Analytical challenges for nanomaterials in risk assessment

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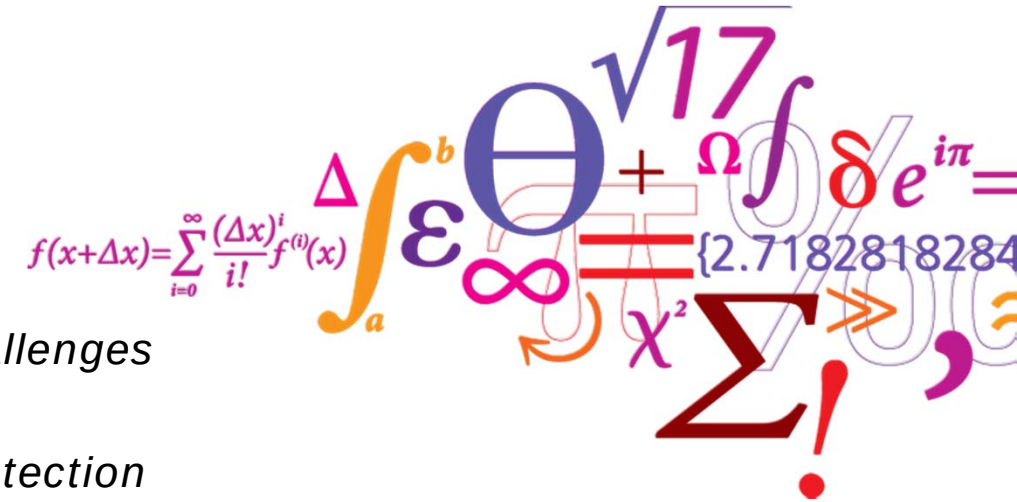
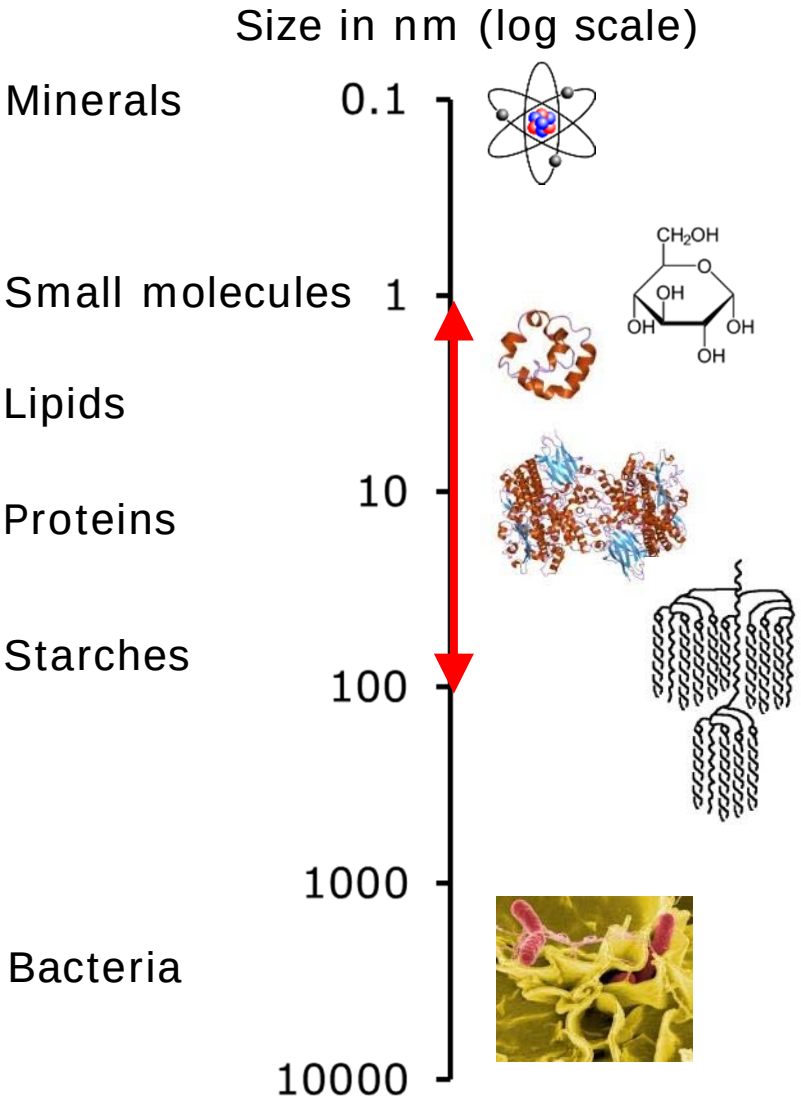
Research Group for Nano-Bio Science



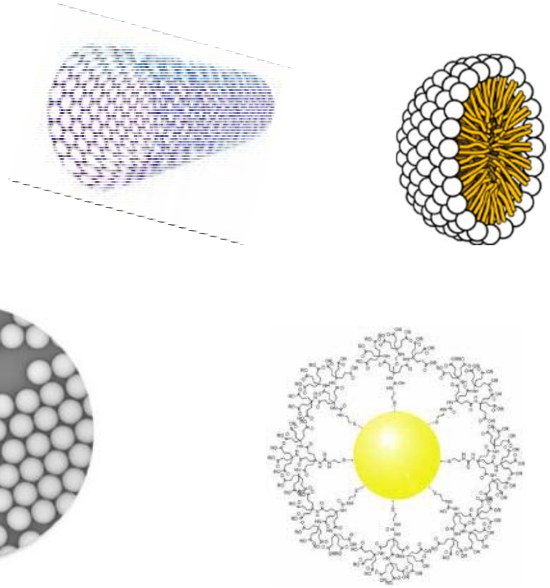
Joint International Symposium
Global Past, Present and Future Challenges in Risk Assessment – Strengthening Consumer Health Protection
30 November / 1 December 2017, Berlin

DTU Food
National Food Institute

The nanoscale



Nanoparticles: discrete piece of material with one or more dimensions in the size range of 1 to 100 nm



The EC recommendation of a Definition of a Nanomaterial (2011/696/EU)

- Recommendation, developed for regulatory purposes
- Review process finalized 2017
- Already used in some regulations (biocides, medical products), intentions to amend existing regulations (e.g. Novel Food Reg.)

"Nanomaterial" means a natural, incidental or manufactured material containing particles, in an unbound state or as an aggregate or as an agglomerate and where, for 50 % or more of the particles in the number size distribution, one or more external dimensions is in the size range 1 nm - 100 nm.

In specific cases…[the] threshold of 50% may be replaced by a threshold between 1 and 50%.

…fullerenes, graphene flakes and single wall CNTs…should be considered as nanomaterials.

The European Commission (2011) 'Commission recommendation of 18 October 2011 on the definition of nanomaterial (2011/696/EU).', *Official Journal of the European Union*, L275(June 2010), pp. 38–40.

Identity of a nanoparticle

Nanoparticle

- **Size** (distribution)
- Chemical composition
- Shape
- Surface contamination
- Crystal structure
- Surface charge
- Porosity
- Surface area
- Agglomeration state
- Surface chemistry
- Solubility

Potential sources of nanoparticles in food

Naturally present (raw ingredients, feed)

Intentionally added (e.g. food additives, novel foods)

Release/migration (e.g. food packaging, processing equipment)

Contaminant from environment

Guidance by EFSA

- Supplementing existing sector-specific guidance for risk assessment, linked to respective EU legislation per sector
- 2016-2018: Update of guidance

European Food Safety Authority

EFSA Journal 2011;9(5):2140

SCIENTIFIC OPINION

Guidance on the risk assessment of the application of nanoscience and nanotechnologies in the food and feed chain[1]

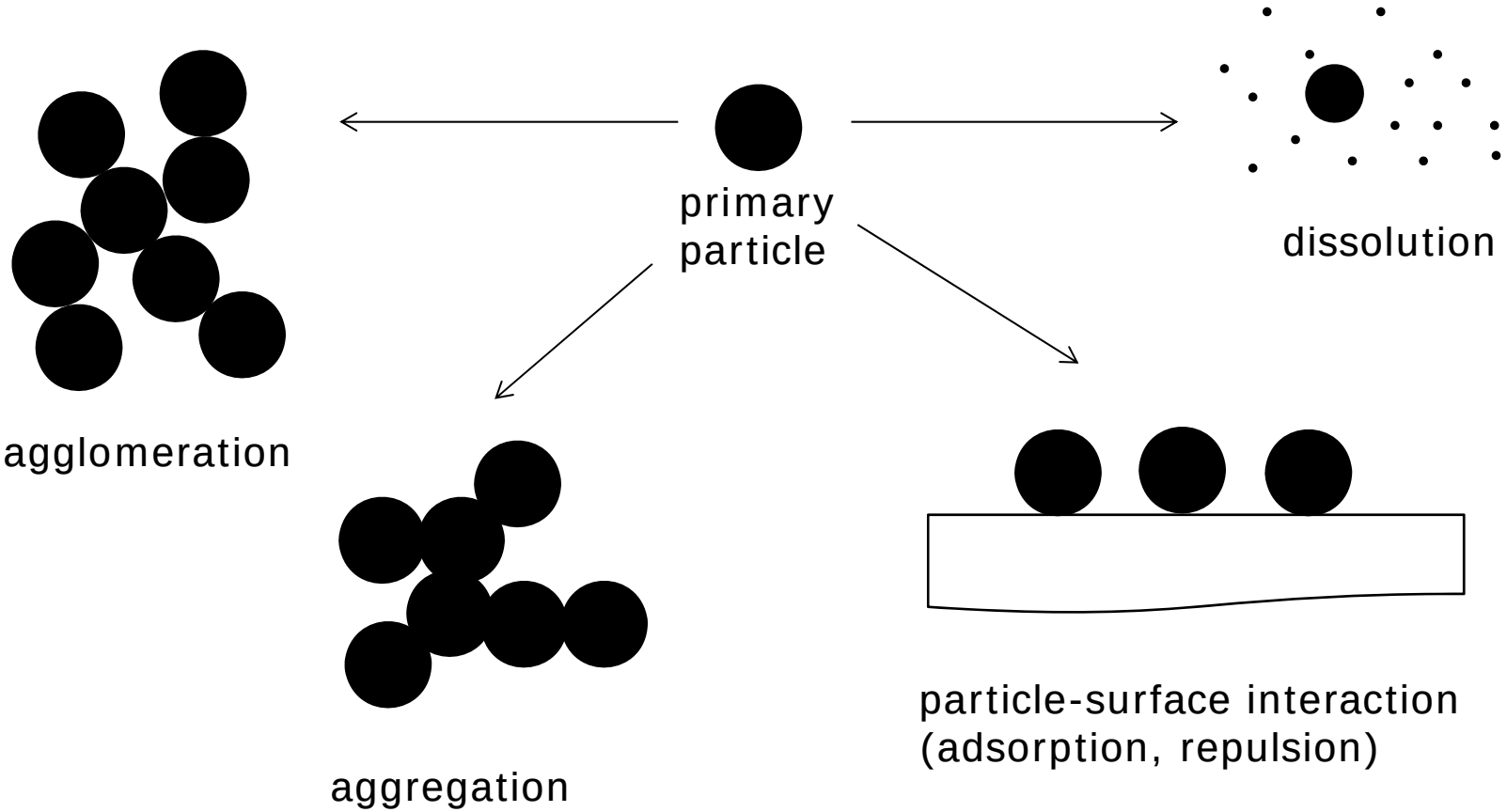
EFSA Scientific Committee[2, 3]

European Food Safety Authority (EFSA), Parma, Italy

Guidance for risk assessment by EFSA

- Adequate **characterization** of an engineered nanomaterial (ENM) is essential for establishing its identity
- The risk of an ENM will be determined by its chemical composition, **physico-chemical properties**, its interactions with tissues, and potential exposure levels
- **Physico-chemical characterization** of ENMs should be considered at several stages (e.g. as manufactured, in the food/feed matrix, in toxicity testing medium, in biological tissues in the human or animal body)

The physico-chemical properties of nanoparticles can change



+ Interaction with matrix constituents

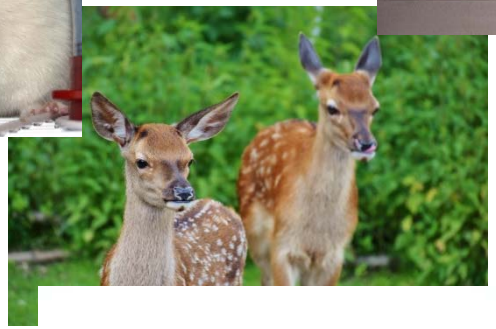
Examples of analyzed samples (2008 – 2017)



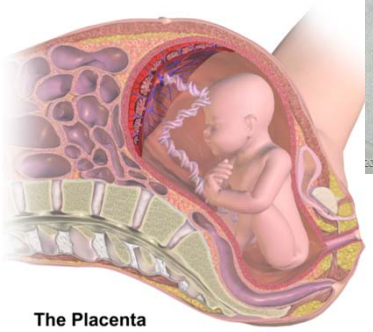
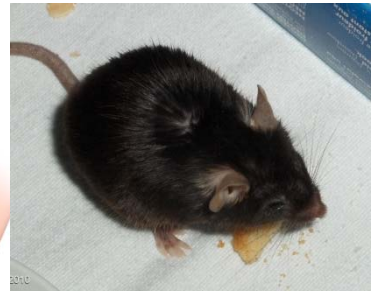










Images courtesy of Pixabay / WikiCommons

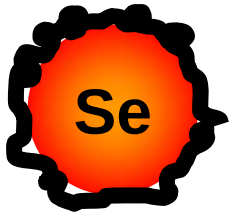
NanoTest project (2008 – 2010)

Testing of adverse effects of nanoparticles relevant to exposure via food

in vivo
(28 day repeated oral exposure)

nanoparticles

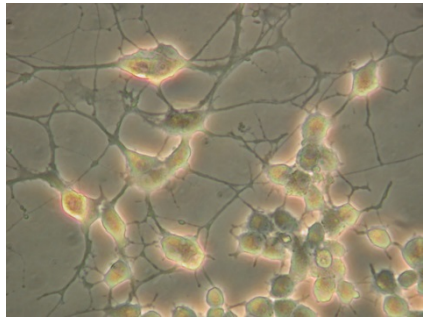
∅ 10 nm +



∅ 20 nm

?

in vitro



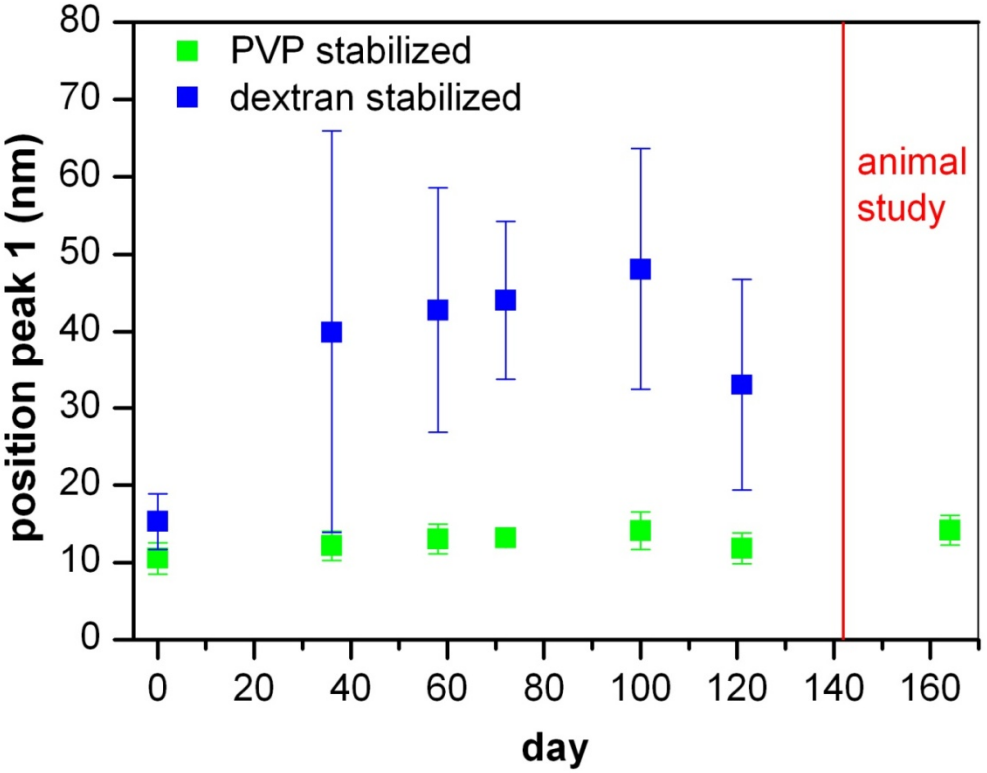
DFFE - Fødevareforskningsprogrammet 2007

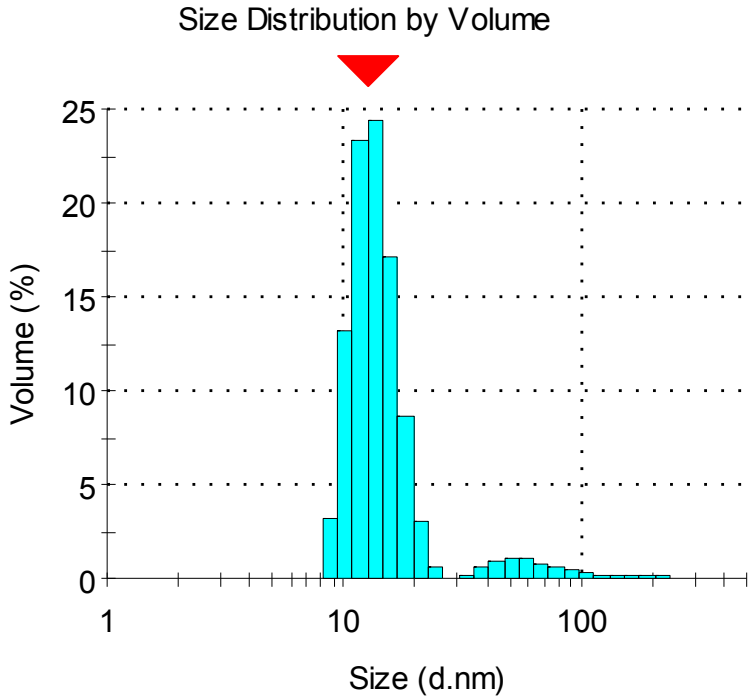
Silver nanoparticles in products related to food and beverages

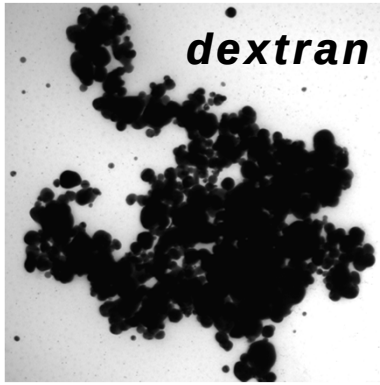
http://www.nanotechproject.org/

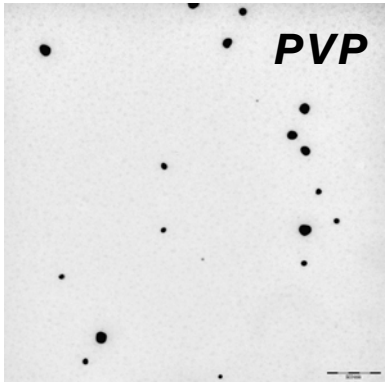
Control of nanoparticle stability

Nanoparticle size distribution monitored by dynamic light scattering









Detection strategy

1. Organ distribution of Ag in the rat

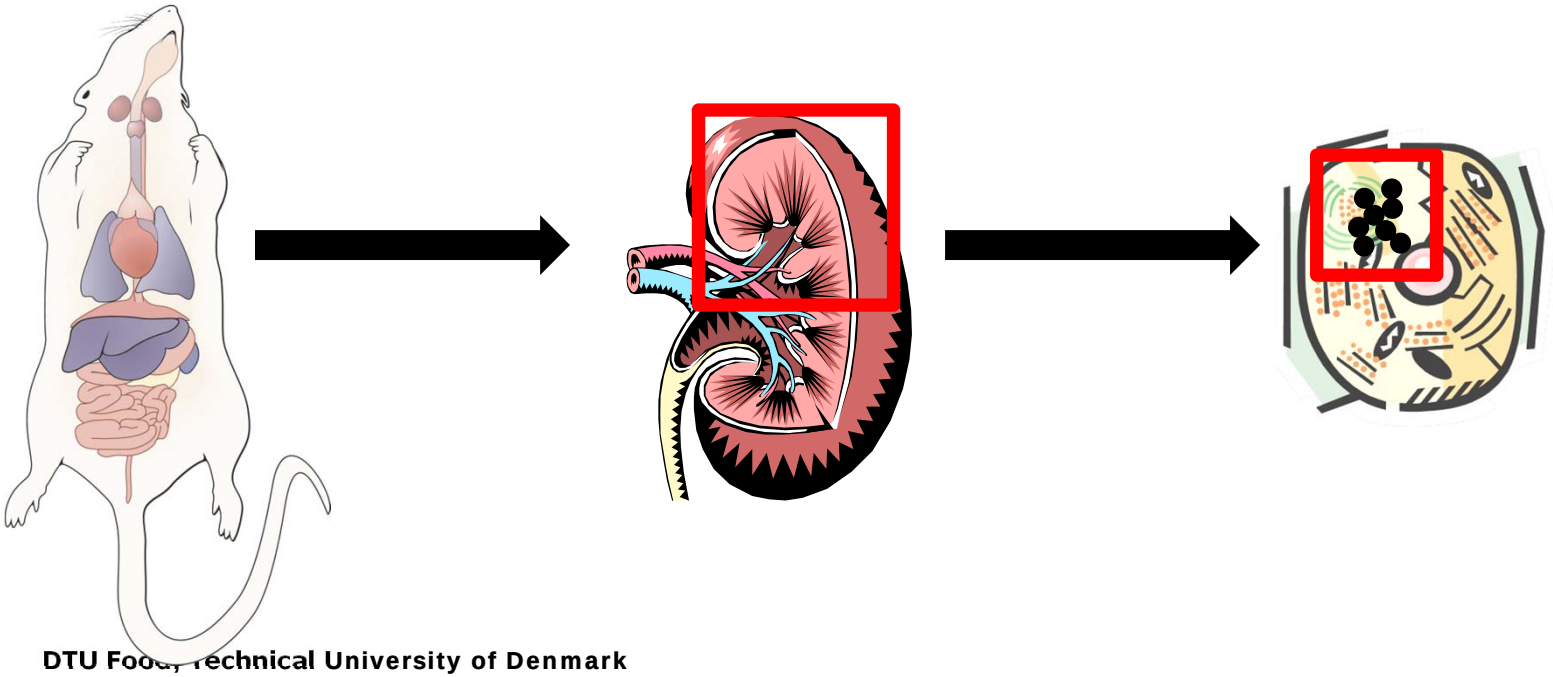
ICP-MS of acid digested tissue

2. Distribution of Ag in selected organs

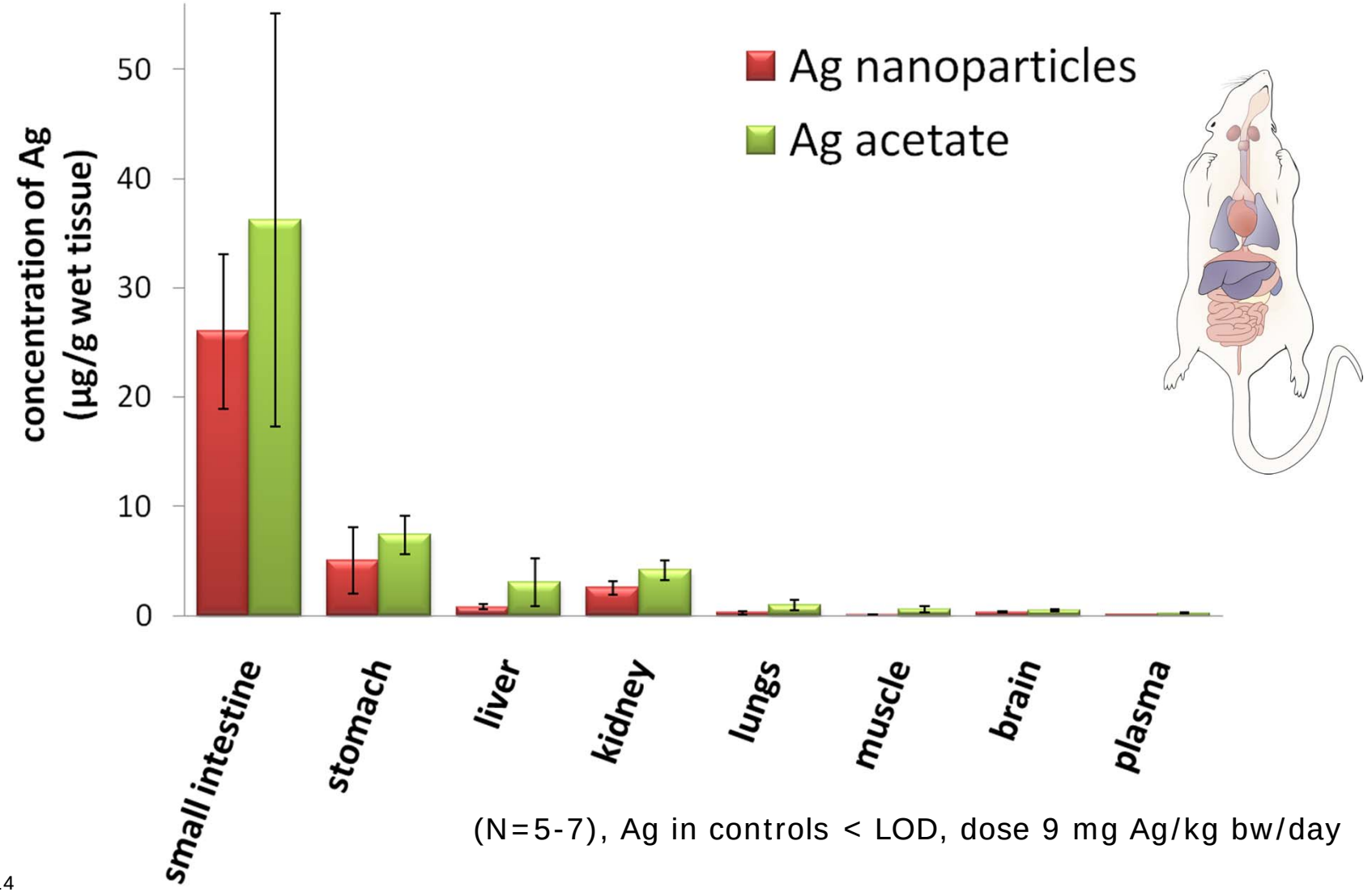
Autometallography (silver enhancement) + light microscopy

3. Are there NPs in rat organs? Where are particles located in the cells?

Transmission electron microscopy of thin sections



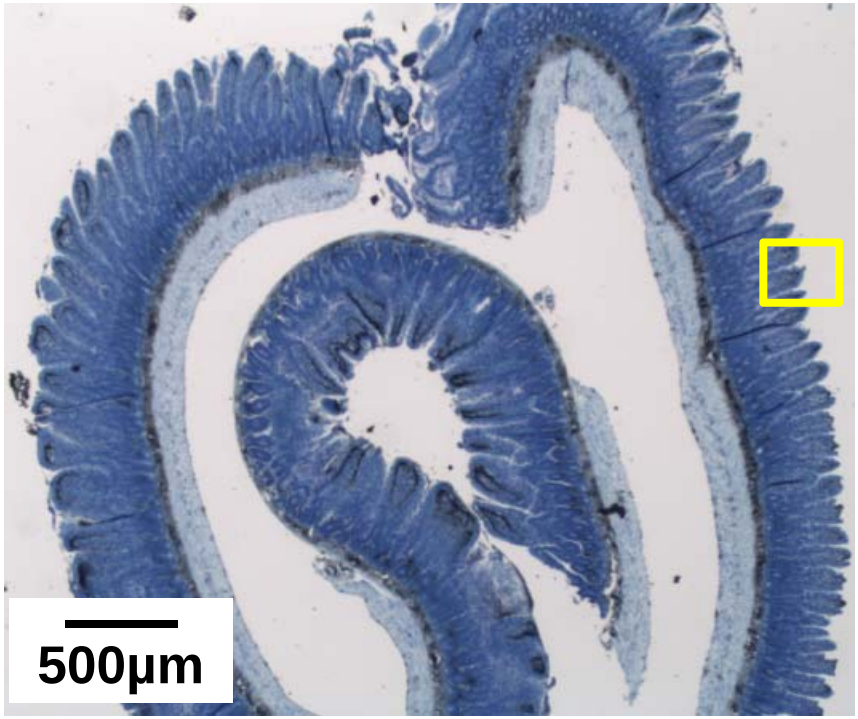
Organ distribution of silver – ICP-MS of nitric acid digested tissue

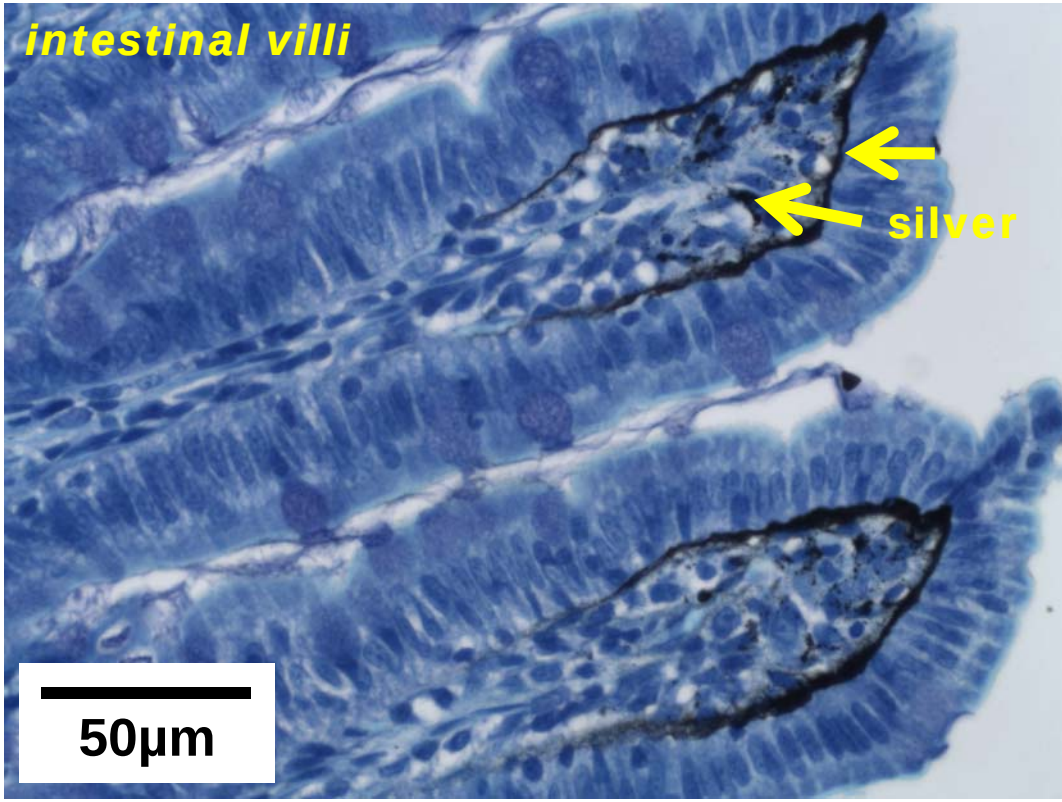


(N=5-7), Ag in controls < LOD, dose 9 mg Ag/kg bw/day

Light microscopy / autometallographic staining

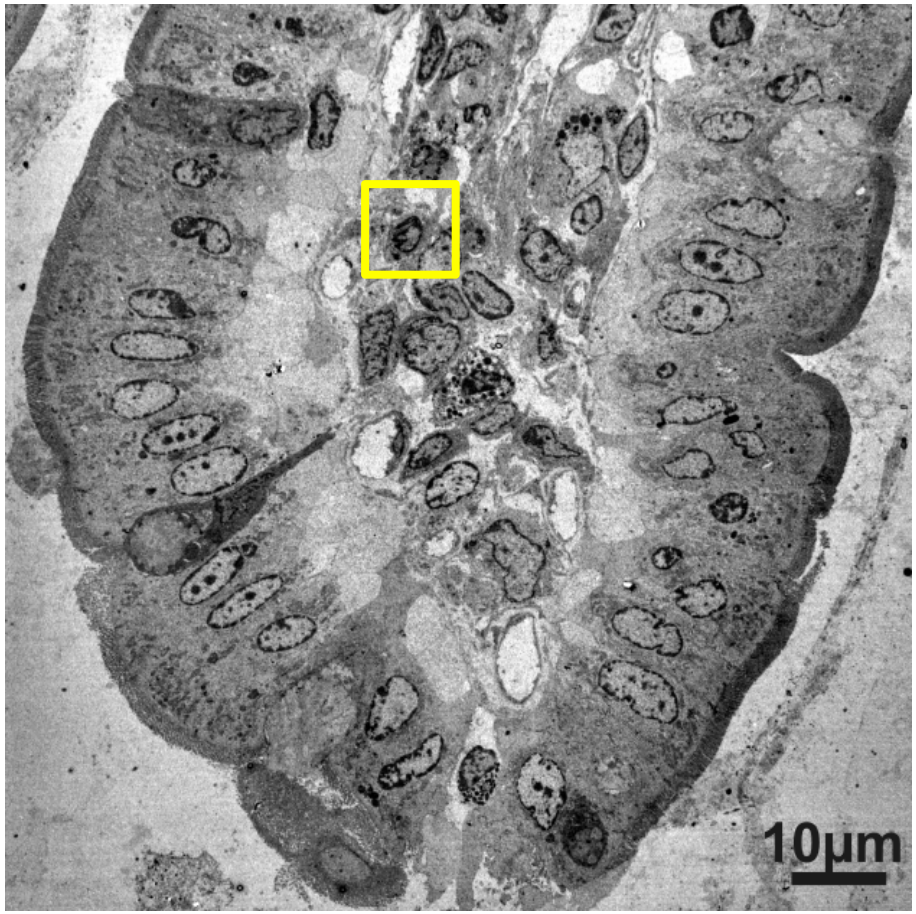
Silver nanoparticle exposed rat: ileum



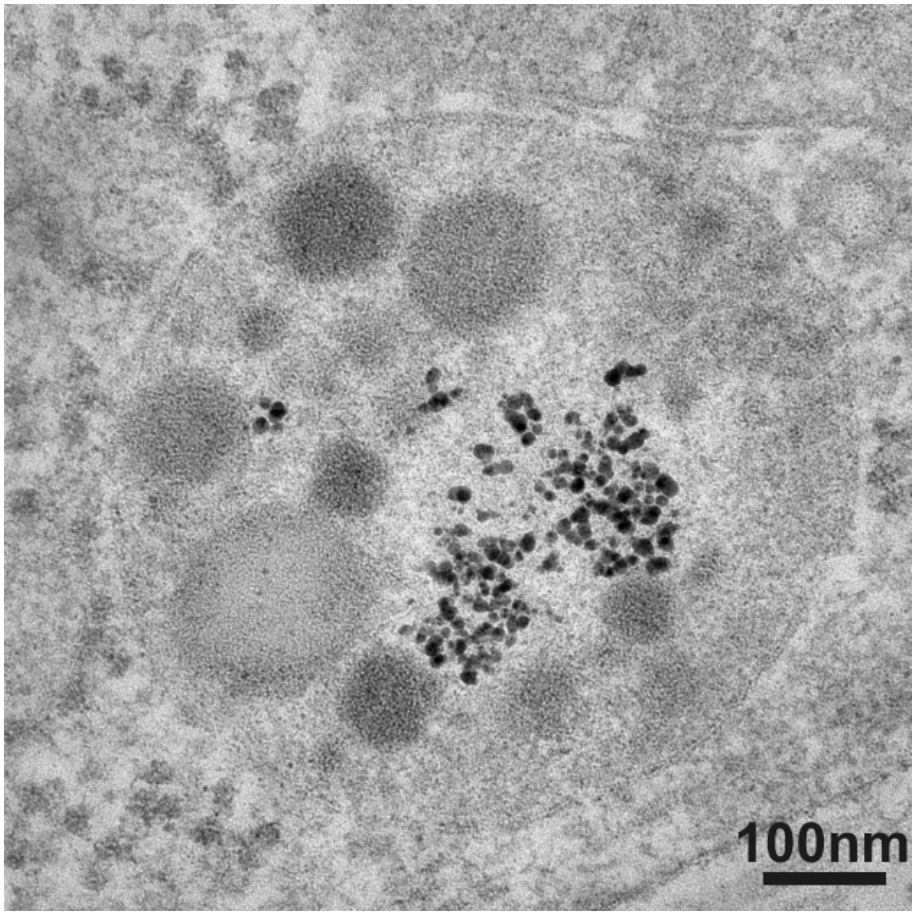


Transmission electron microscopy

Silver nanoparticle exposed rat: ileum



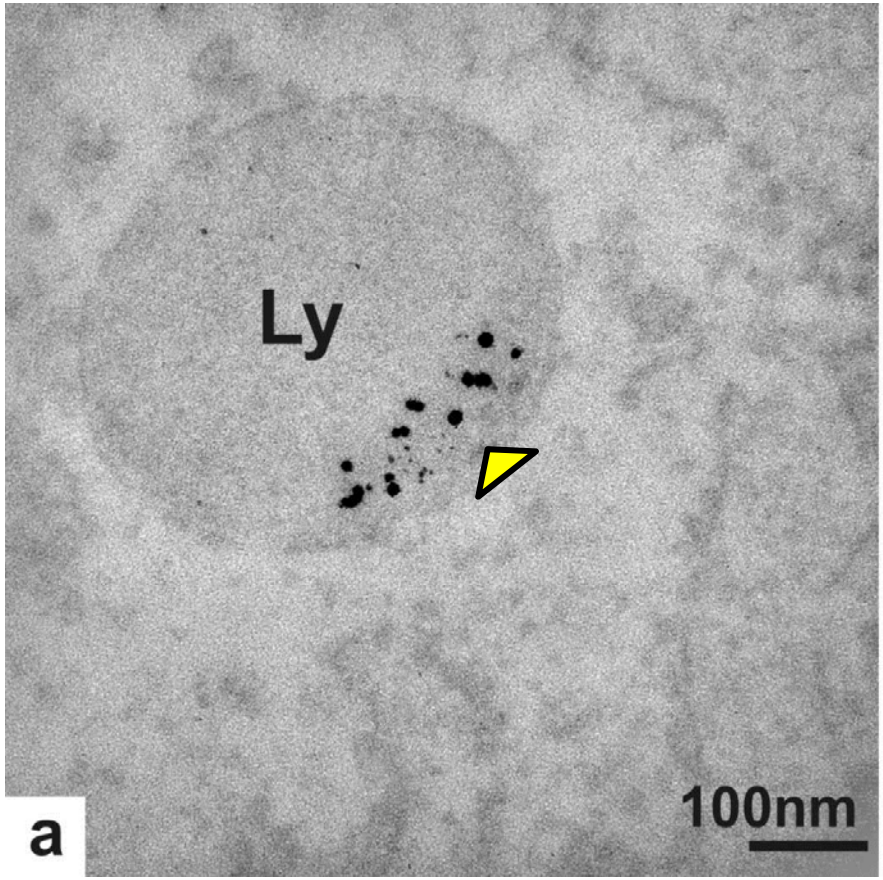
intestinal villus



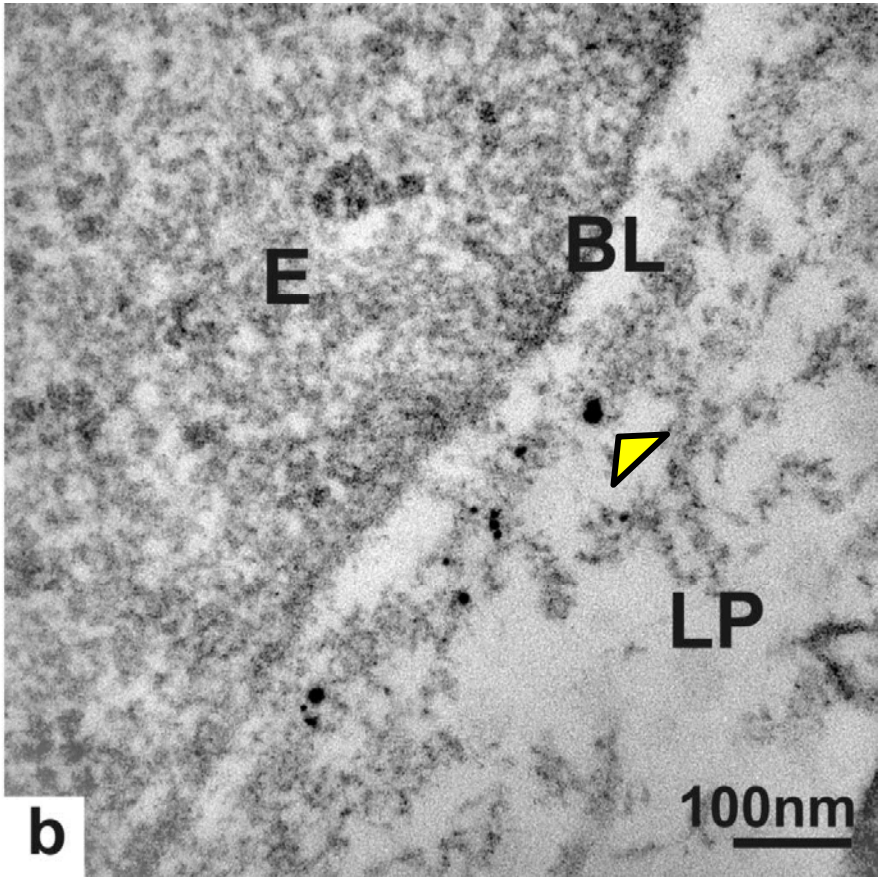
lysosome containing particles

Transmission electron microscopy

***Silver acetate* exposed rat: ileum**

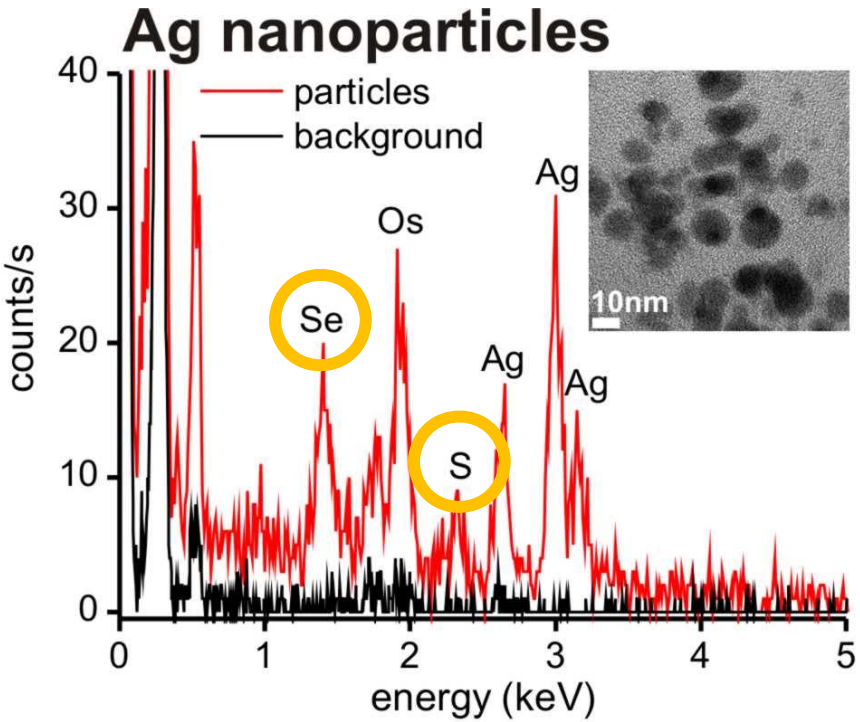


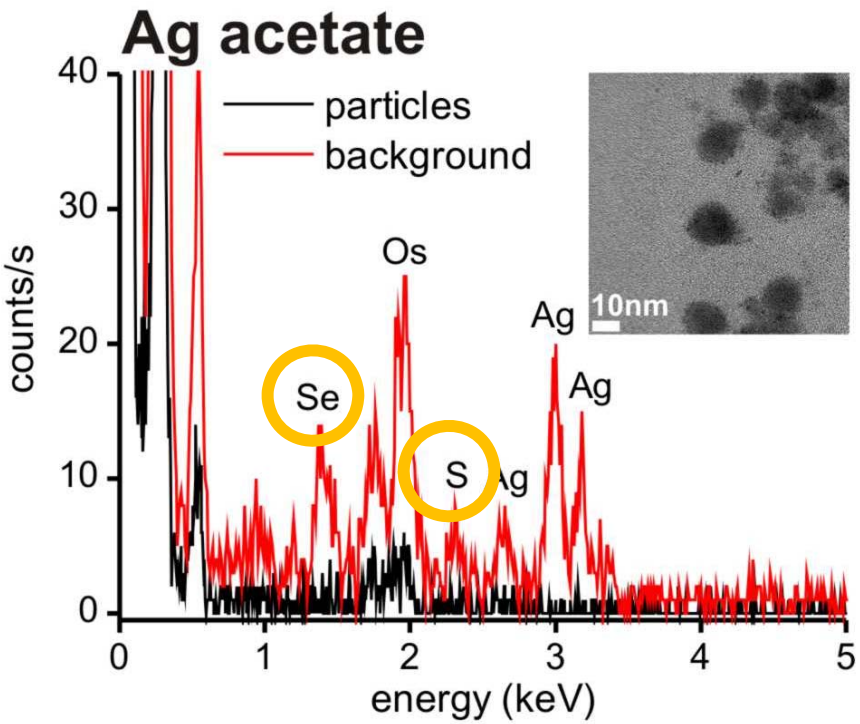
lysosome containing particles



particles in the basal lamina

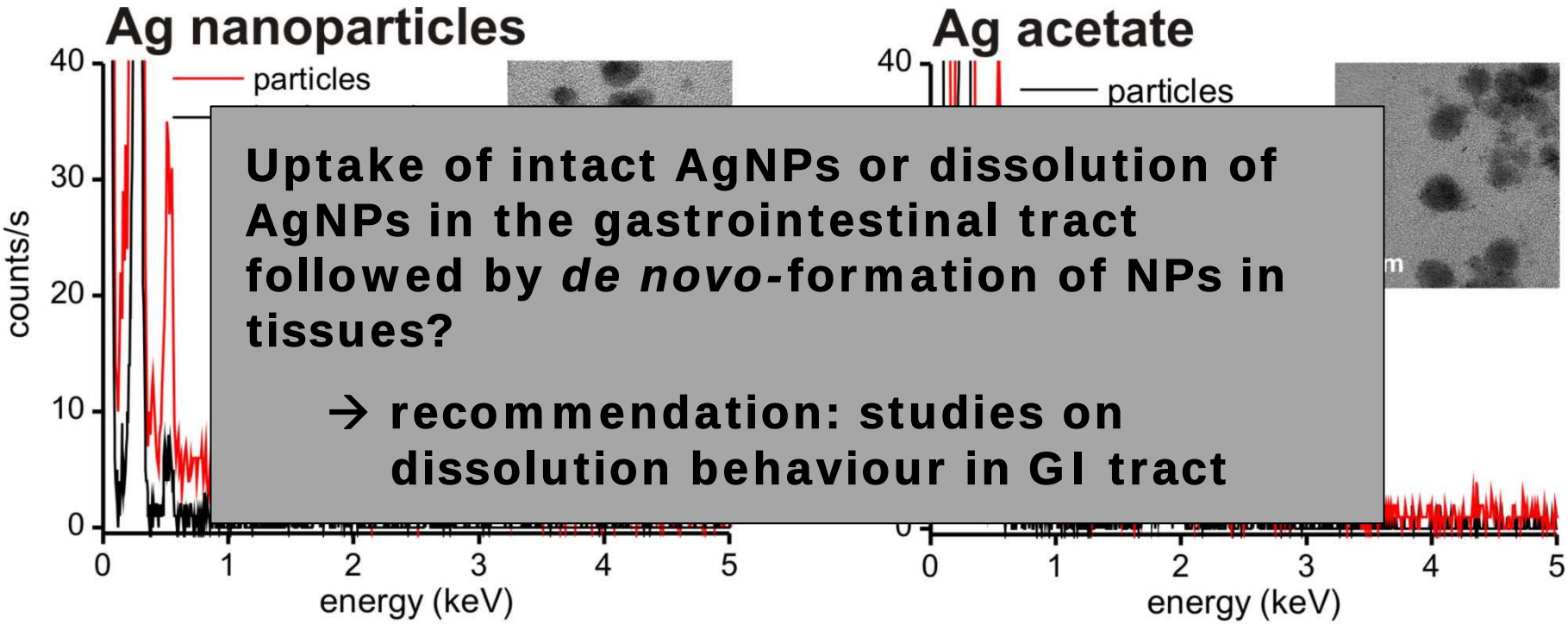
Transmission electron microscopy + energy dispersive X-ray spectroscopy (EDX)





particles have similar size and shape and consist of silver, sulfur and selenium (both, after exposure to AgNPs and Ag acetate)

Transmission electron microscopy + energy dispersive X-ray spectroscopy (EDX)

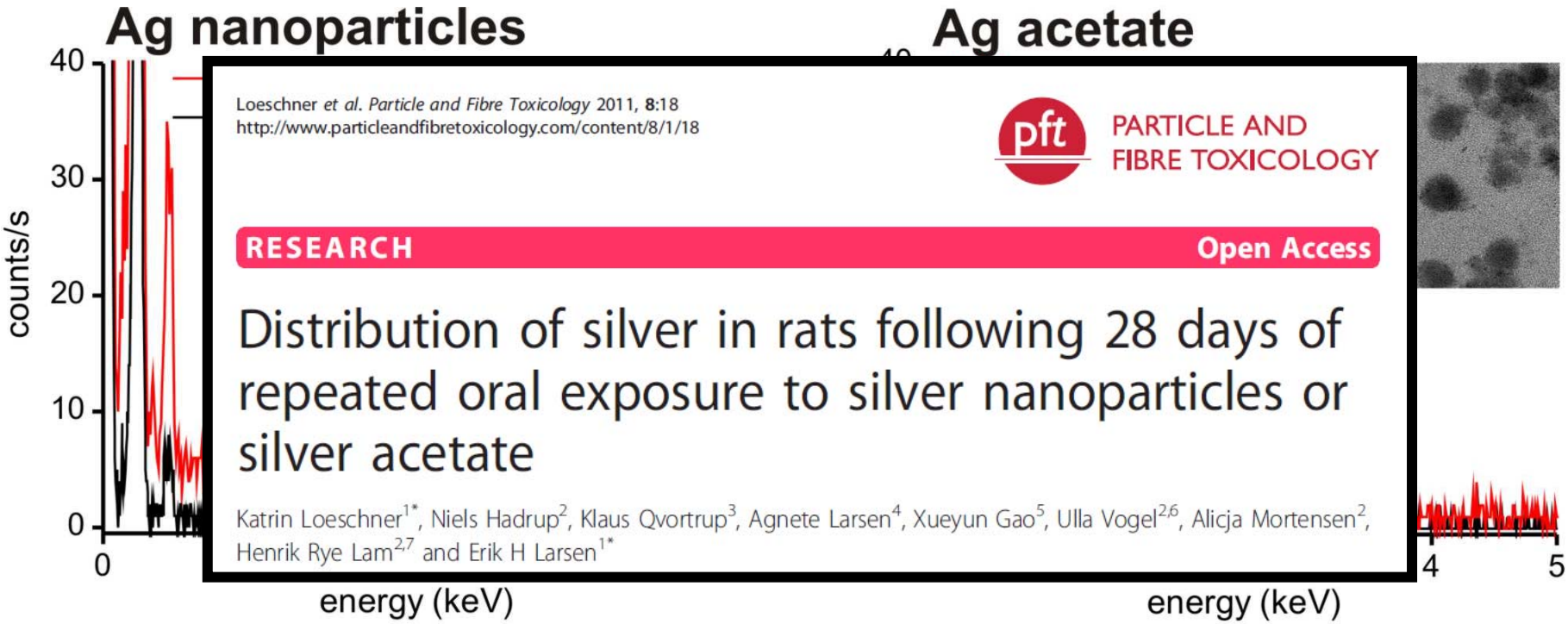


Uptake of intact AgNPs or dissolution of AgNPs in the gastrointestinal tract followed by *de novo*-formation of NPs in tissues?

→ recommendation: studies on dissolution behaviour in GI tract

particles have similar size and shape and consist of silver, sulfur and selenium (both, after exposure to AgNPs and Ag acetate)

Transmission electron microscopy + energy dispersive X-ray spectroscopy (EDX)



Loeschner *et al. Particle and Fibre Toxicology* 2011, **8**:18
http://www.particleandfibretoxicology.com/content/8/1/18

PARTICLE AND FIBRE TOXICOLOGY

RESEARCH Open Access

Distribution of silver in rats following 28 days of repeated oral exposure to silver nanoparticles or silver acetate

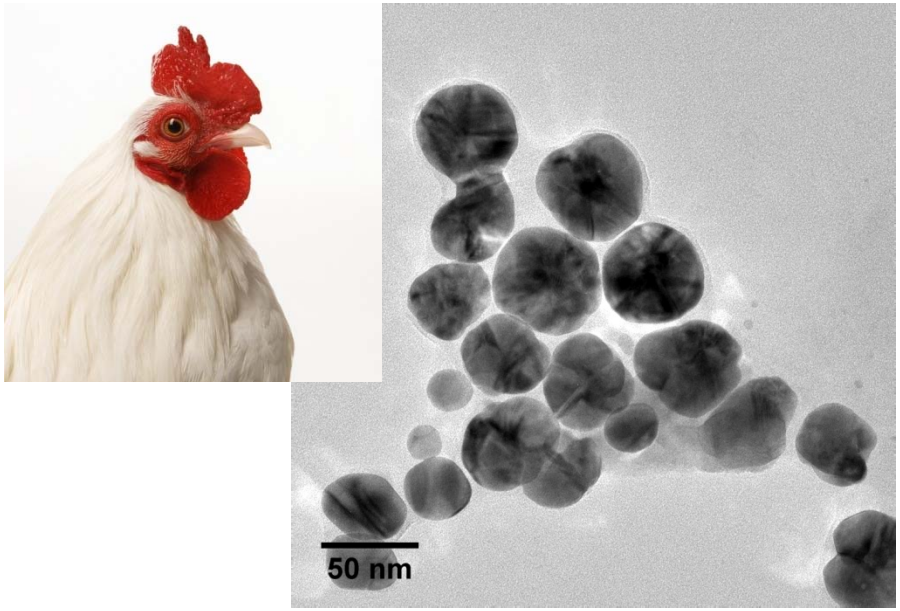
Katrin Loeschner[1*], Niels Hadrup[2], Klaus Qvortrup[3], Agnete Larsen[4], Xueyun Gao[5], Ulla Vogel[2,6], Alicja Mortensen[2], Henrik Rye Lam[2,7] and Erik H Larsen[1*]

particles have similar size and shape and consist of silver, sulfur and selenium (both, after exposure to AgNPs and Ag acetate)

NanoLyse Project (2010-2013)

"Nanoparticles in Food: Analytical methods for detection and characterisation"

Silver nanoparticles *in lean meat*

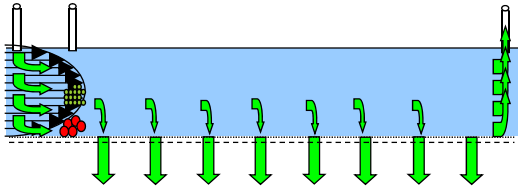


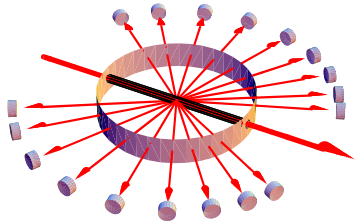
NANO LYSE

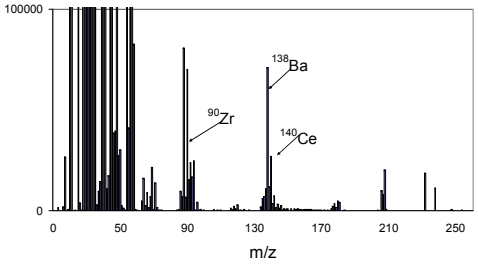
SEVENTH FRAMEWORK PROGRAMME

http://www.nanolyse.eu

The analytical platform







asymmetric flow field-flow fractionation

(AF⁴)

optical detection
multi angle (MALS) and dynamic light scattering (DLS), UV-vis absorption

inductively coupled plasma mass spectrometry

(ICP-MS)

particle separation
according to their size (1nm – few µm)

particle detection
(fractogram)

size determination

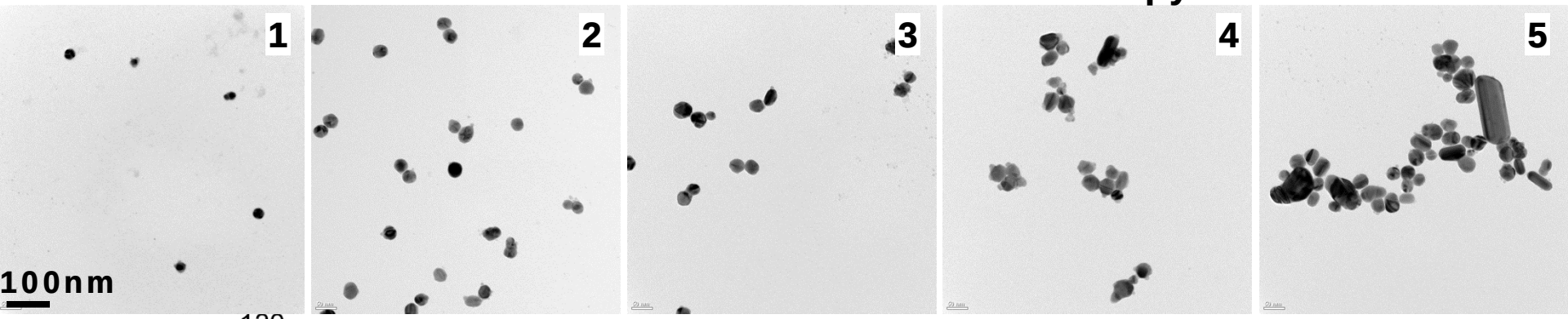
elemental detection
chemical identity

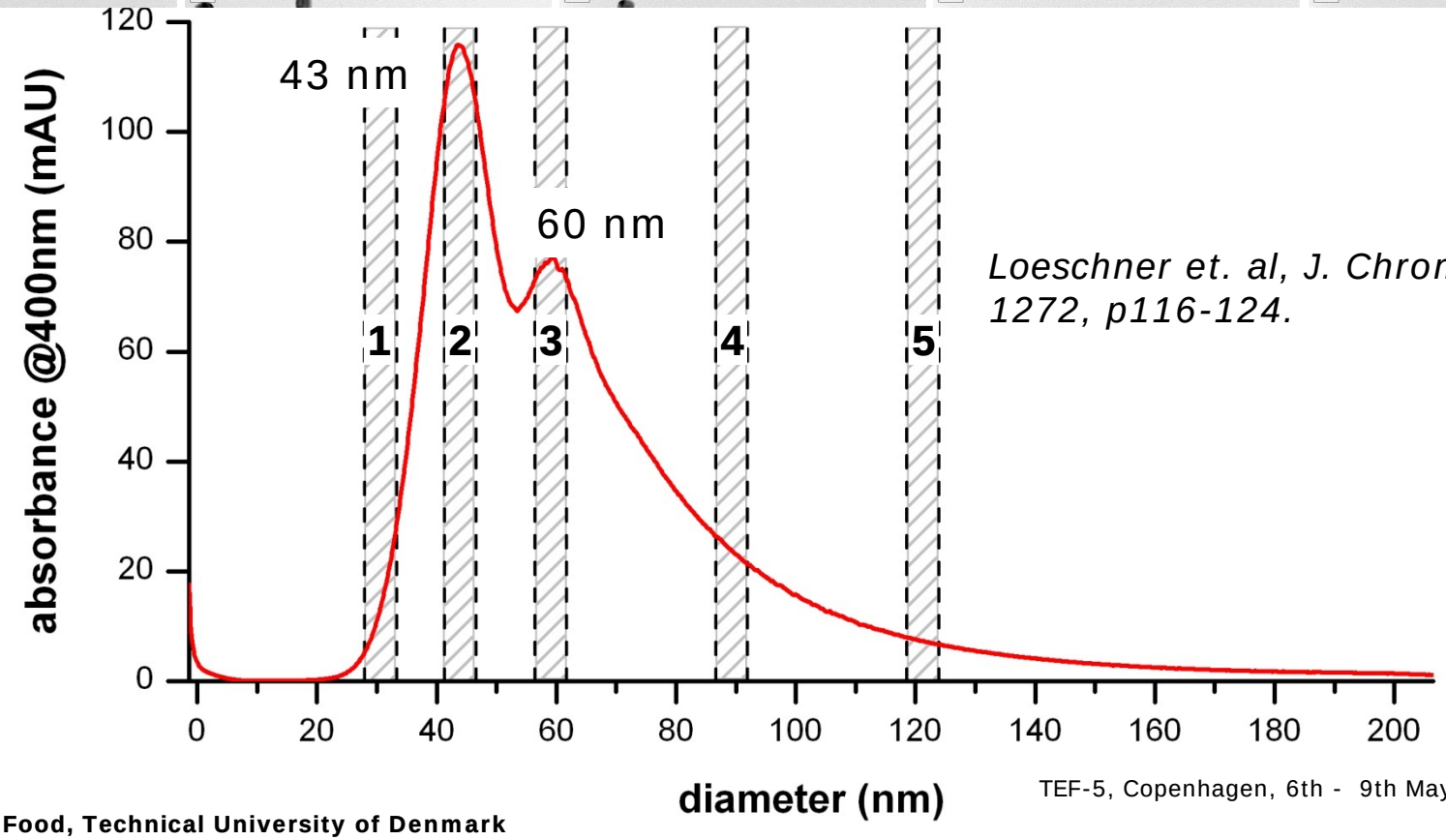
quantification

mass fraction

Separation of pristine AgNPs by AF⁴

+Fraction collection for transmission electron microscopy





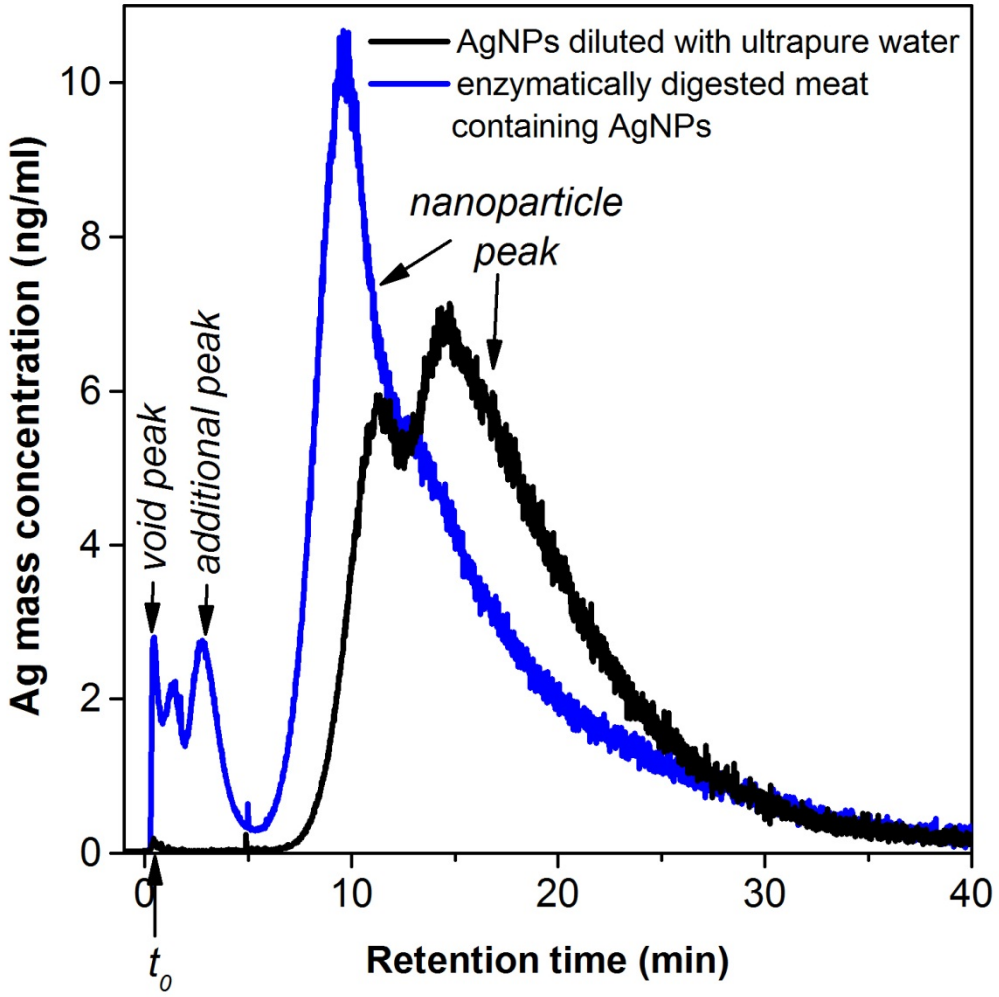
Loeschner et. al, J. Chrom. A (2013), 1272, p116-124.

Sample preparation

Water extraction or **filtration** - not efficient (strong affinity AgNPs/meat) → digestion of the meat matrix is essential

Enzymatic digestion - Proteinase K enzyme/meat (dry matter) ratio 1:5; succesful

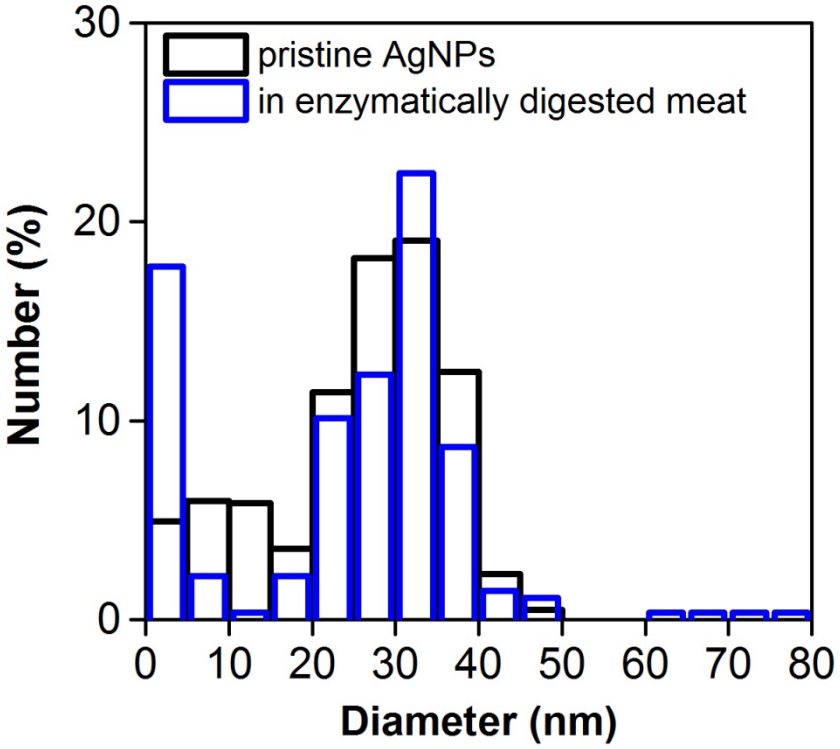
Separation of AgNPs from enzymatic digest by AF⁴-ICP-MS

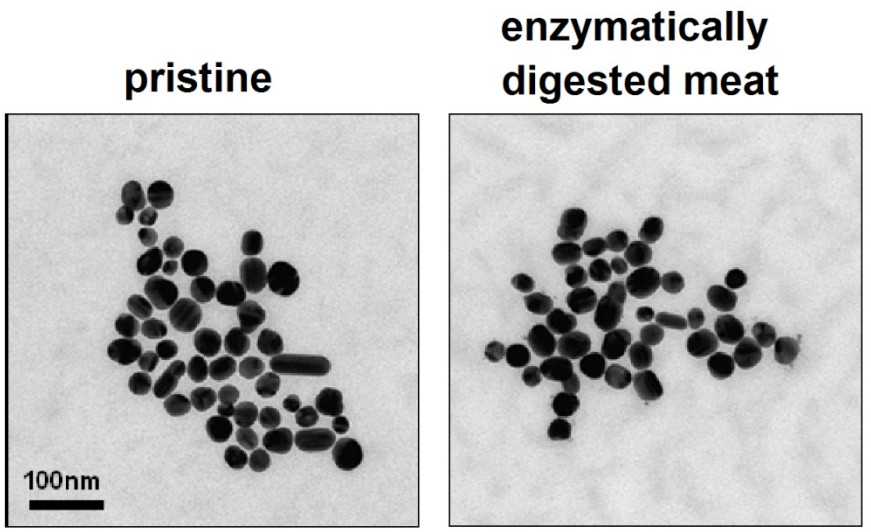


- Significant nanofraction (~80%) recovered
- Formation of additional peaks
- Pre-elution (~ 2 min) in comparison to pristine AgNPs

→ **Change of the NP size distribution (10 nm smaller NPs by dissolution)?**

TEM analysis of AgNPs before and after enzymatic digestion





No indication for a change of the particle size distribution!

Changed elution behavior of the NPs!

Anal Bioanal Chem (2013) 405:8185–8195
DOI 10.1007/s00216-013-7228-z

RESEARCH PAPER

Detection and characterization of silver nanoparticles in chicken meat by asymmetric flow field flow fractionation with detection by conventional or single particle ICP-MS

Katrin Loeschner · Jana Navratilova · Carsten Købler · Kristian Mølhave · Stephan Wagner · Frank von der Kammer · Erik H. Larsen

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enzymatically digested meat

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Food Chemistry 181 (2015) 78–84

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Analytical Methods

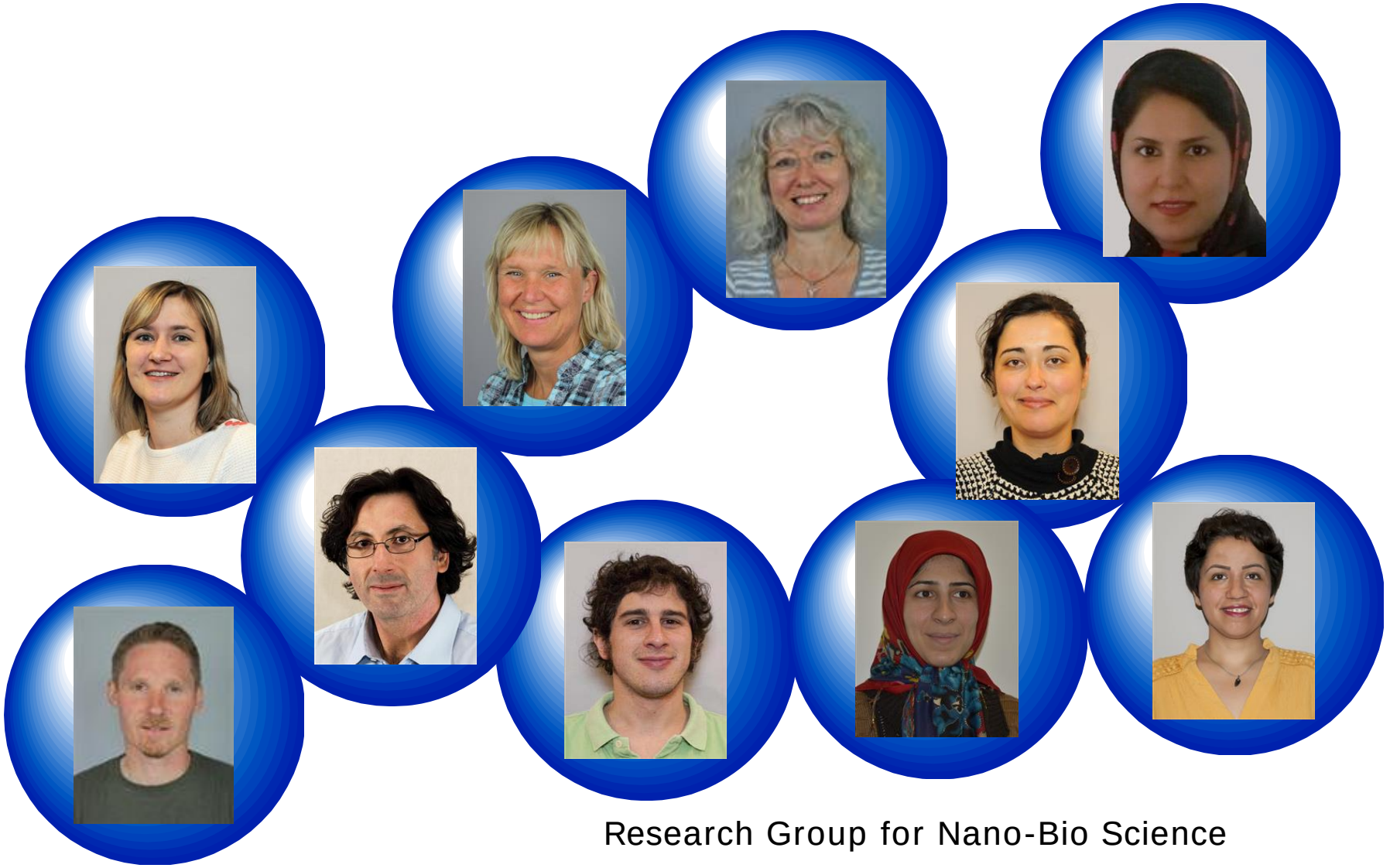
In-house validation of a method for determination of silver nanoparticles in chicken meat based on asymmetric flow field-flow fractionation and inductively coupled plasma mass spectrometric detection

Katrin Loeschner [a,*], Jana Navratilova [a], Ringo Grombe [b], Thomas P.J. Linsinger [b], Carsten Købler [c], Kristian Mølhave [c], Erik H. Larsen [a]

Conclusions and outlook

- Analysis of NMs in food and biological samples is tricky but possible (AgNPs are a good example)
- Sample preparation is often the most challenging step
- A combination of analytical techniques is usually required
- Current and future work:
  - screening of different foods for NPs
  - migration of NPs from food contact materials
  - nanoplastics
  - exploring (the limitations) of single particle ICP-MS
  - validated methods for food surveillance (TiO_2...)

Thank you for your attention!!!



Research Group for Nano-Bio Science