Fifth Internation Conference

NANOSAFE 2016

In vitro **human digestion test to monitor the**

dissolution of silver nanoparticles

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Background

Increasing industrial production and use of commercial goods

containing silver nanomaterials ➡️ **Likely human oral exposure**

Risk assessment model

in conditions simulating human ingestion

Quantification of

bioaccessibility/-**availability** ➡️ **products of biotransformation**

- dissolved ions
- aggregates/agglomerates
- nanosized particles

The model

In vitro human digestion model:

It simulates the human digestion in the oral, gastric and intestinal compartments with salt and protein composition, pH differences and transit times alike the *in vivo* digestion

Dynamic process

5 minutes

pH 6.8

120 minutes

pH 2.5

120 minutes

pH 6.5
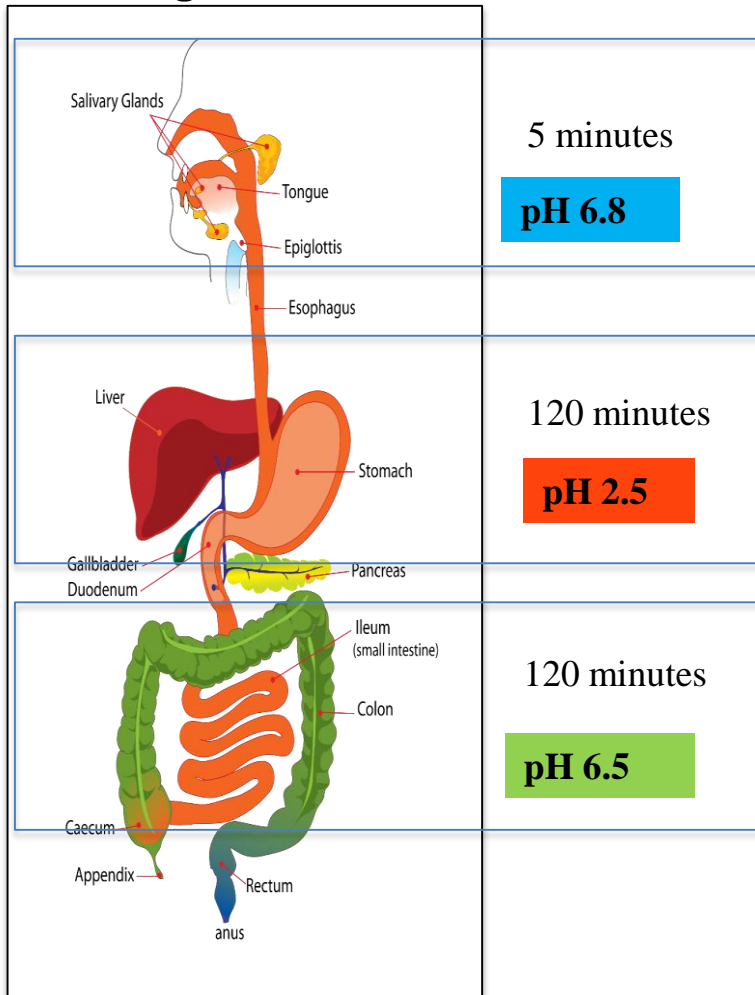
=> **useful** analytical tool

to measure bioaccessibility and

bioavailability of drugs or food

contaminants

Versantvoort et al, *Food Chem. Toxicol.*, 2005

Our aim

- <u>Size</u>
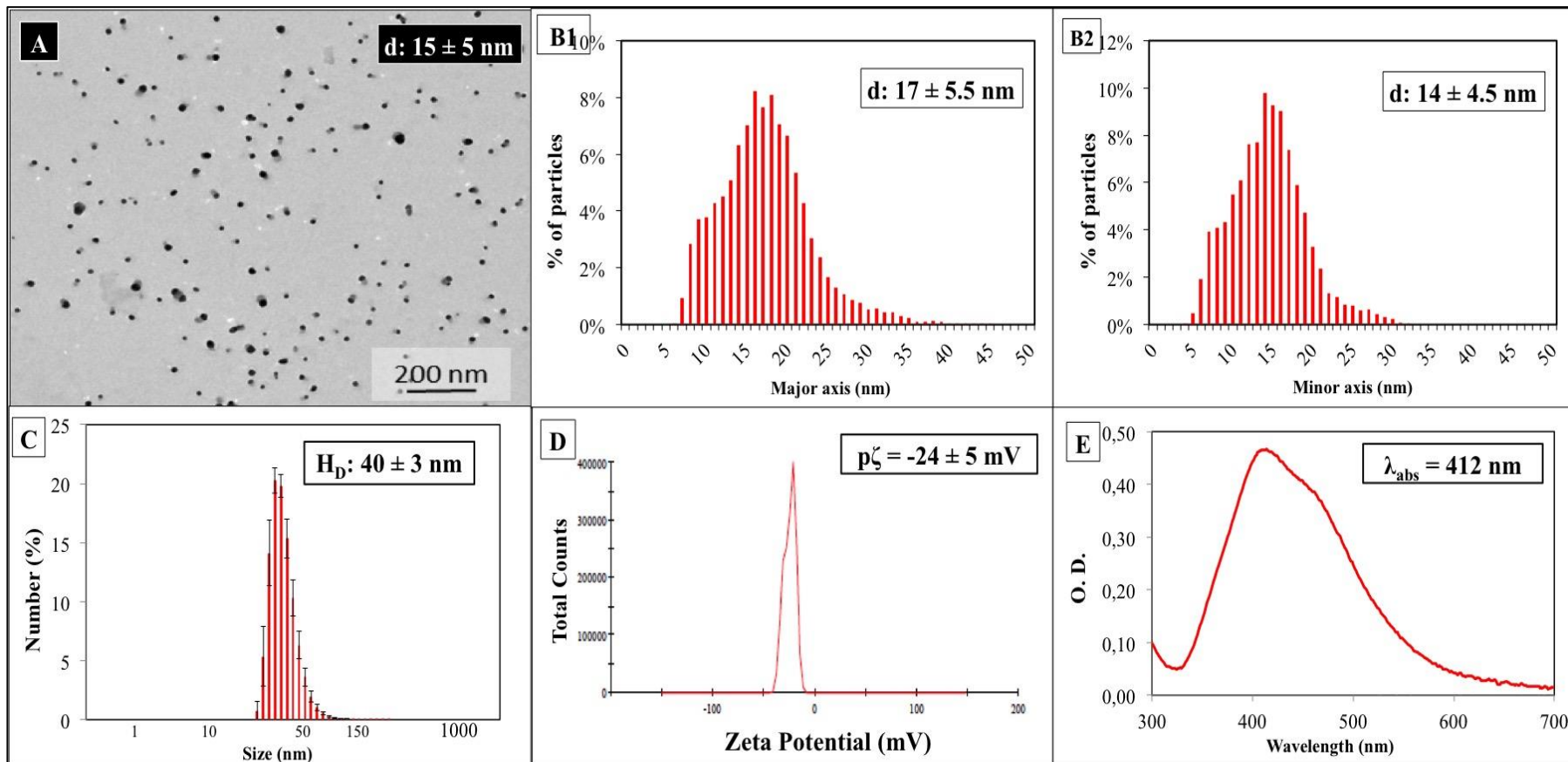
- <u>Dissolution</u>

- <u>Agglomeration</u>

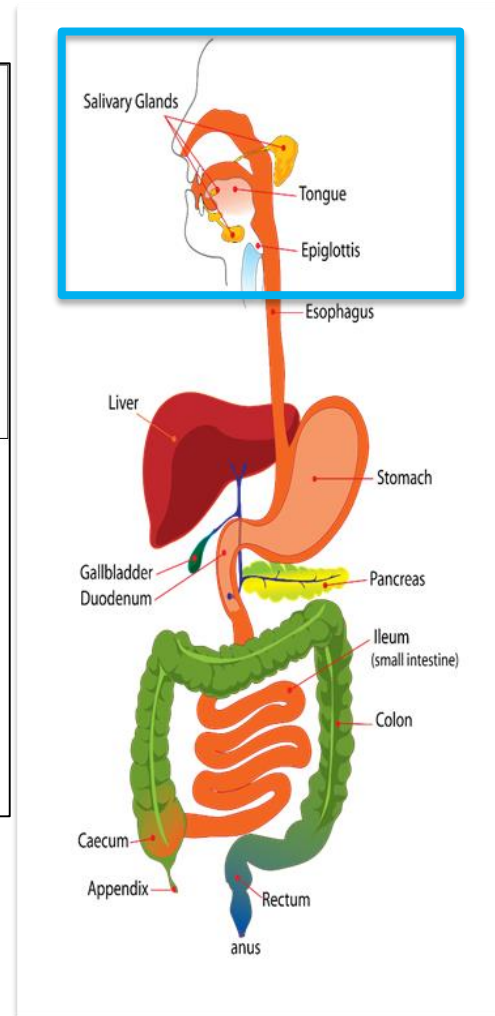→ **sorrounding enviroment**
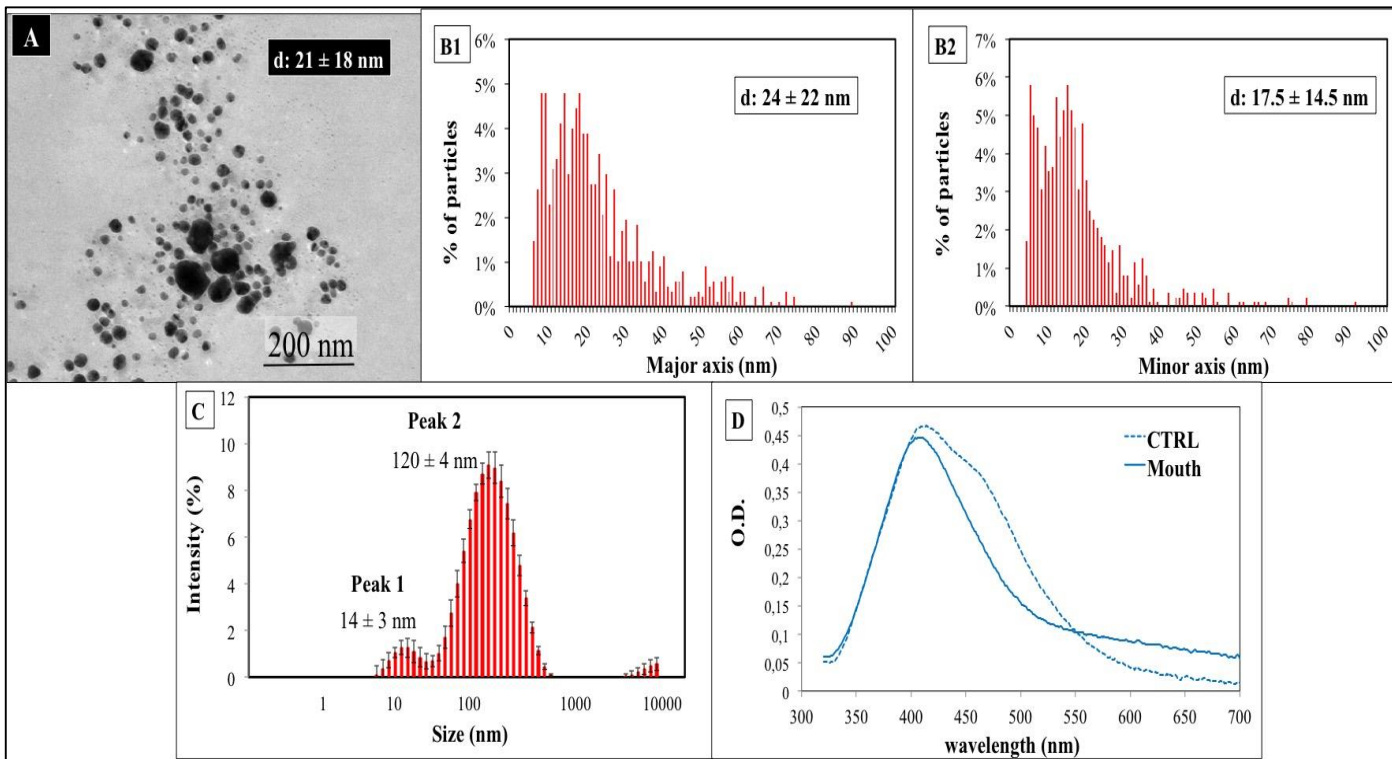
dependent properties

Our approach

- **NM300k** (klein *et al*, 2011): reference nanoparticles in many European projects in nanoregulatory context

- Pre-Standard Operational Procedures (**SOPs**)

  - **material preparation**

  - **probe sonication**

  - **TEM grids**

  - **matrix juices**

  - **instrument use**

- Multi-technique approach: **to gain complementary information**

  - **TEM**

  - **DLS**

  - **UV-Vis**

  - **UF/ICP-AES**
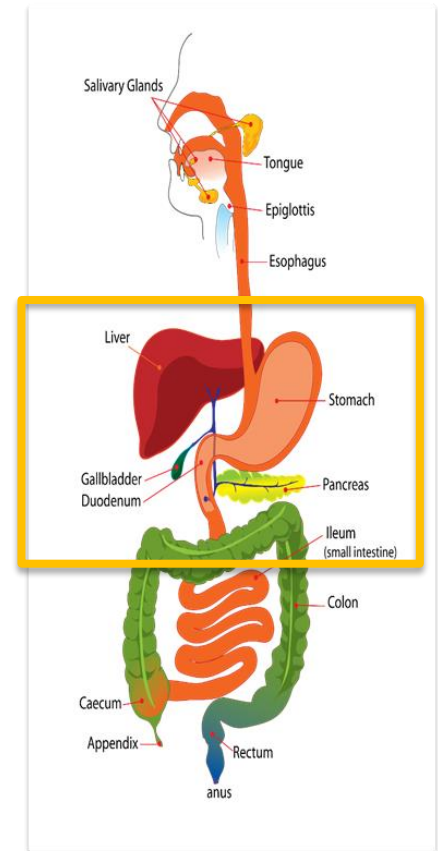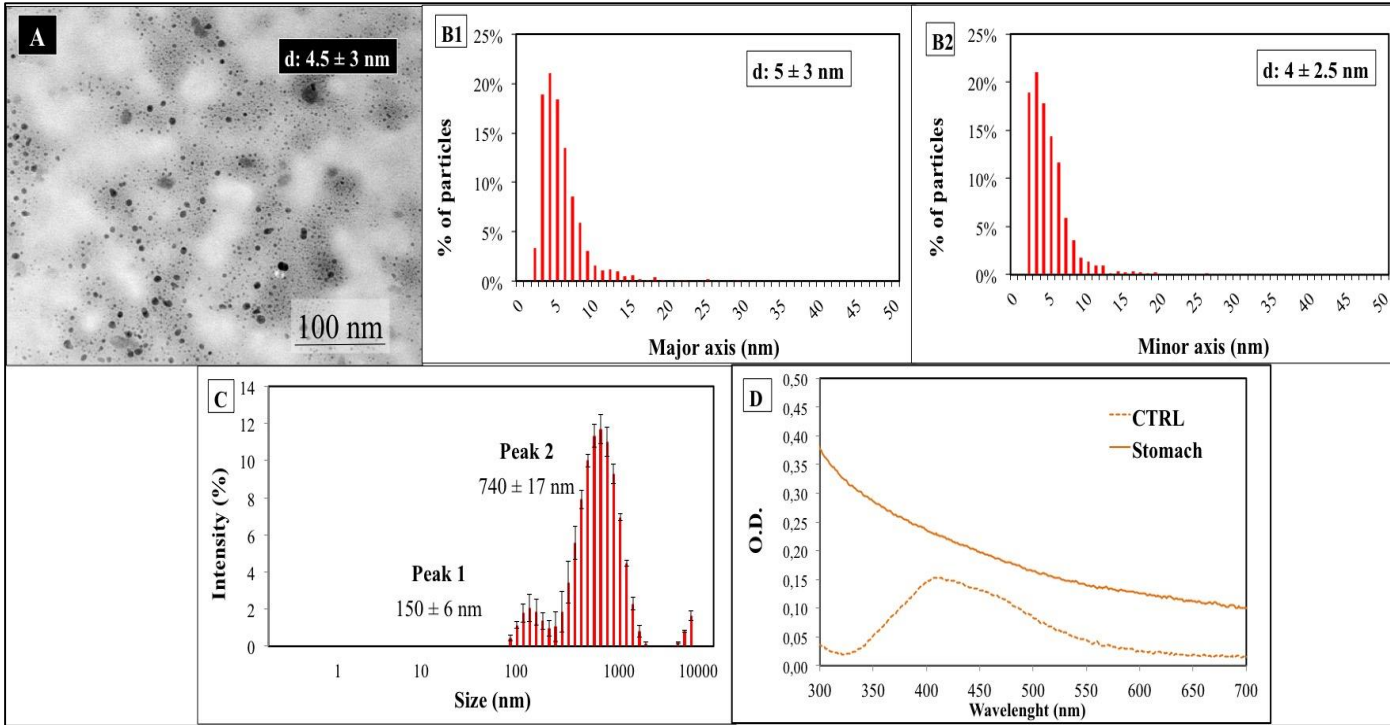
NM300k in dispersion medium

A — d: 15 ± 5 nm — 200 nm

B1 — % of particles vs Major axis (nm) — d: 17 ± 5.5 nm

B2 — % of particles vs Minor axis (nm) — d: 14 ± 4.5 nm

C — Number (%) vs Size (nm) — H_D: 40 ± 3 nm

D — Total Counts vs Zeta Potential (mV) — $p\zeta$ = -24 ± 5 mV

E — O. D. vs Wavelength (nm) — λ_{abs} = 412 nm
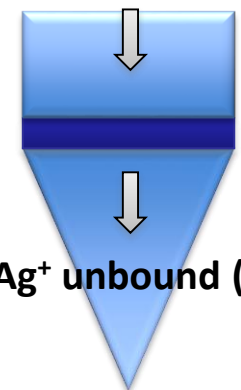
NM300k in mouth

- **NPs almost show the <u>primary size</u> and <u>tend to form</u> <u>agglomerated</u> structures**

NM300k in stomach

- **NPs strongly reduce the mean diameter, as also evidenced by the lack of plasmonic peak, and dissolve**

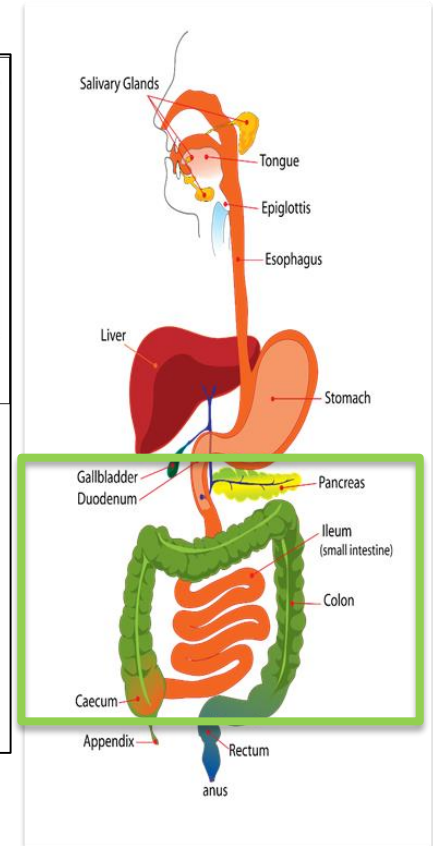- **Big agglomerates embedded in organic matrix**
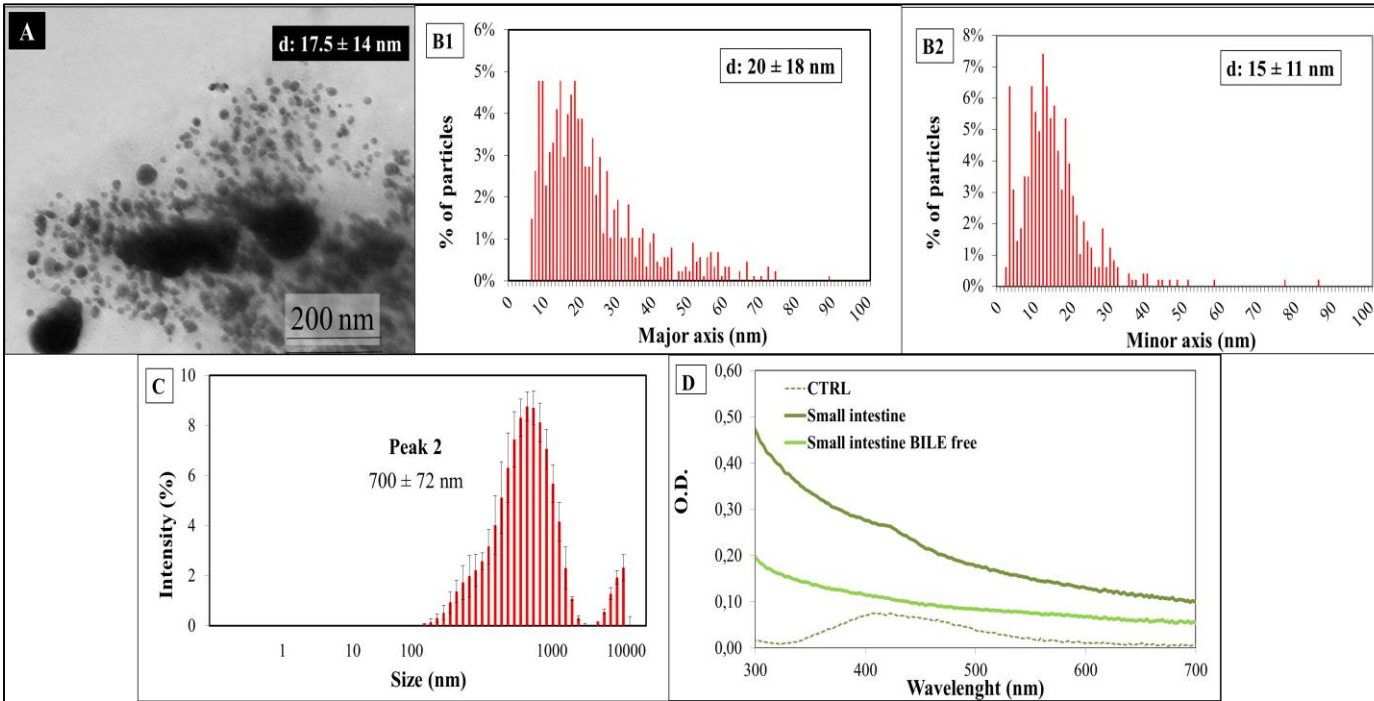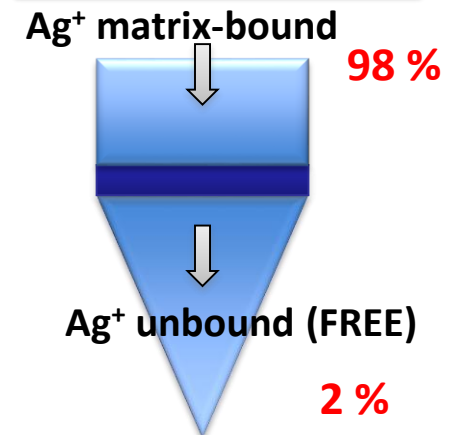
Ag+ matrix-bound
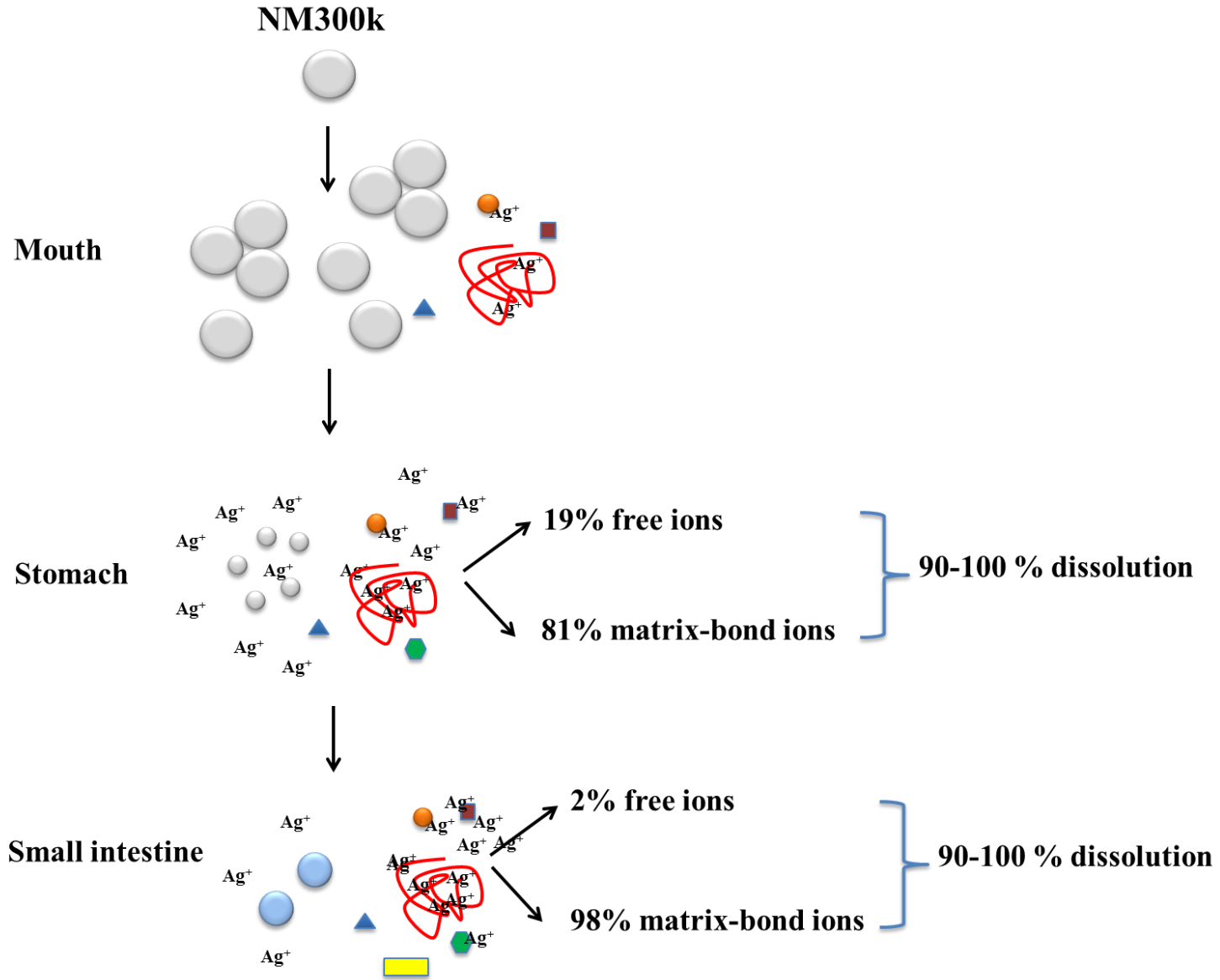
81%

Ag+ unbound (FREE)

19%

NM300k in small intestine

- Absence of UV signal for inorganic nanoparticles

- 2% of free ions <u>available for intestinal adsorption</u>

- Presence of <u>big aggregates and few nanosilver salts</u> of different

  nature

Ag$^+$ matrix-bound

98 %

Ag$^+$ unbound (FREE)

2 %

NM300k

Mouth

Ag⁺

Ag⁺

Ag⁺

Stomach

Ag⁺ Ag⁺
Ag⁺
Ag⁺
Ag⁺
Ag⁺
Ag⁺
Ag⁺
Ag⁺
Ag⁺
Ag⁺
Ag⁺

19% free ions

81% matrix-bond ions

90-100 % dissolution

Small intestine

Ag⁺
Ag⁺
Ag⁺
Ag⁺
Ag⁺ Ag⁺
Ag⁺
Ag⁺
Ag⁺
Ag⁺
Ag⁺
Ag⁺
Ag⁺
Ag⁺

2% free ions

98% matrix-bond ions

90-100 % dissolution

Pristine NM300K   Digestive proteins   Biotransformed NPs or nanosized salts

Ag⁺ ions   Organic Matrix   Inorganic matrix   Bile   Bove *et al*, submitted

Evaluation of test predictability (1)

This *in vitro* dissolution test may be relevant for the risk assessment of AgNPs:

1. majority of the initial NPs is dissolved in ions => NP exposure levels are similar to those of corresponding saline form

2. ions appear to be mostly bound to the matrix => they may follow the same excretion pathway of the saline silver

Evaluation of test predictability (2)

read-across based hypothesis

Ag excreted (*in vitro*)	Ag excreted (*in vivo*)	Literature
98%	**60%** **(faeces)**	**60-99%** (Bergin *et al,* 2016)

Ag absorbed (*in vitro*)	Ag absorbed (*in vivo*)	Literature
2%	**0.06%** **(blood)**	**0.4-10%** (Loeschner *et al,* 2011; van der Zande *et al*, 2012)
	0.02% **(urines)**	
	1.92 % **(tissues)**	

Bove *et al*, submitted

Conclusions

The dissolution test may be a valid analytical tool for

nanoregulation:

=> It allows to quantify the silver nanoparticles

biotransformation, through read-across of saline form