New approaches for studying nanomaterials in food: focus on field-flow fractionation and spICP-MS

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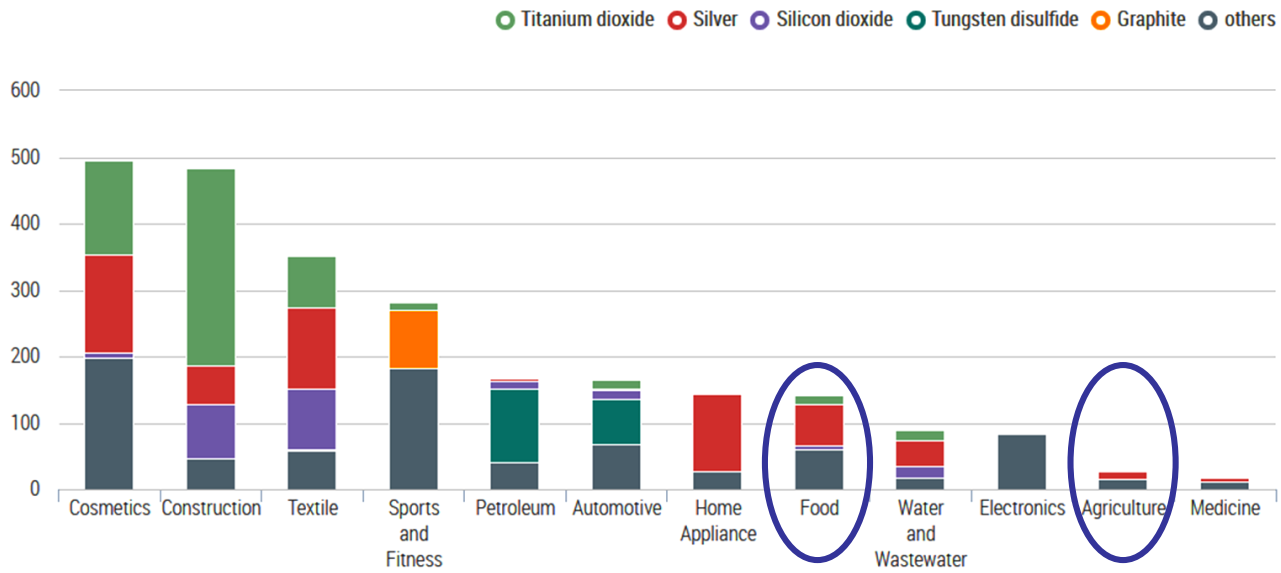
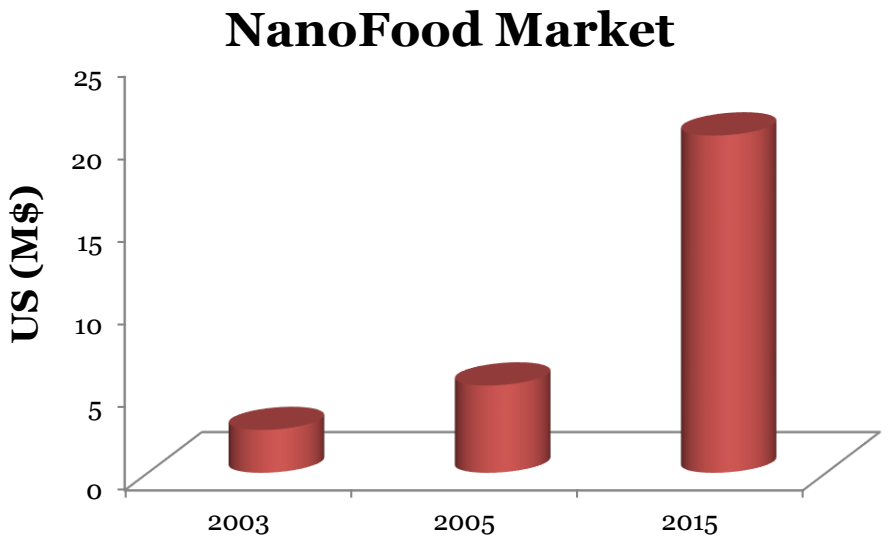
Nanomaterials in the food sector: a growing market





Peters et al. 2016, NanoStat

Nanomaterials in the food sector: a growing market

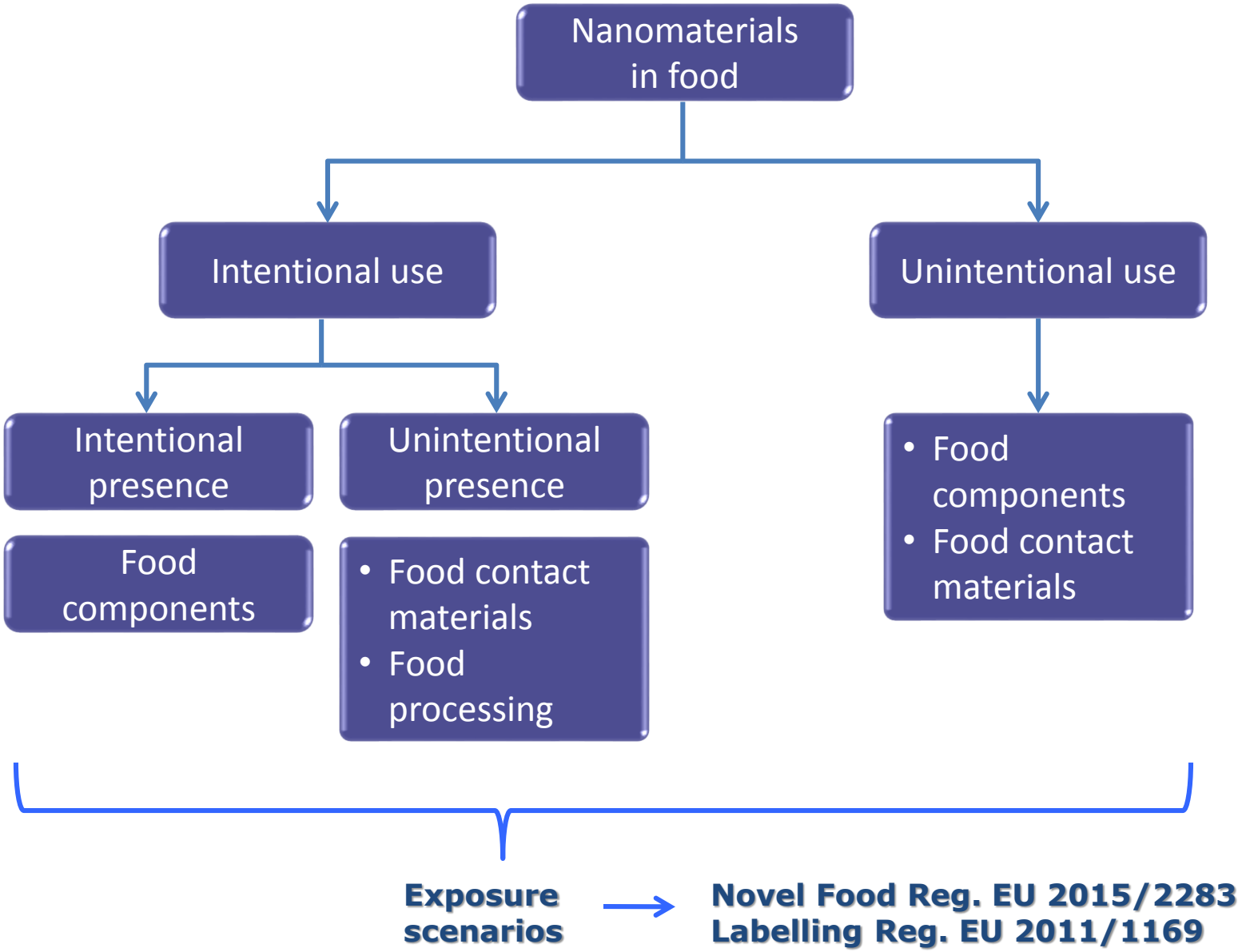
Applications

- Nano-sized or nano-incapsulated additives
- Nanostructured ingredients
- Nano-improved food contact materials
- Nanotechnology-based devices (e.g. nano-filtration)
- Nanosensors

Advantages

- Increased absorption and bioavailability
- Improved organolectic properties, consistence and aspect
- Antimicrobial action
- Improved processing efficiency and safety
- Treachability of food conditions during transport and storage

Nanomaterials in food



Guidelines for exposure assessment

- Prepared by EFSA scientific committee under request by EC
- Adopted April 6th 2011, published May 10th 2011
- Concerns risk assessment for the following classes of products/applications:
    1. direct consumption (human, cattle)
    2. farming (es. pesticides)
    3. food/feed contact materials
- Provides practical reccomendation for risk assessment in all agroindustrial sectors dealing with the use nanomaterials (included food/feed additives, enzymes, flavours, food contact materials, new foodstuff, pesticides)
- Addresses to those corcerned and risk assessers
- Rational decision process

Decision tree for exposure assessment

Type of nanomaterial application (eg. ingredient, additive, pesticide, food contact material, etc.

Directly added

Presence due to migration/transferring

Quantify migration/transferring

Identify (possibly quantify) engineered nanomaterials or their (non-nano) degradation forms in food, cattle feed.

Identify (possibly quantify) engineered nanomaterials or their (non-nano) degradation forms in food simulants, food, cattle feed.

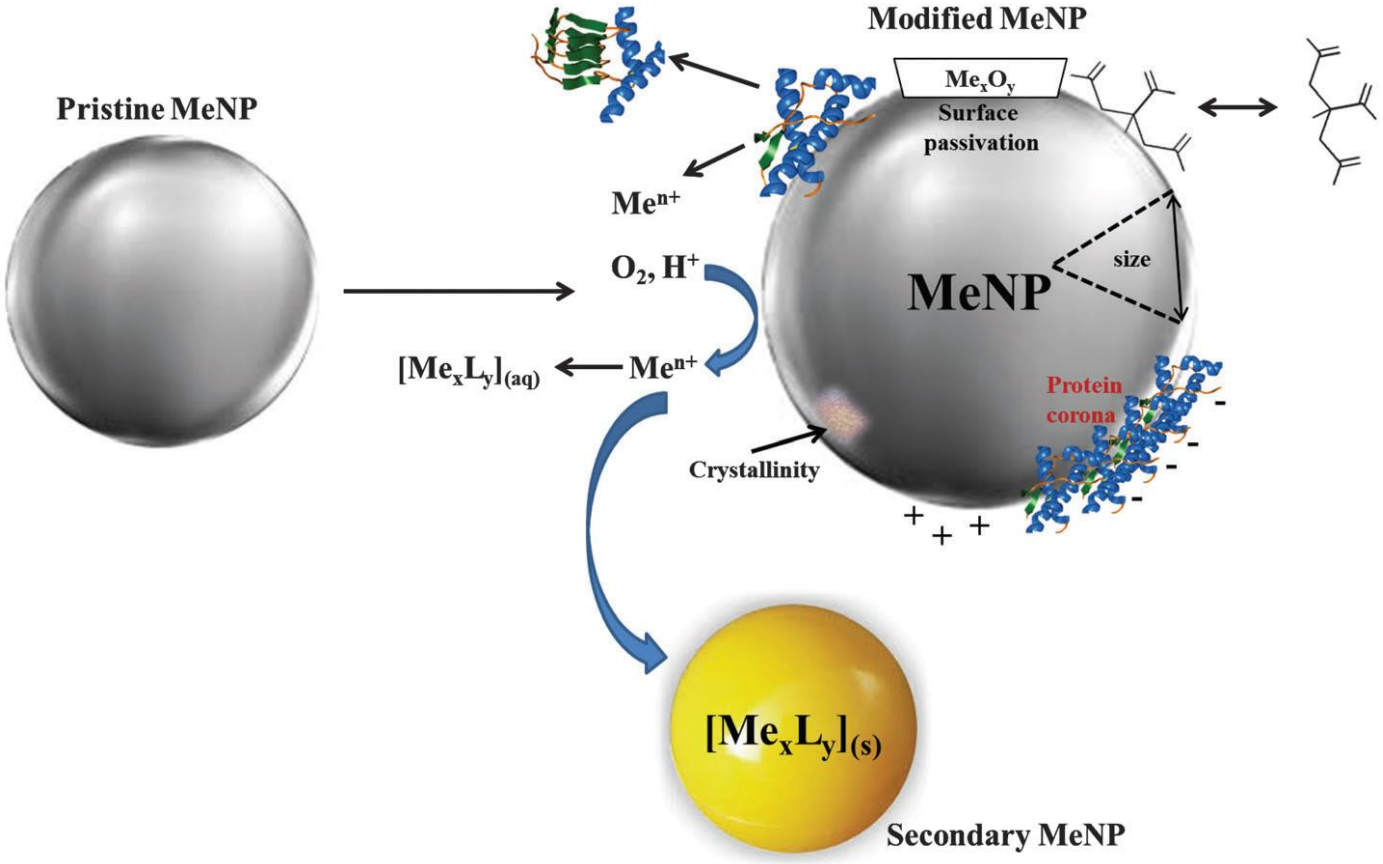
Are there still engineered nanomaterials?

Yes

Nanoforms must be included in the procedure of exposure assessment

No

Nanoforms can be excluded from the procedure of exposure assessment



Nanomaterials in complex organic matrix are ***never*** expected to be like in the pristine form!

Food/feed

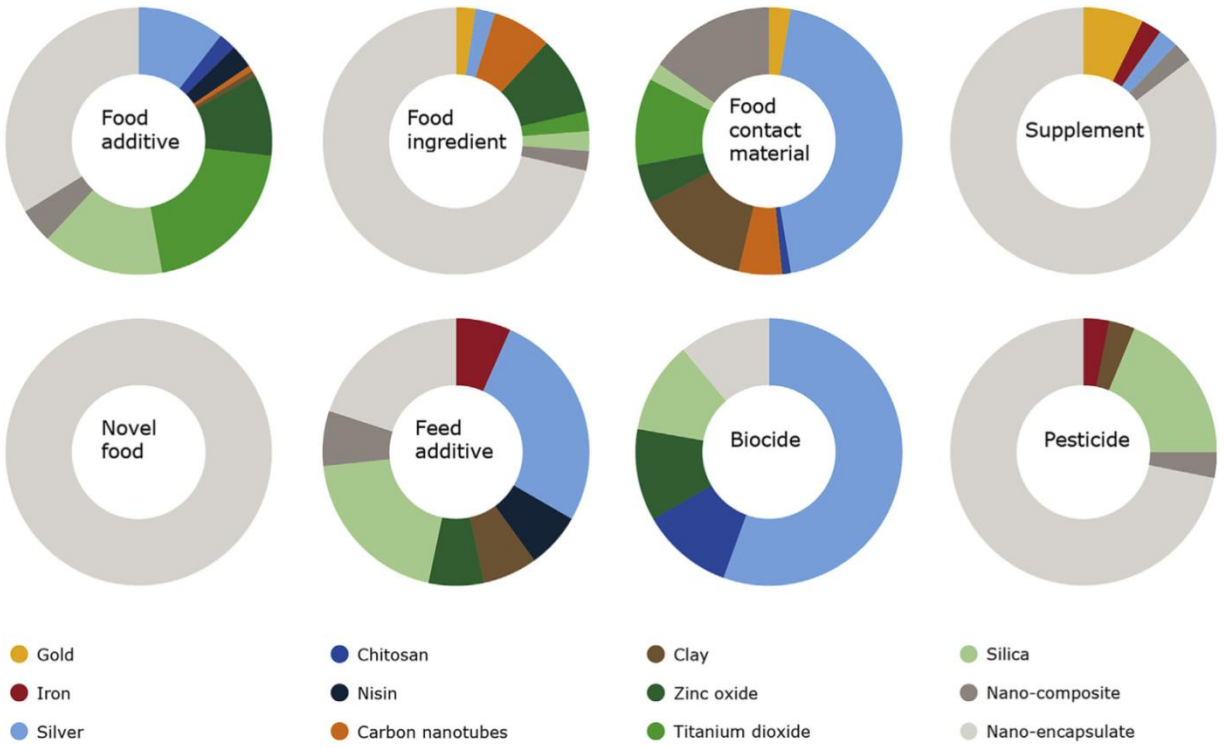
- **Any kind of biological substance**
- **Food supplements**

Food simulants

Union Guidelines on Regulation EU 2014

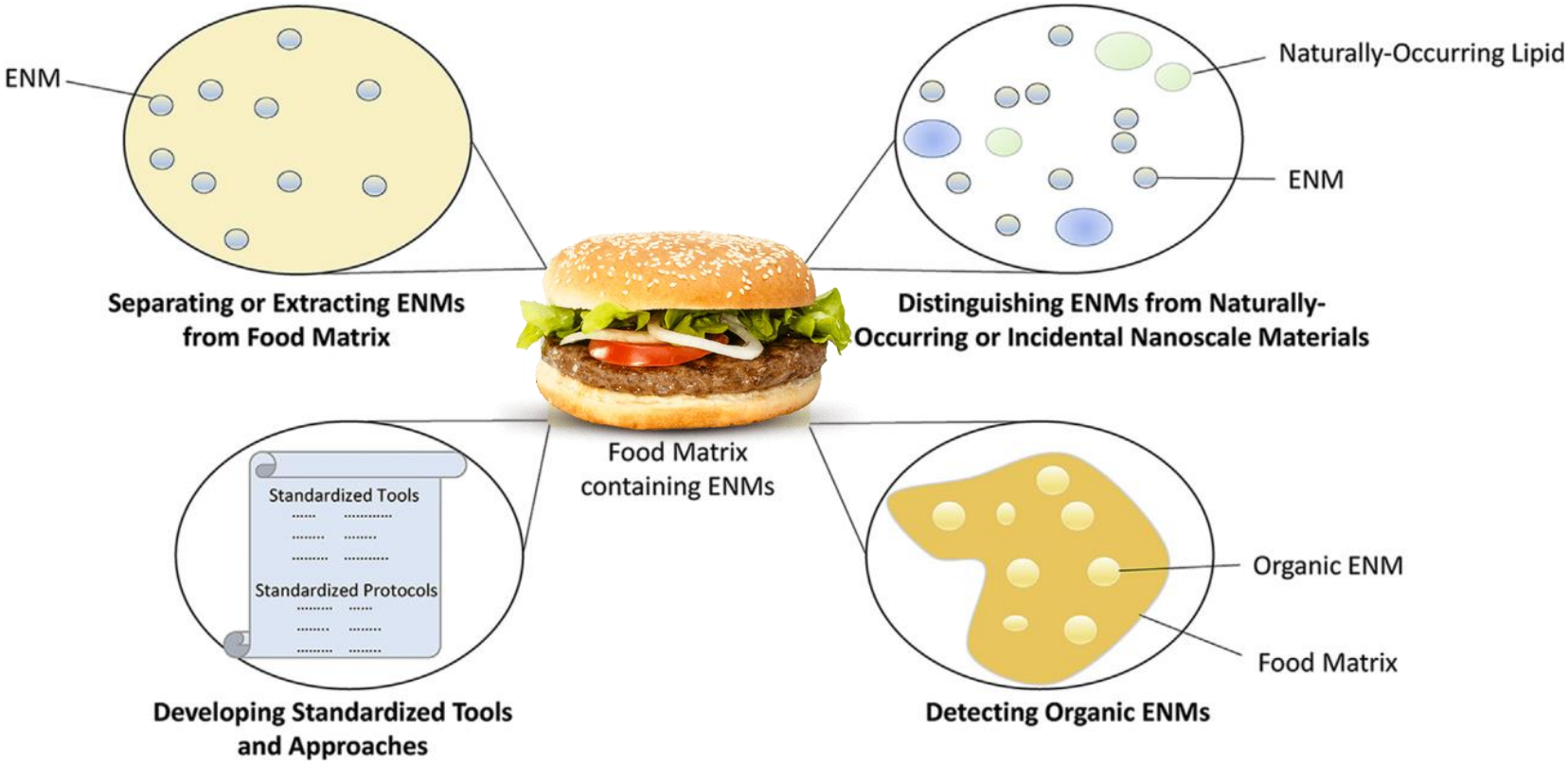
- **A Ethanol 10%**
- **B Acetic acid 3 %**
- **C Ethanol 20%**
- **D1 Ethanol 50%**
- **D2 Vegetal oil (Isooctane)**

Which type of nanomaterial?



- **Titanium dioxide TiO_2 E171**
- **Amorphous silica SiO_2 E551**
  (Na-Al, Na-Ca silicates) (E552, E559)
- **Metallic silver Ag E174**
- **Zinc oxide ZnO_2**
- **Nanoclays (cloisite, MMT)**

Analytical challenges



Analytical tools

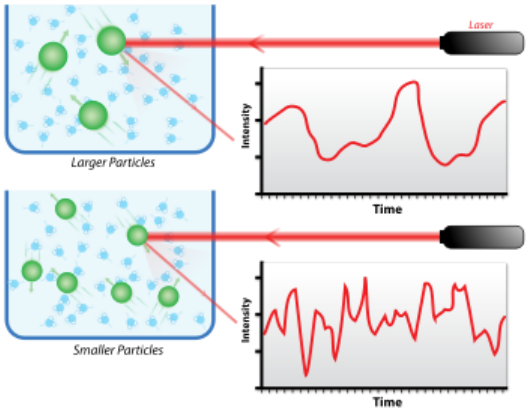
- **Nanomaterials have multiple and interdependent physicochemical properties**
- **Nanomaterials are defined by their size: this is the main property to be determined, then quantity (chemical composition can be preparatory for both)**

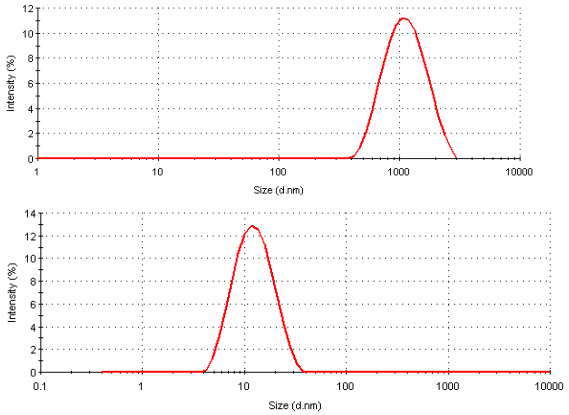
Particles' size:

- defined in multiple ways
- measured by multiple techniques

SEM, AFM, TEM
XRD
DLS
SEC, HDC, FFF (-Uv, MALS, IR, ICP-MS)
spICP-MS

Dynamic light scattering



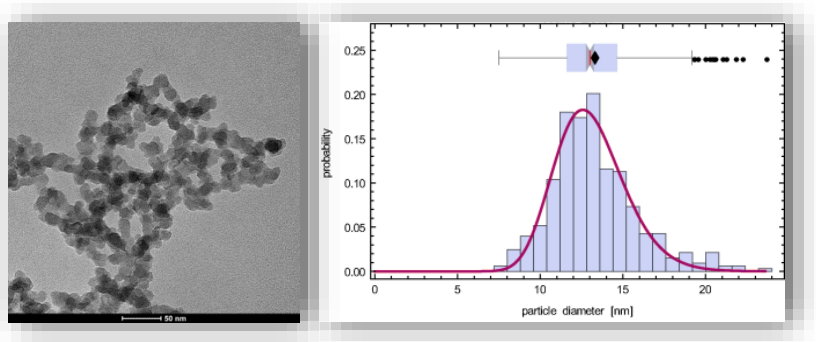


- Cheap, fast
- Wide size range (0.3 nm – 10 µm)

- Poor overall sensitivity
- Big particles mask the presence of the smaller ones

Good for preliminary screening

Transmission electron microscopy



You see what you get!!

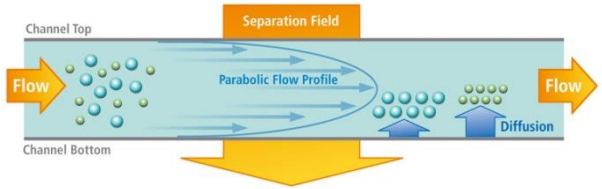
- Primary technique for regulatory testing
- Wide size range (0.2-0.3 nm to µm)
- Multi-property determination (size, morphology, composition by EDAX)

Good for final validation

- Low sample size/representativity
- Difficulty to distinguish nanomaterials from the matrix
- Expensive

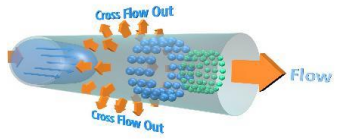
Field-flow fractionation (FFF)

- Family of techniques for the physical separation of particles based on their hydrodinamic diameter

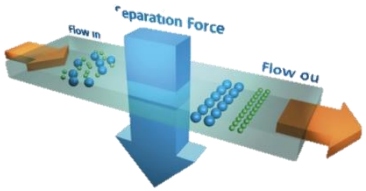


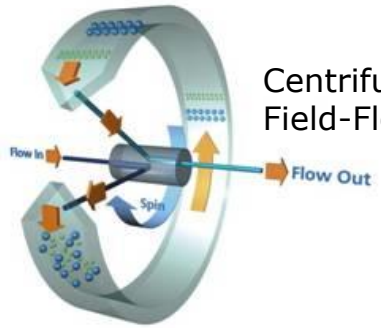
Separation based on hydrodynamic diameter in a velocity field

Hollow Fiber Flow Field-Flow Fractionation



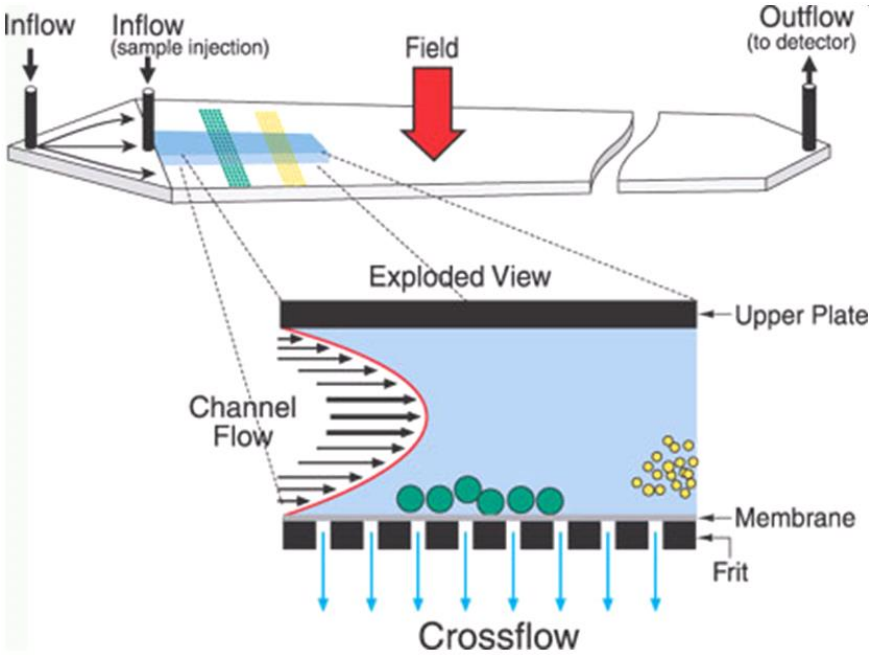
Symmetric/Asimmetric Flow Field-Flow Fractionation





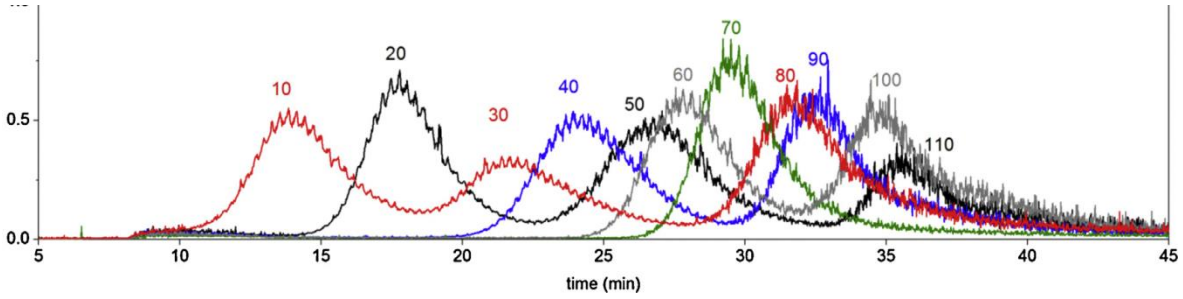
Centrifugal Flow Field-Flow Fractionation

Asymmetric Flow Field-Flow Fractionation (AF4)

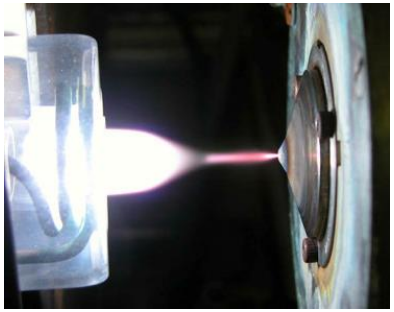


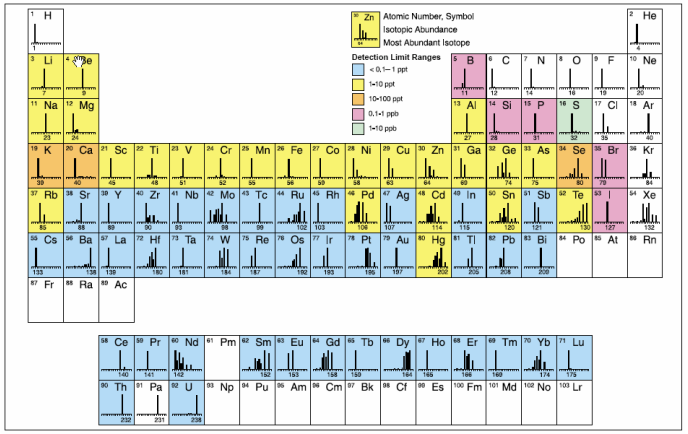
- Wide size range (1-300 nm)
- Good resolving power
- Separation from the matrix
- Good representativity

- A detector is needed



Inductively Coupled Plasma – Mass Spectrometry (ICP-MS)





ICP-MS

Quantitative determination of elements from ppm to ppt levels

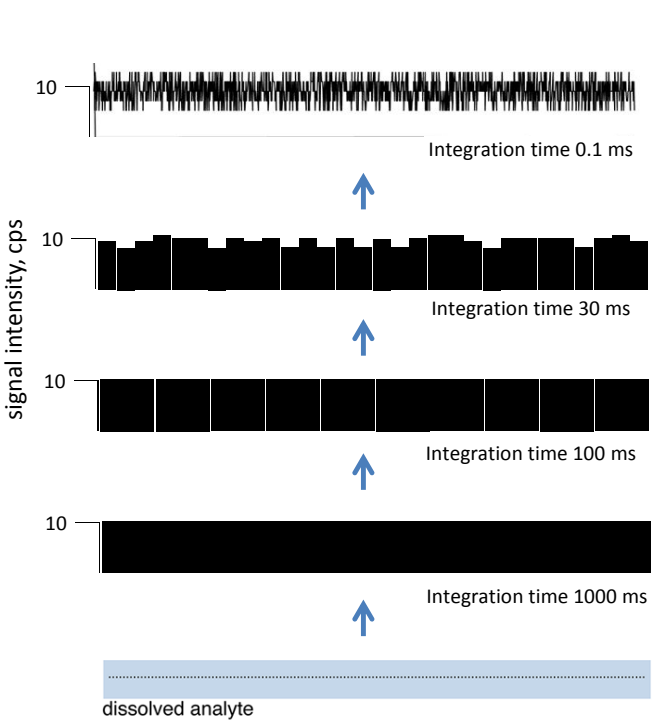
- Very high sensitivity
- High specificity

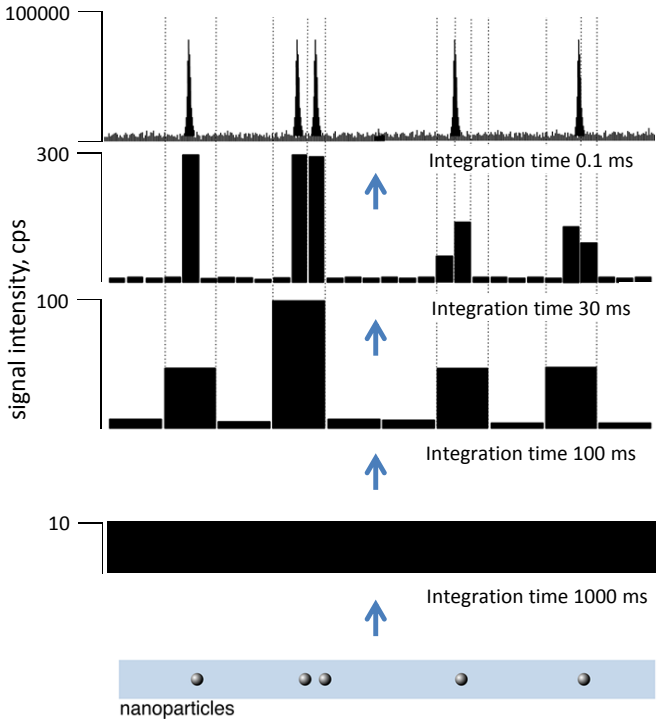
- No distinction between particles, dissolved species and matrix
- Expensive

Single particle (sp) ICP-MS

Single particle acquisition mode

Normal acquisition mode





- Advantages of ICP-MS

\+ Nanoparticles information:

- Number concentration
- Mass
- Size
- Mass concentration

Monoelemental
(no geometric diameter)

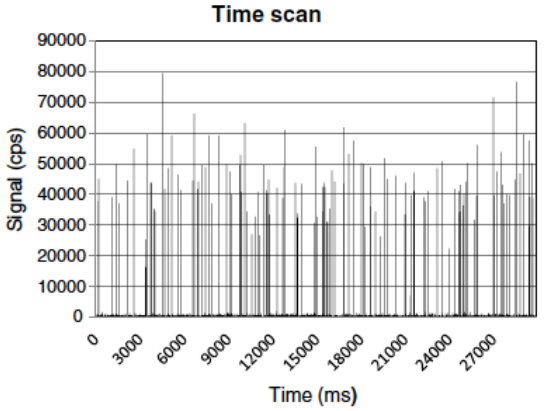
Silver nanoparticles (60 nm) in chicken meat

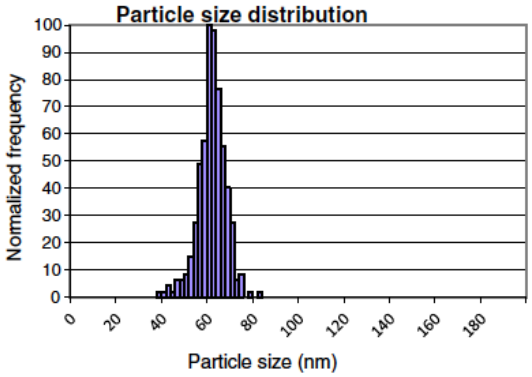
NANOLYSE

Interlaboratory comparison for spICP-MS method validation

AgNPs spike
5-10-25 mg/kg

Enzymatic digestion

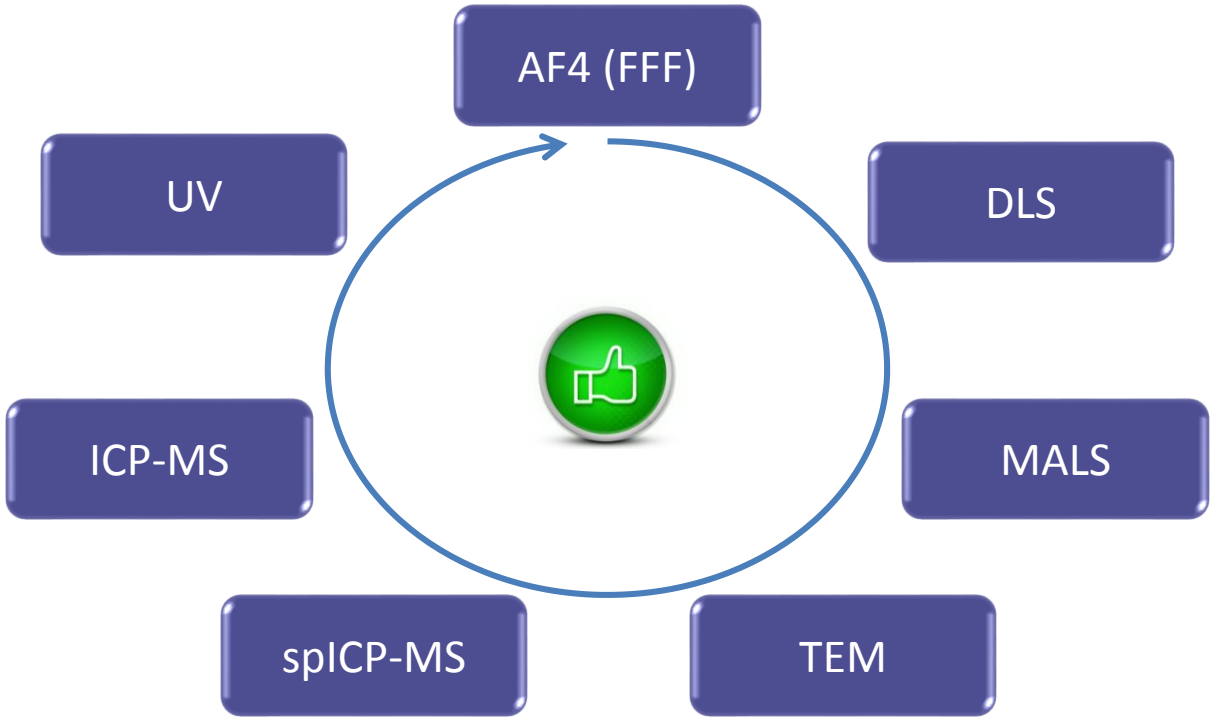




Parameter	Conc. (mg/kg)	Trueness (%)	RSD_r (%)	$RSD_{interlabr}$ (%)
Particle diameter	5	98	0,8	5,2
(n= 21)	10	98	1,2	5,6
	25	99	1,8	5
Particle number concentration	5	92	14	18
(n= 21)	10	95	9,6	12
	25	91	6,4	7,5
Particle mass concentration	5	101	11	16
(n= 21)	10	98	7,2	9,9
	25	100	6,7	8,9

Linearity range: 0.5 mg/kg – 50 mg/kg
LOD 0.05 mg/kg

Combined and coupled techniques



Multi-technique approaches offer the most complete and robust information:

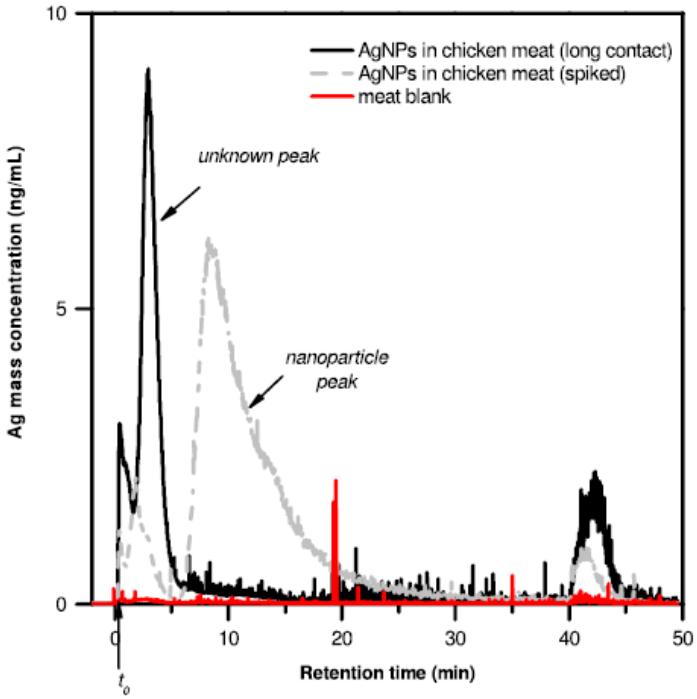
Combined techniques ***off-line***
Coupled techniques ***on-line***

Silver nanoparticles (40 nm) in chicken meat

AF4-ICP-MS (coupled)
- Hydrodynamic diameter
- Size distribution based on mass concentration

Fractions collection

spICP-MS (combined)
- Mass-equivalent diameter
- Size distribution based on number concentration



Loeschner et al., 2013 and 2015

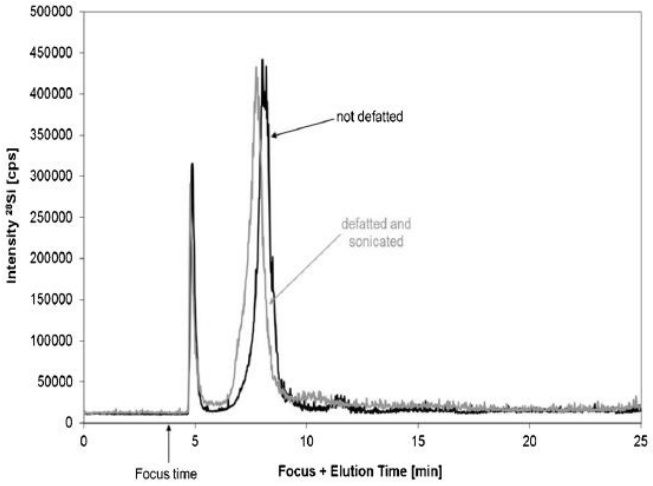
Silica nanoparticles (12 nm) in commercial coffee creamer

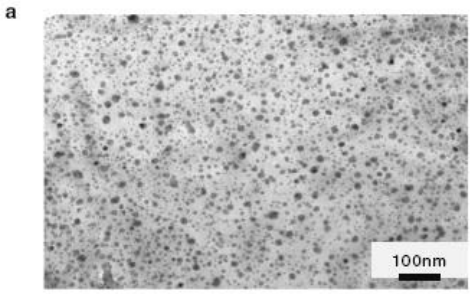
Sample defatting (solvent extraction)

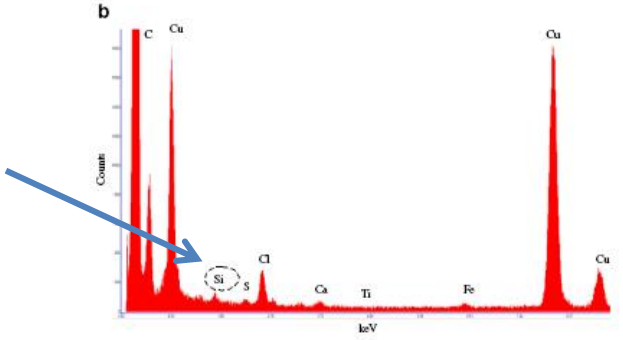
AF4-ICP-MS (coupled)
- Hydrodynamic diameter
- Size distribution based on mass concentration

Fractions collection

TEM-EDAX (combined)
- Geometric diameter
- Morphology
- Elemental composition



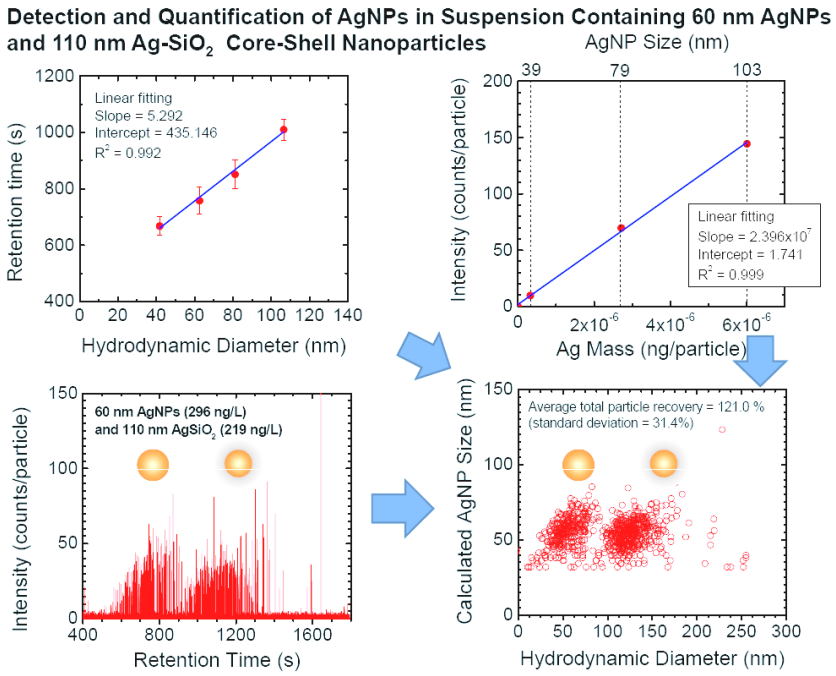




Heroult et al., 2014

AF4-spICP-MS (coupled)

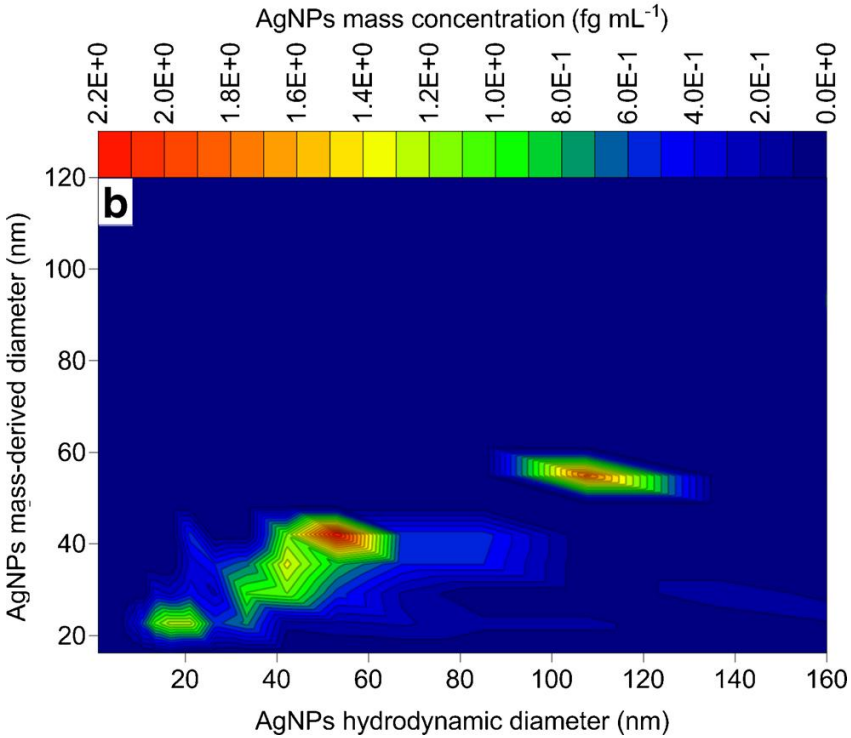
Silver nanoparticles in water (EPA)



An Huynh et al., 2016

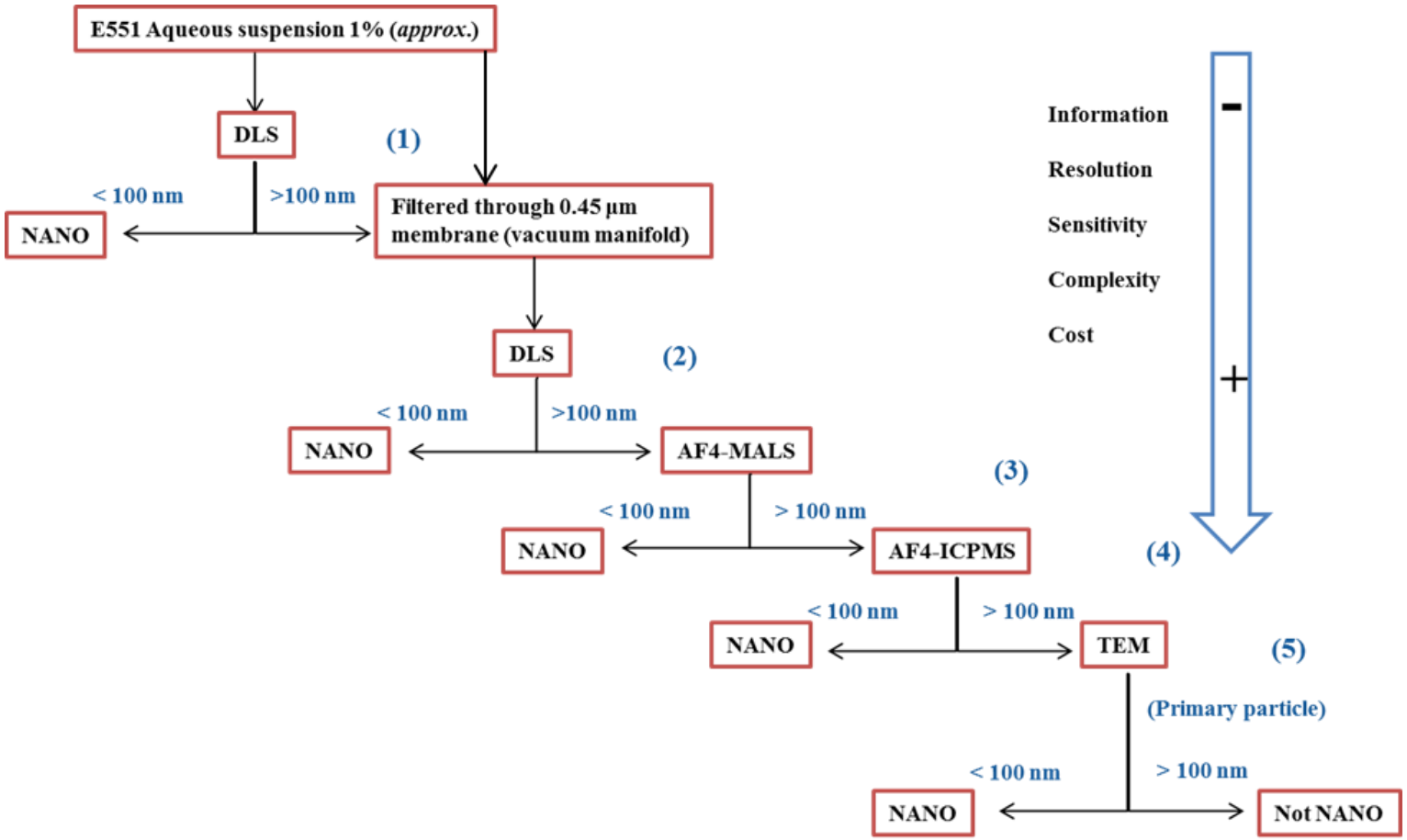
HDC-spICP-MS (coupled)

Silver nanoparticles in blood



Roman et al., 2016

Silica nanoparticles (20, 40, 60, 80, 100, 150 nm) standard suspensions



Barahona et al, 2016

THANK YOU FOR THE ATTENTION

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