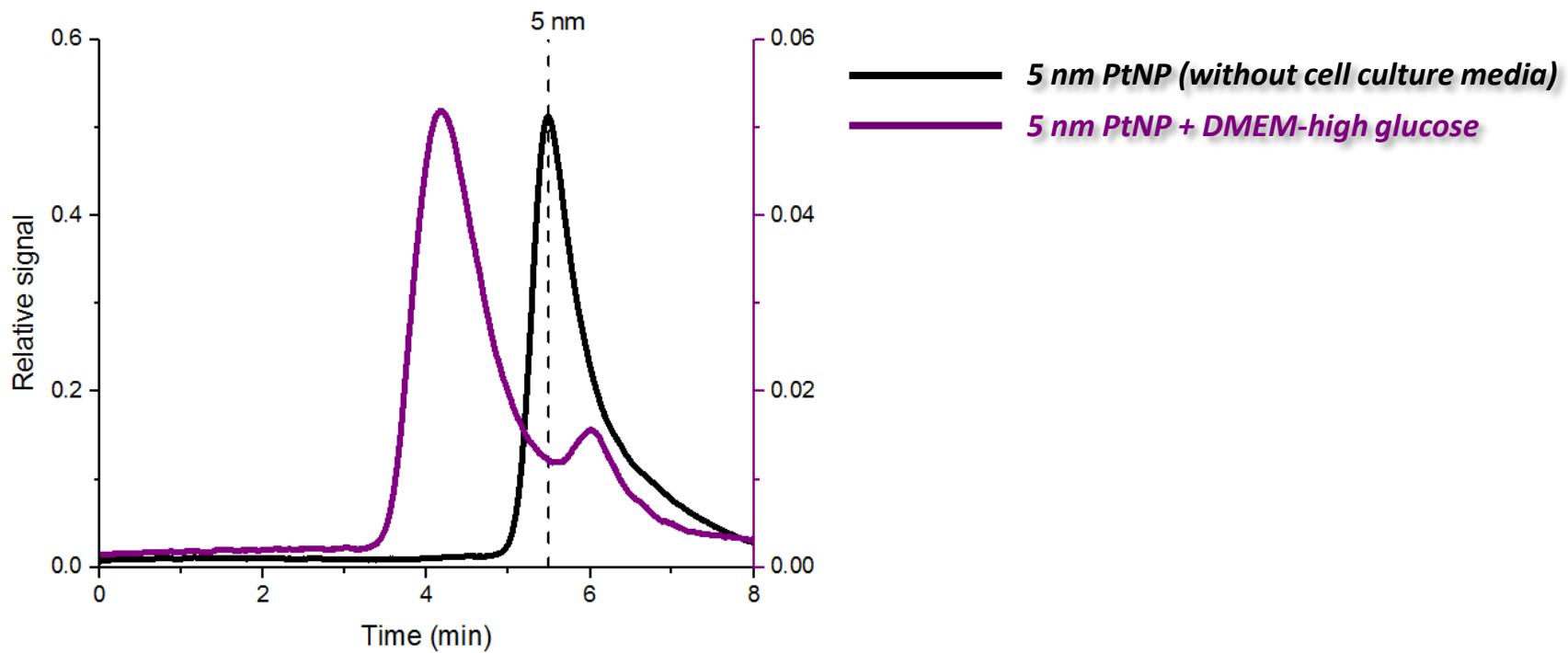
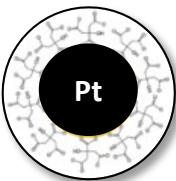
## Other (g/liter)

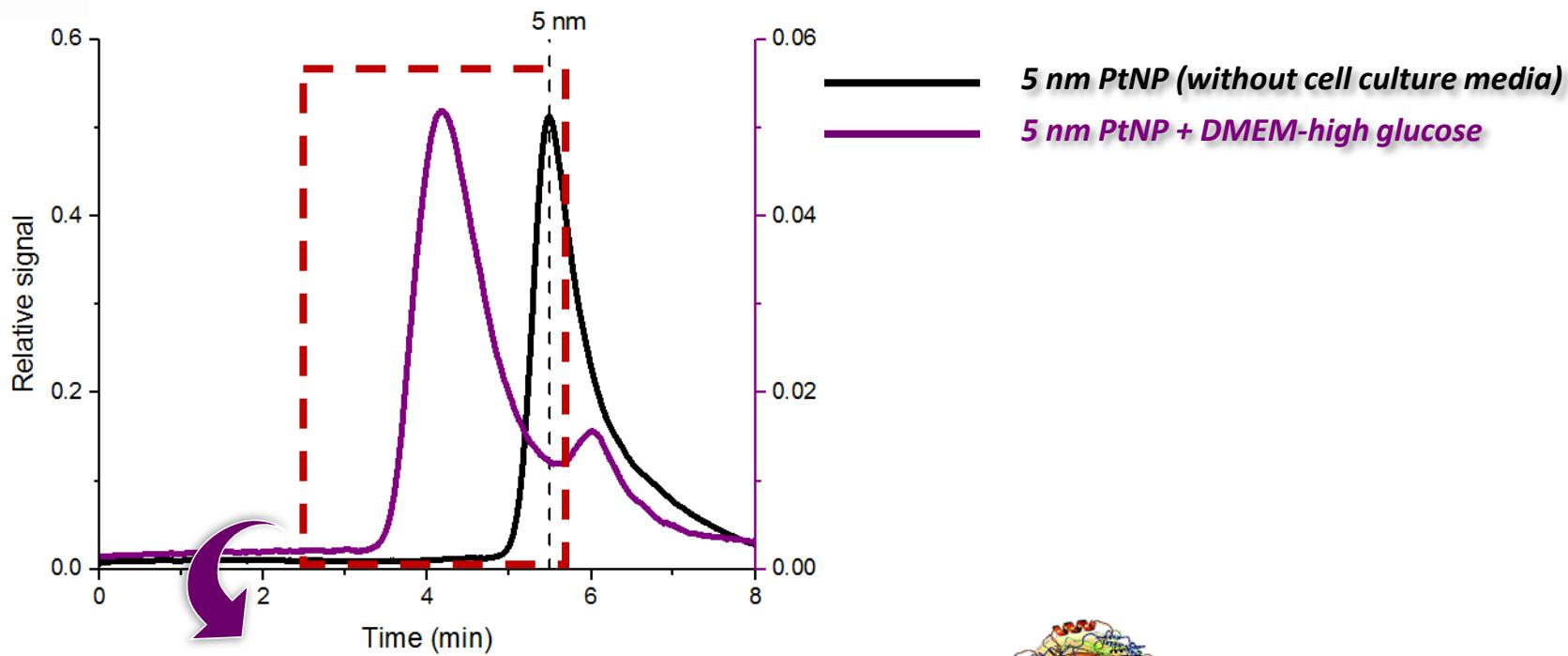
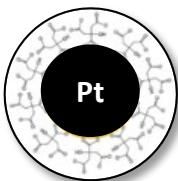
D-Glucose	4.50000
Phenol Red, Sodium Salt	0.01500
Sodium Pyruvate	0.11000



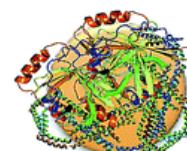
## Supplements:

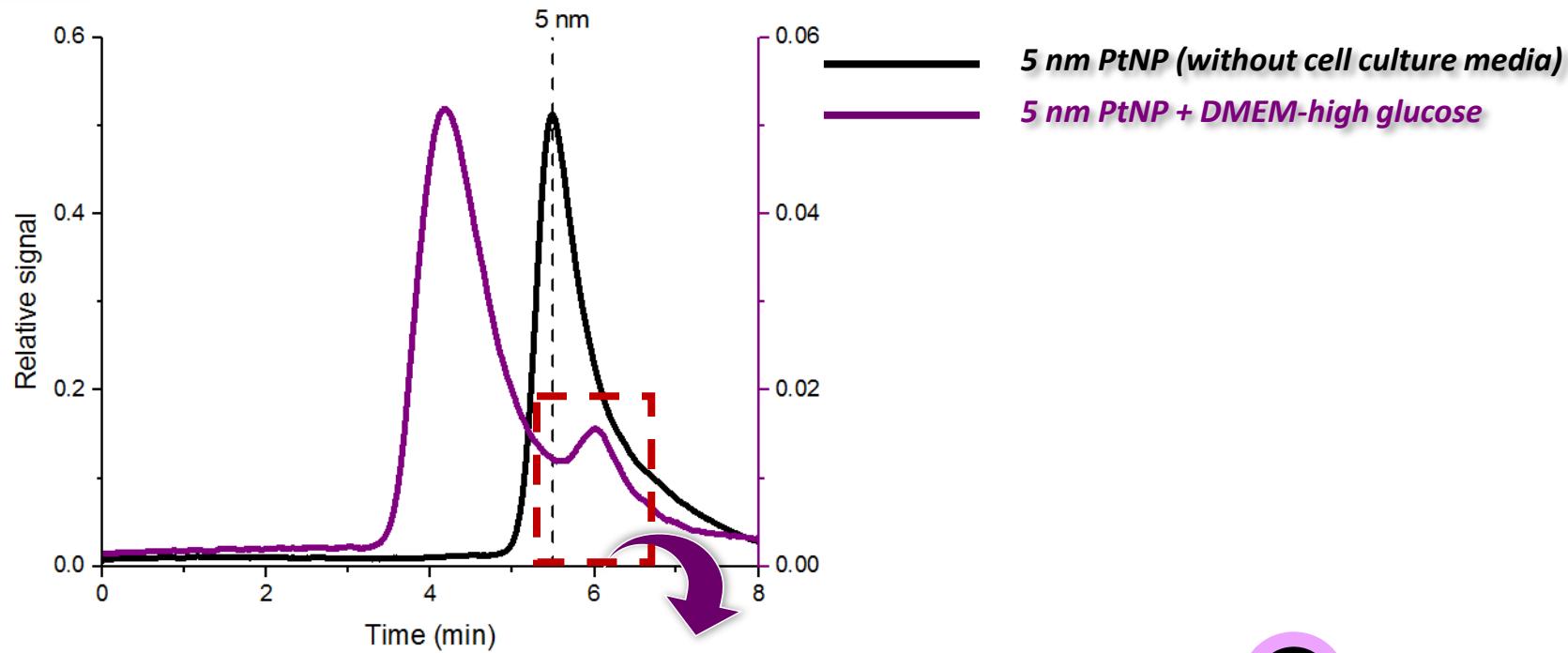
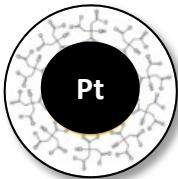
- Fetal bovine serum (FBS) (10 %)
- Penicillin
- Streptomycin





*Why do the retention time and relative signal decrease?*

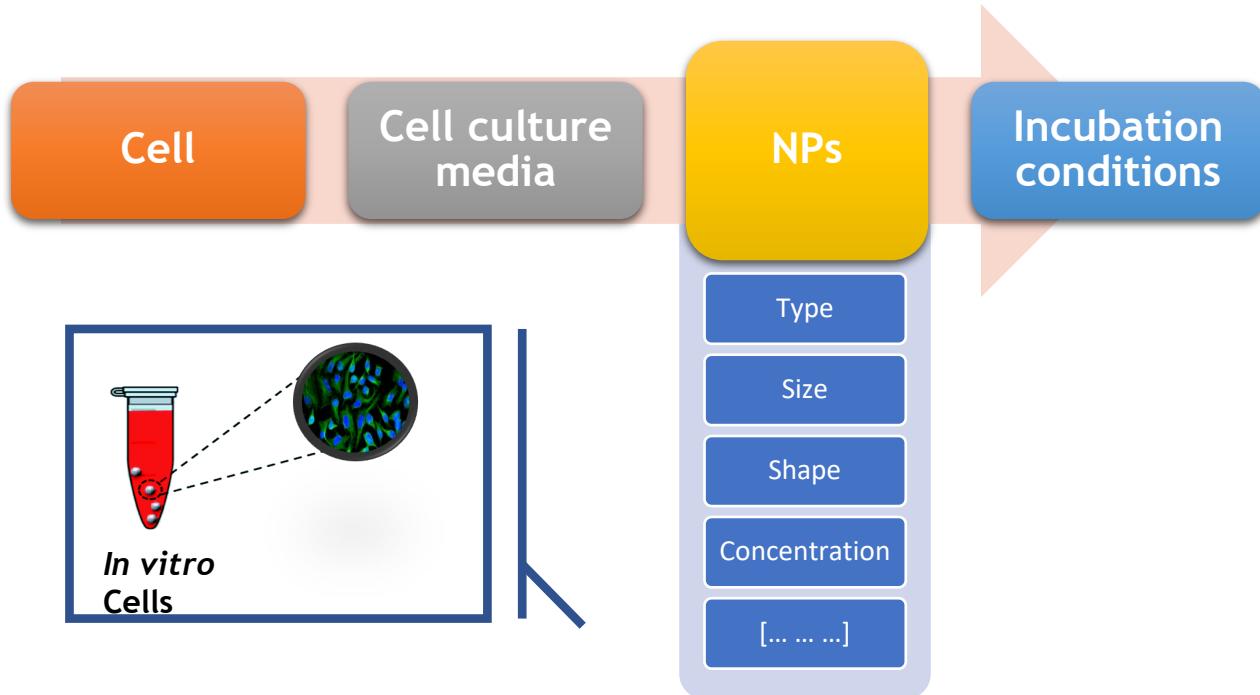




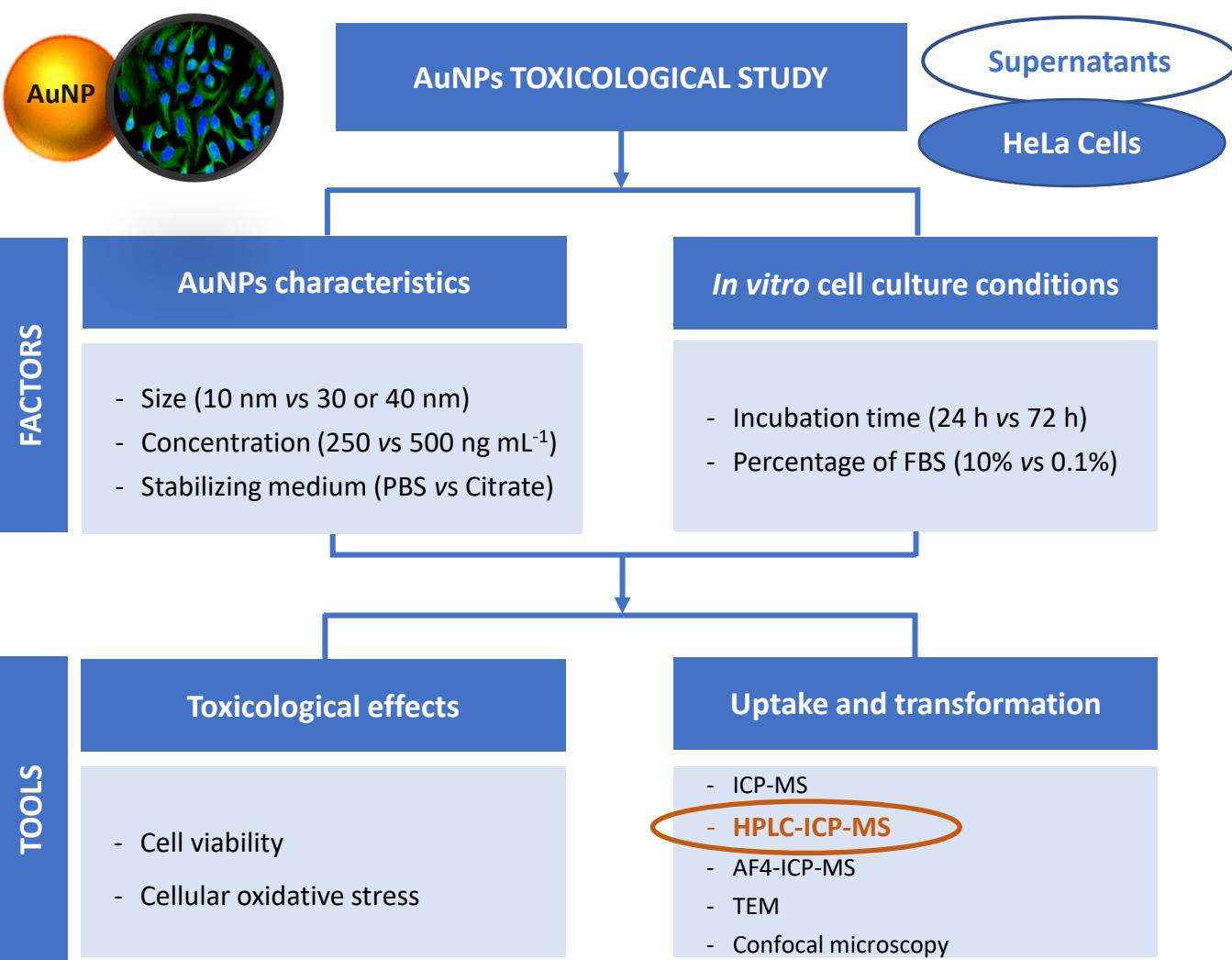
*Why does a second additional peak appear?*

Pt

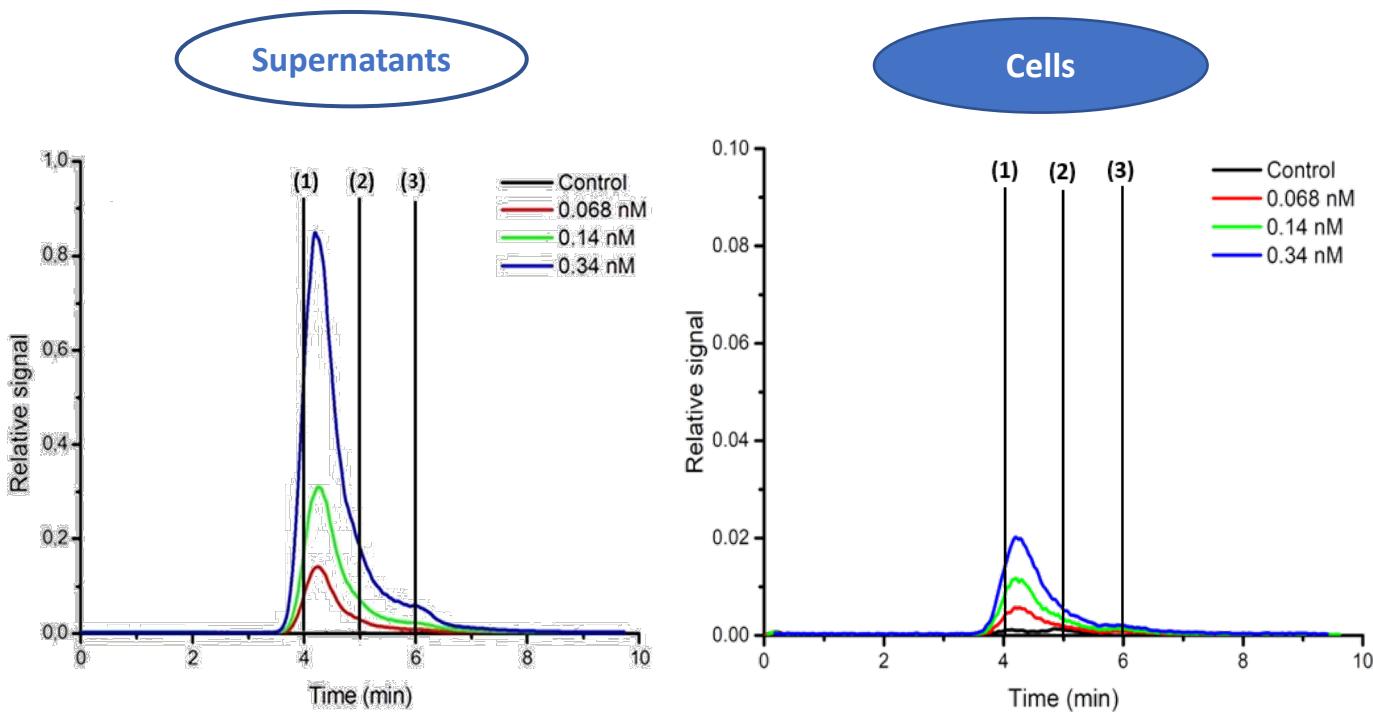
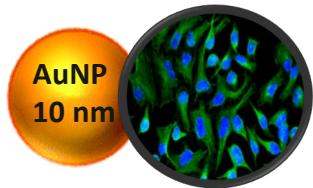
# Toxicological studies

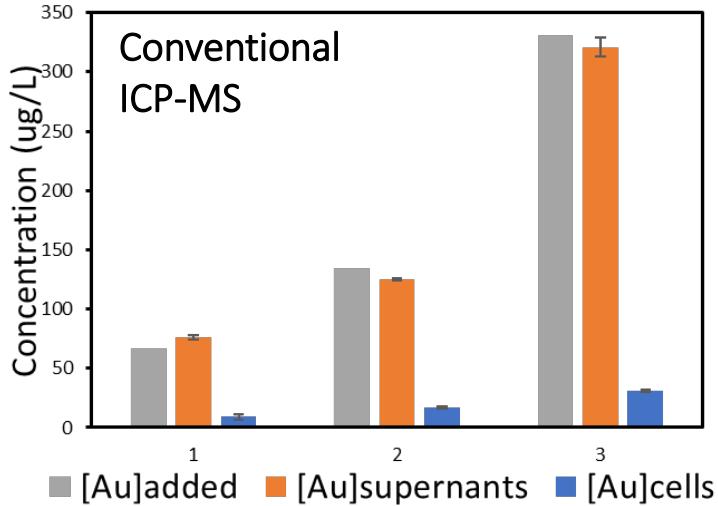


- ✓ Collaboration with the research group of biochemistry-UCLM (Dr. Rosario Serrano)



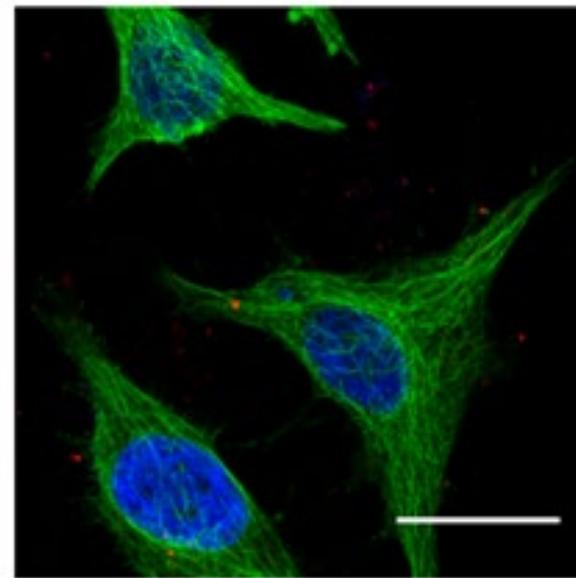
# HPLC-ICP-MS



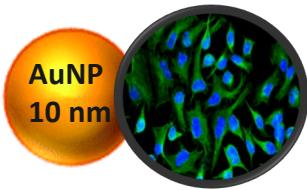


Confocal  
microscopy

Nuclei: blue  
Cytoskeletons: green  
AuNPs: red

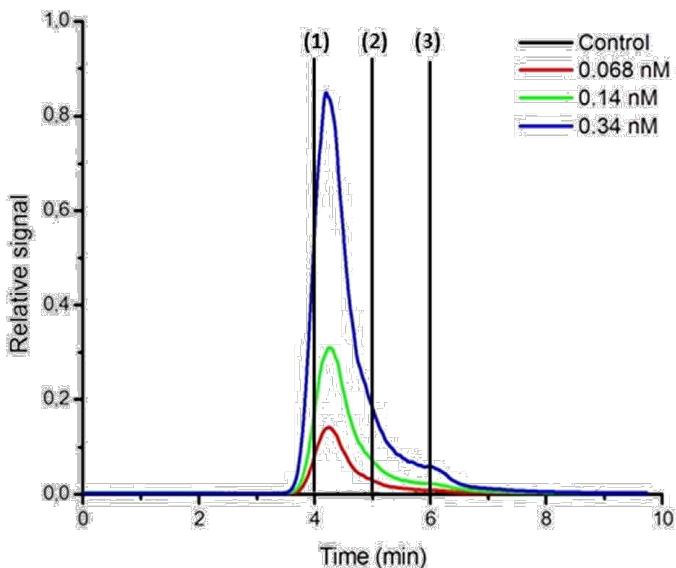


# HPLC-ICP-MS

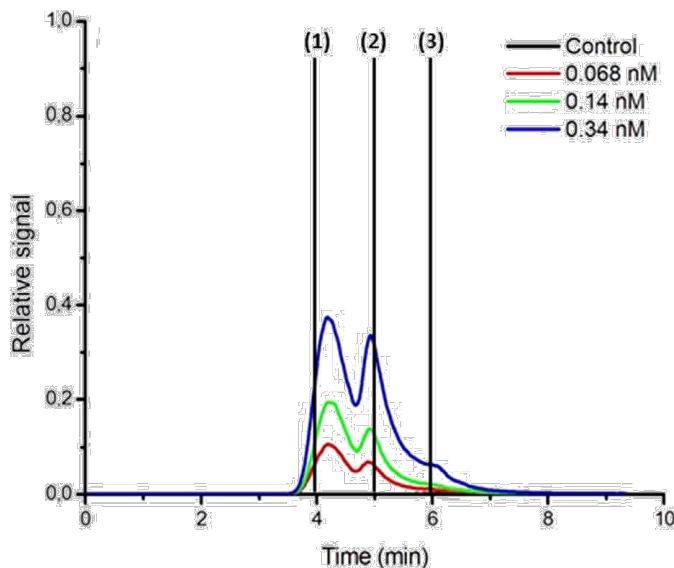


Supernatants

24 h



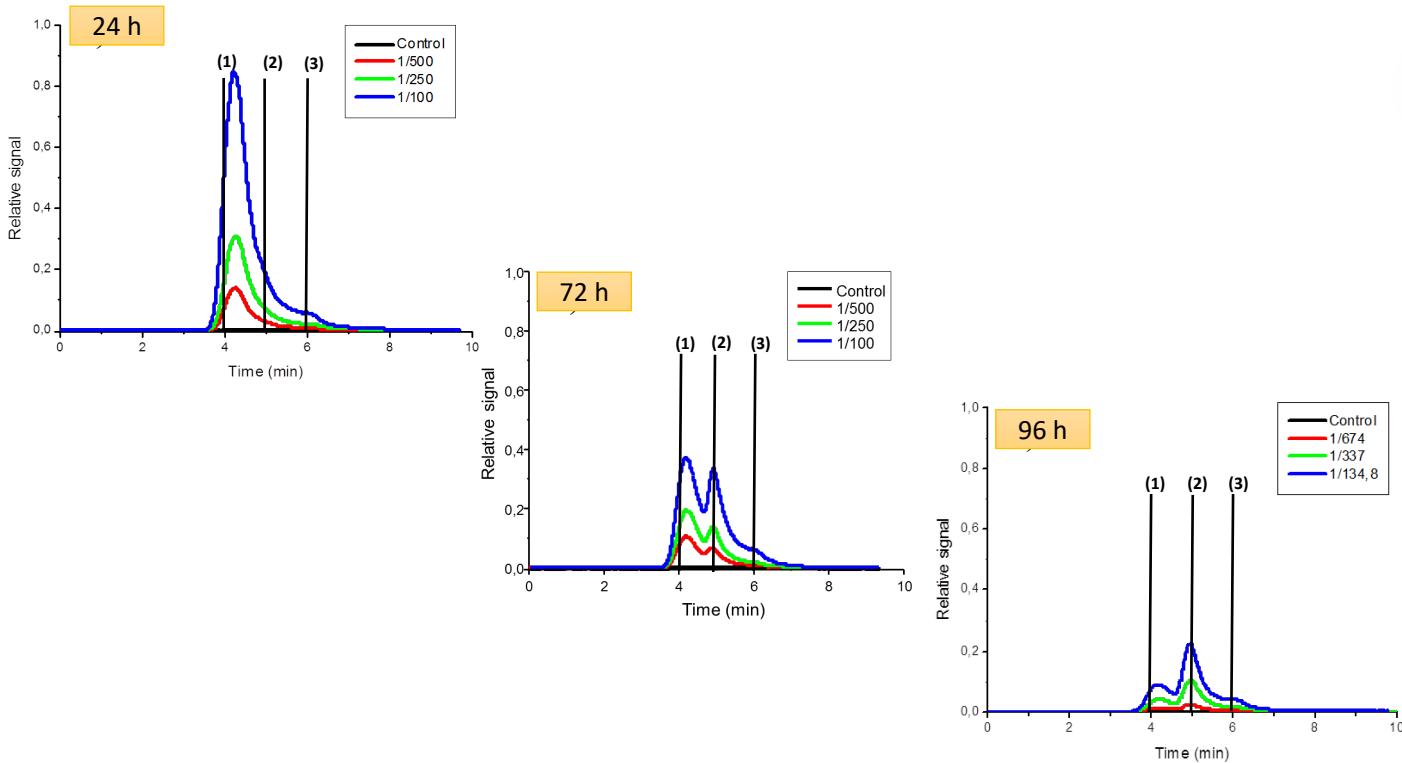
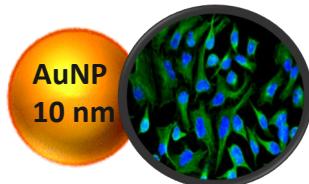
72 h



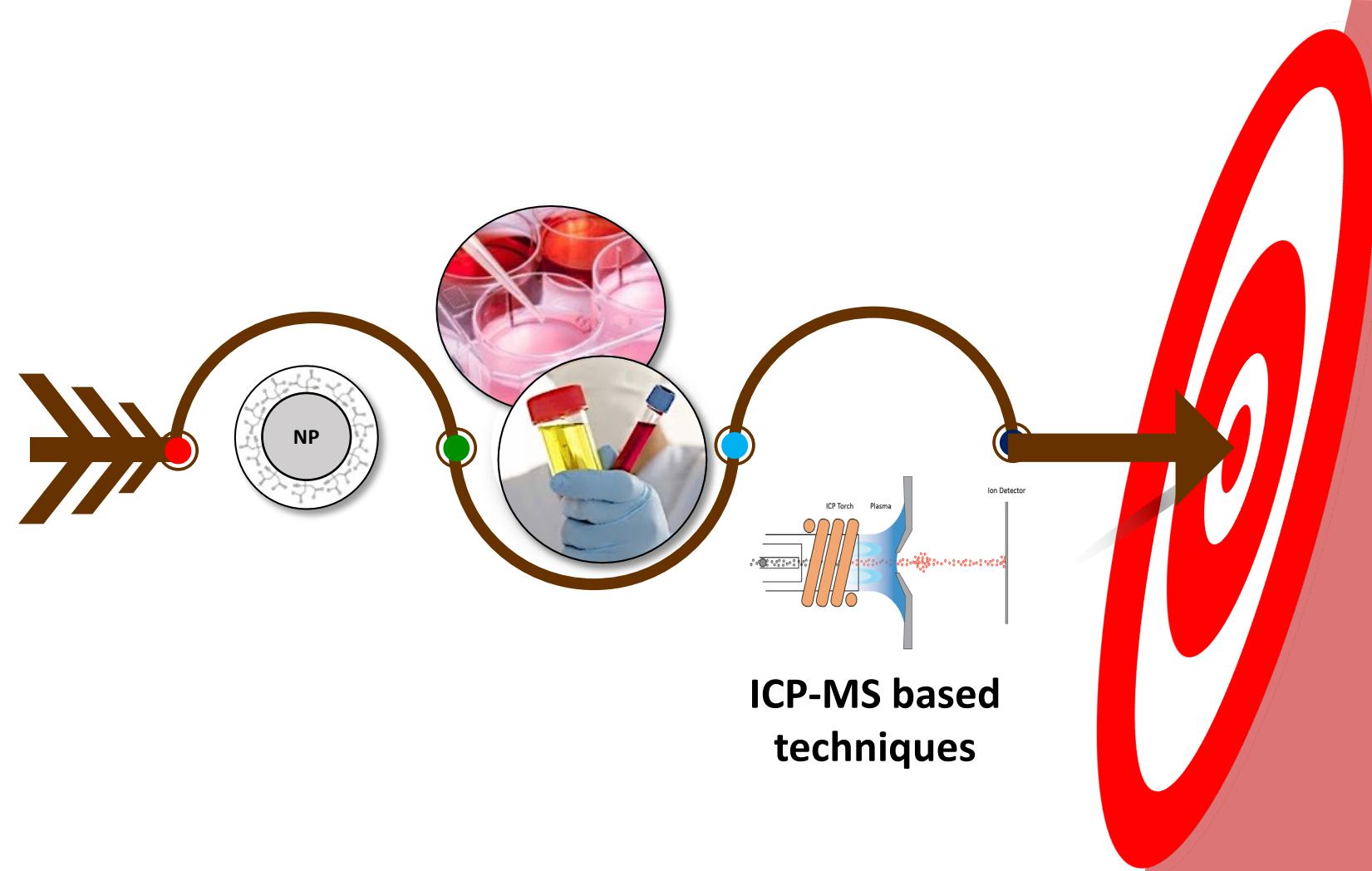
(1) 10 nm AuNPs in DMEM; (2)  $\text{Au}^{3+}$  in DMEM; (3)  $\text{Au}^{3+}$  in mobile phase

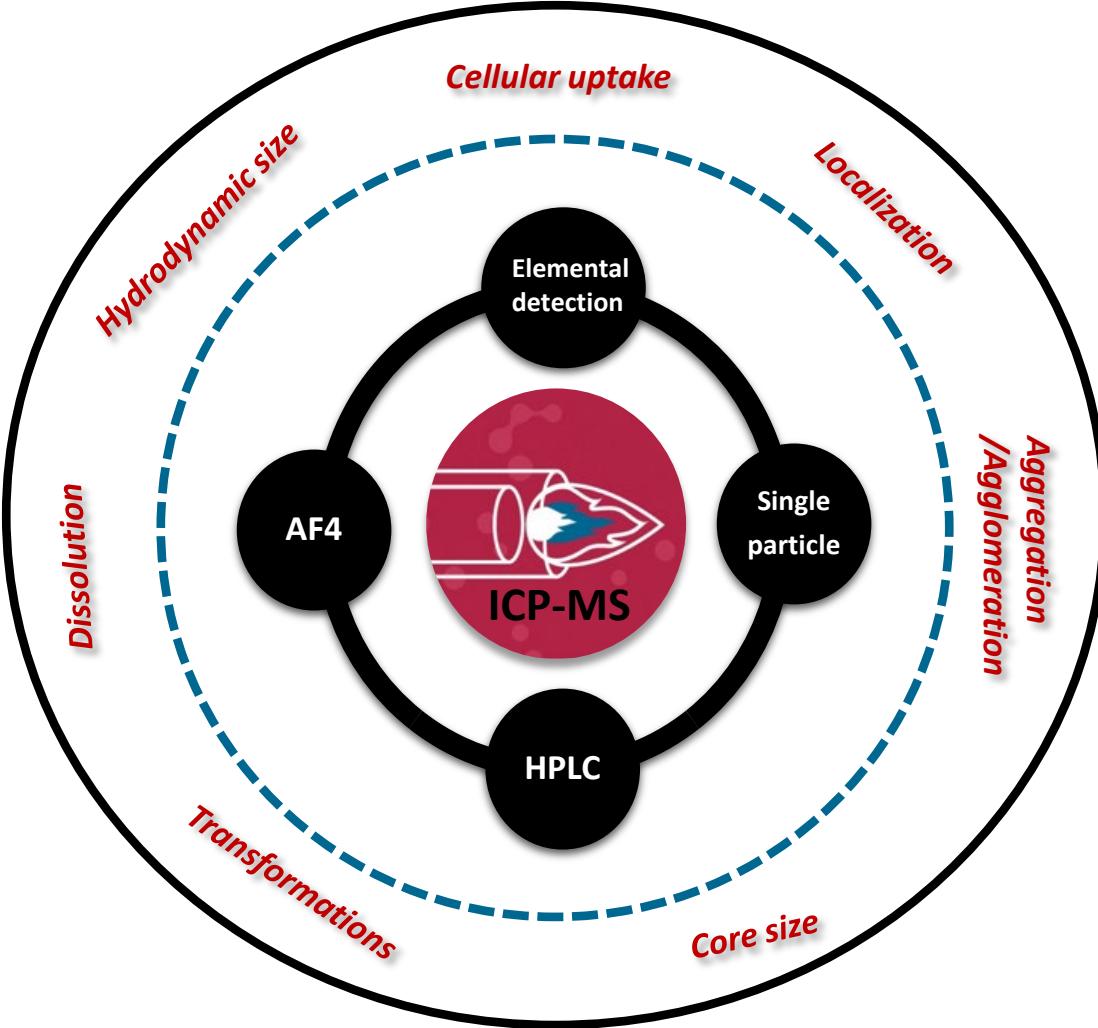
Supernatants

# HPLC-ICP-MS

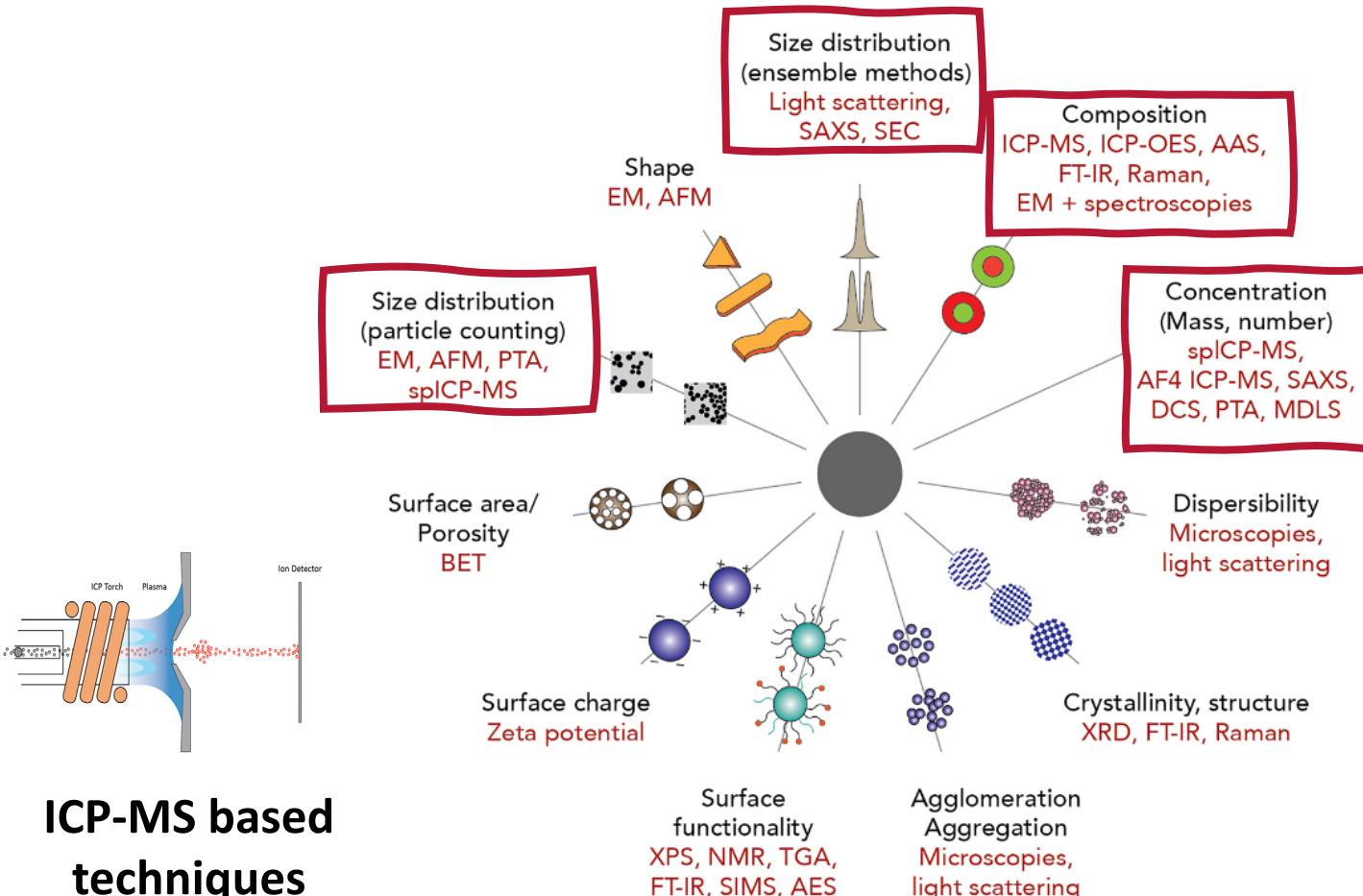


(1) 10 nm AuNPs in DMEM; (2)  $\text{Au}^{3+}$  in DMEM; (3)  $\text{Au}^{3+}$  in mobile phase





# Characterization



## ICP-MS based techniques



↑ *Number of NMs*

↑ *Diversity of NMs*

*Transformations*

*Characterization/Quantification  
(chemical/physical parameters)*

*Need for appropriate/validated analytical  
methods/techniques*

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YES

NO

# Gracias



<http://saman.uclm.es/>

## Acknowledgement for financial support:



Junta de Comunidades de  
**Castilla-La Mancha**

