

Dissolution of Silver nanoparticles and fate of the released silver ions in hepatocytes revealed by a synchrotron nanoprobe

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Silver Nanoparticles (AgNPs)

Wilson Center (USA)

www.nanotechproject.org/cpi



442 products contain AgNP



BlueMoonGoods
™ Fresh Box
Silver
Nanoparticle
Food Storage
Containers

BACTERICIDAL EFFECT:

Release of toxic Ag(I) in the vicinity of bacteria



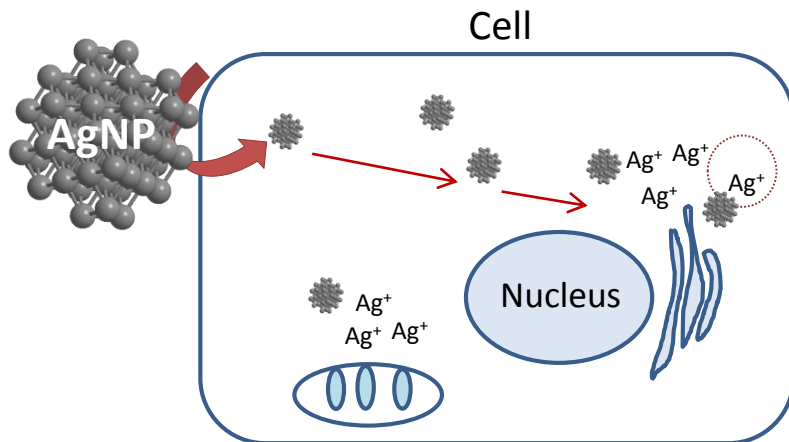
Xiu et al. NanoLetters (2012)

CYTOTOXIC EFFECT:

- AgNPs enter cells
- Release of toxic Ag(I)
- Correlated to *in vitro* dissolution



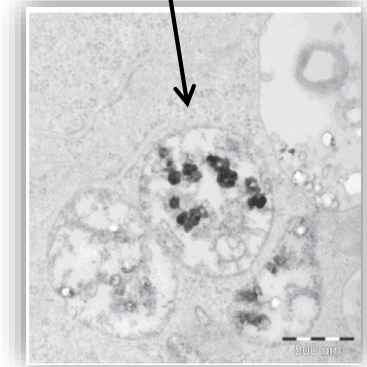
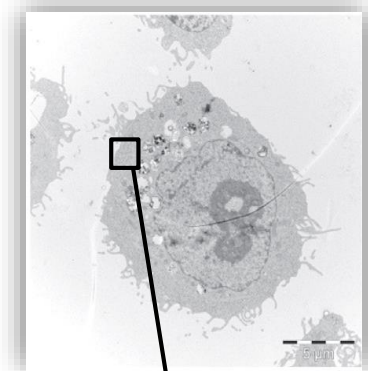
Liu et al. ACS Nano (2012)



?

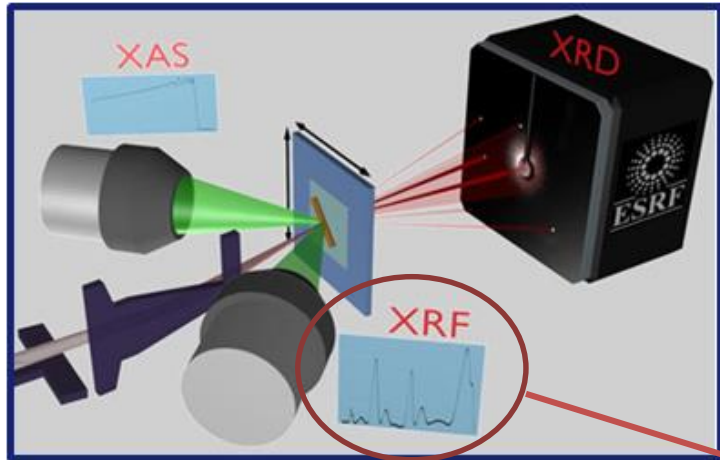
Intracellular dissolution
Ag(I) trafficking

Hepatocytes
(liver target organ)



Gliga et al. P&FT (2014)

Synchrotron Nanoprobe



- X-ray beam focussed to sub-micrometric size
- Multi-analytical platforms

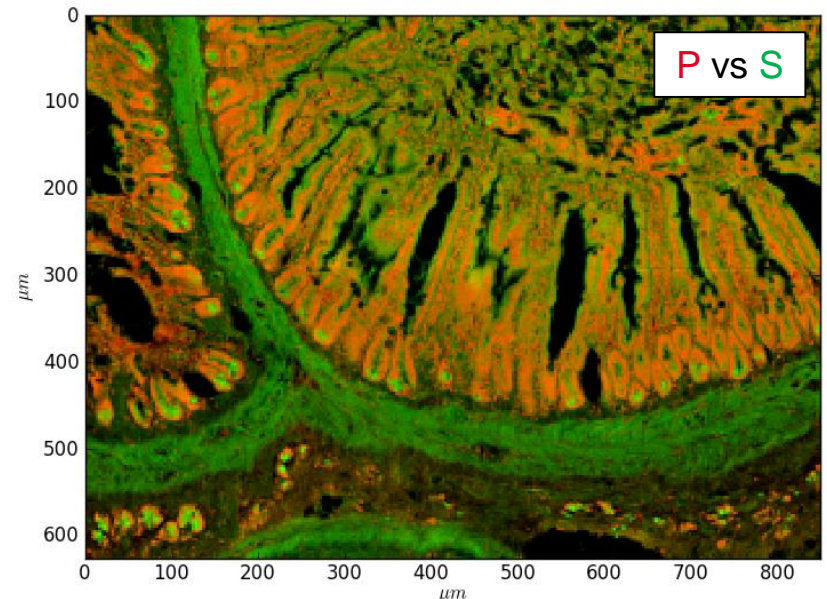


ID16B @ESRF

Resolution: $50 \times 50 \text{ nm}^2$

X-Ray Fluorescence (XRF) imaging

- Hyperspectral imaging (1 XRF spectrum/pixel)
- Provides elemental distributions



Collaboration with Anna Bencsik, ANSES, Lyon

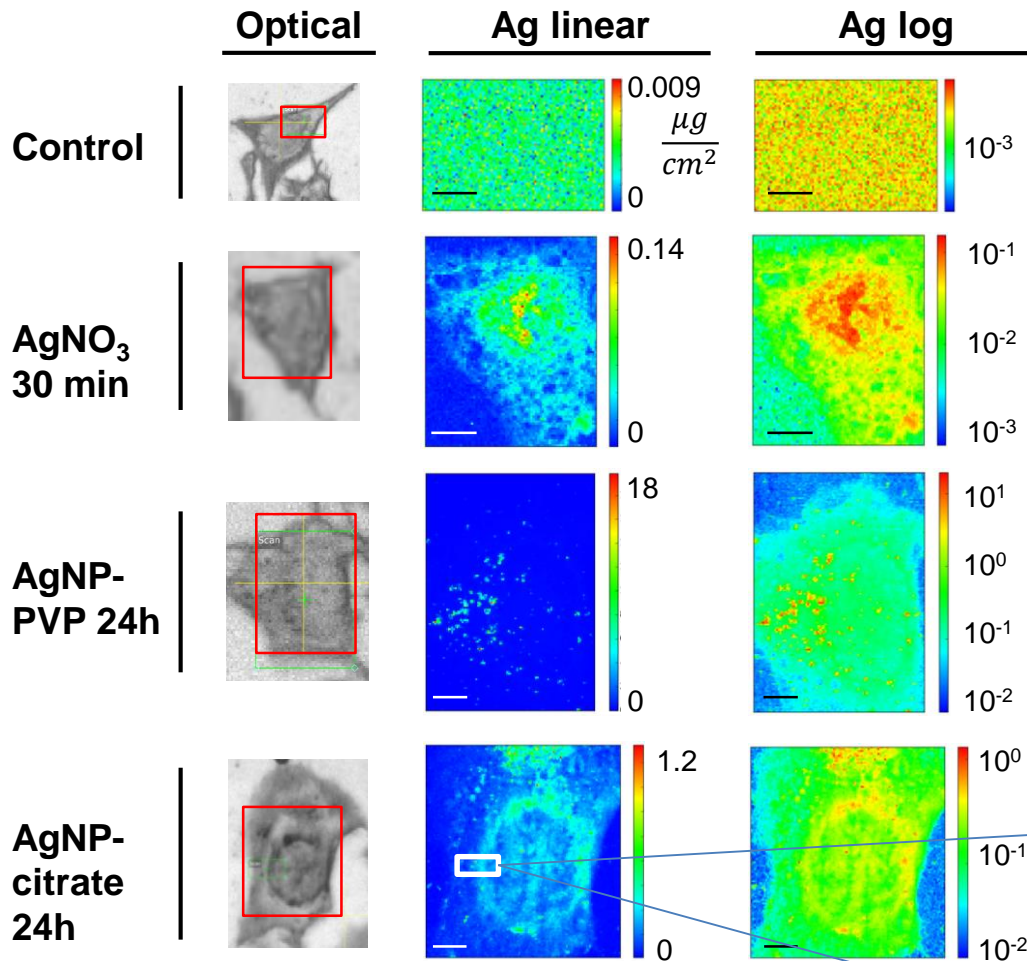
ID21 @ESRF. Resolution: $500 \times 500 \text{ nm}^2$

Visualization and quantification of Ag(I)

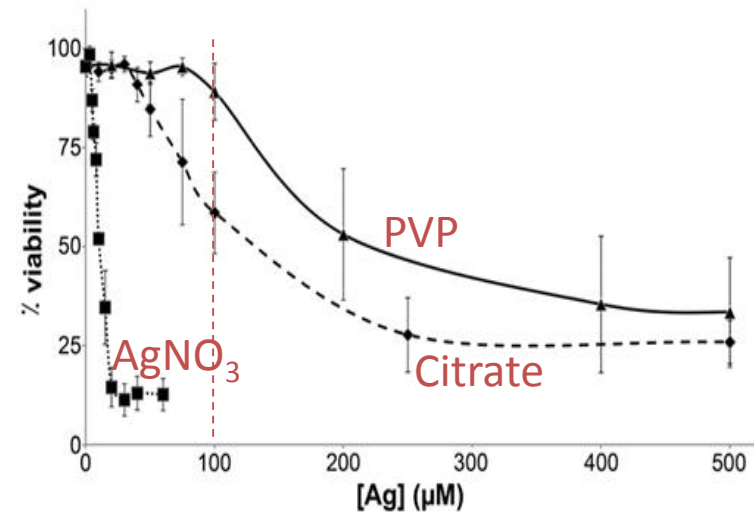
Intracellular dissolution
in HepG2 hepatocytes



Cytotoxicity

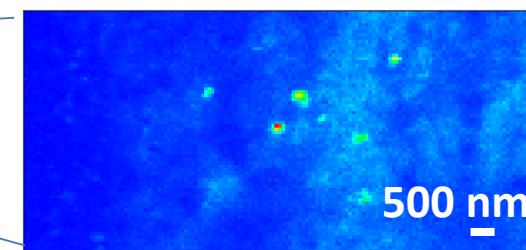


Scale bars = 5 μm

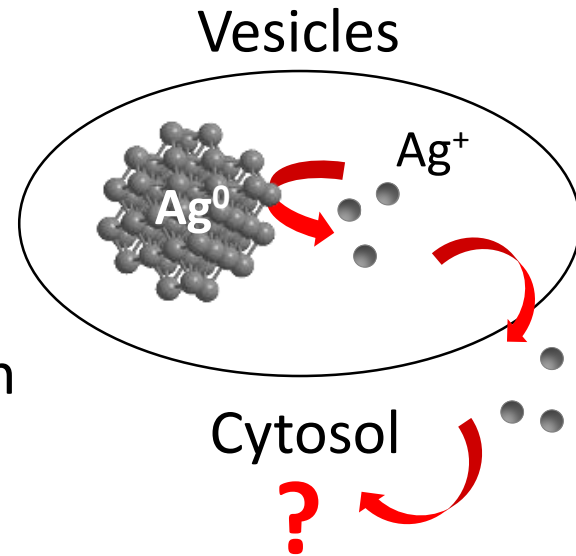
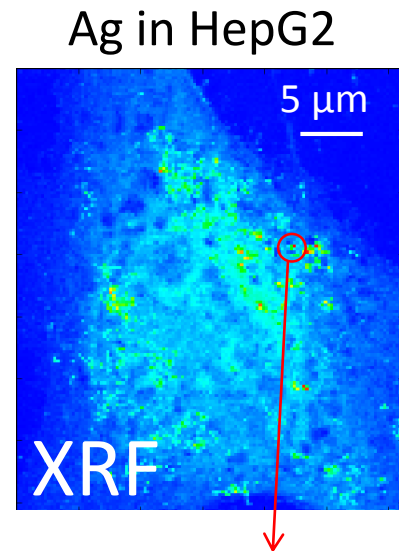
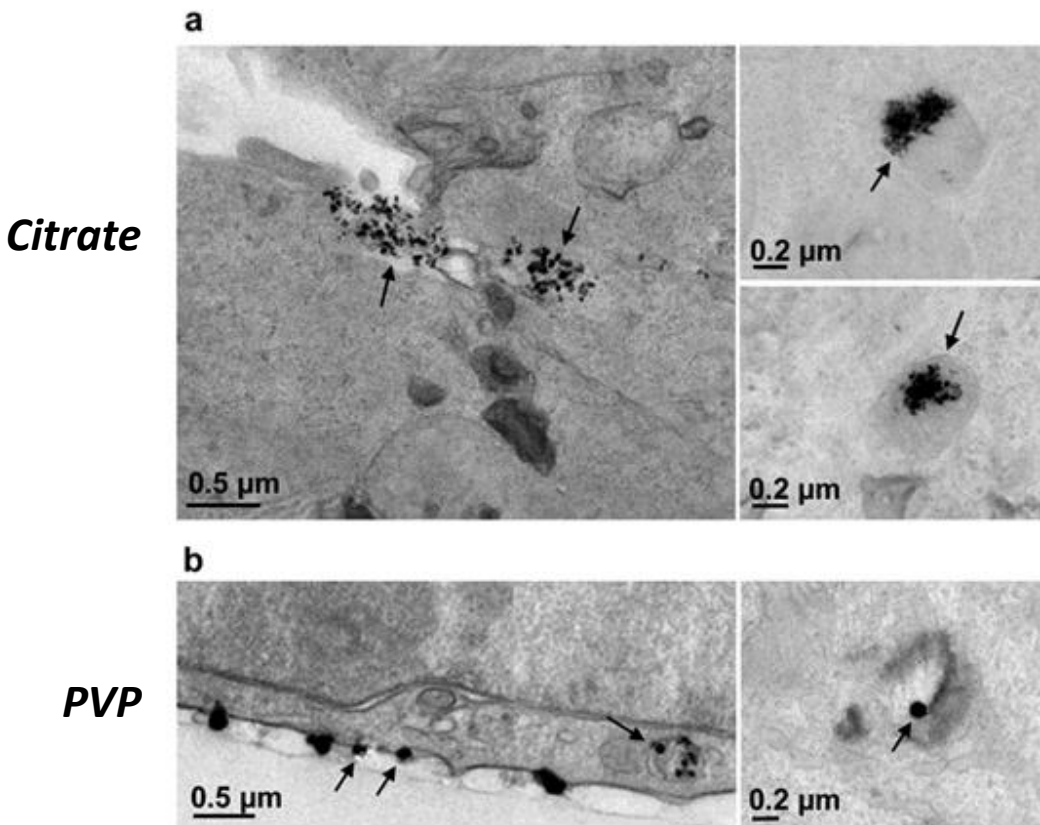


Correlation between
intracellular dissolution
and cytotoxicity

70x70 nm² resolution



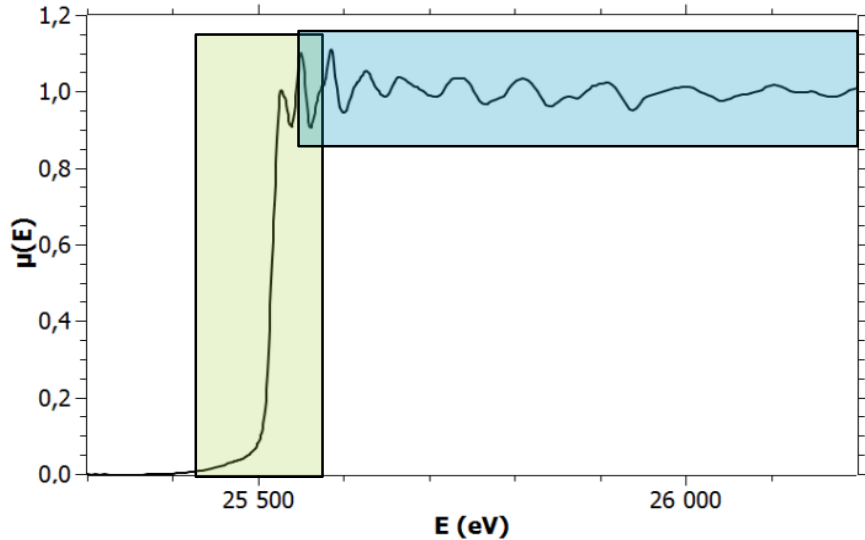
TEM observations



NanoXRF + TEM reveal that AgNPs dissolve in endocytic vesicles and release Ag in cytosol

➔ Chemistry of released Ag(I) ?

X-ray Absorption Spectroscopy (XAS)



XANES: X-ray Absorption Near-Edge Structure
EXAFS: Extended X-ray Absorption Fine Structure

XANES analysis:

linear combination of spectra of reference compounds



- Measure fraction of dissolved Ag
- Retrieve Ag(I) species

EXAFS analysis:

Ab initio fitting of spectra

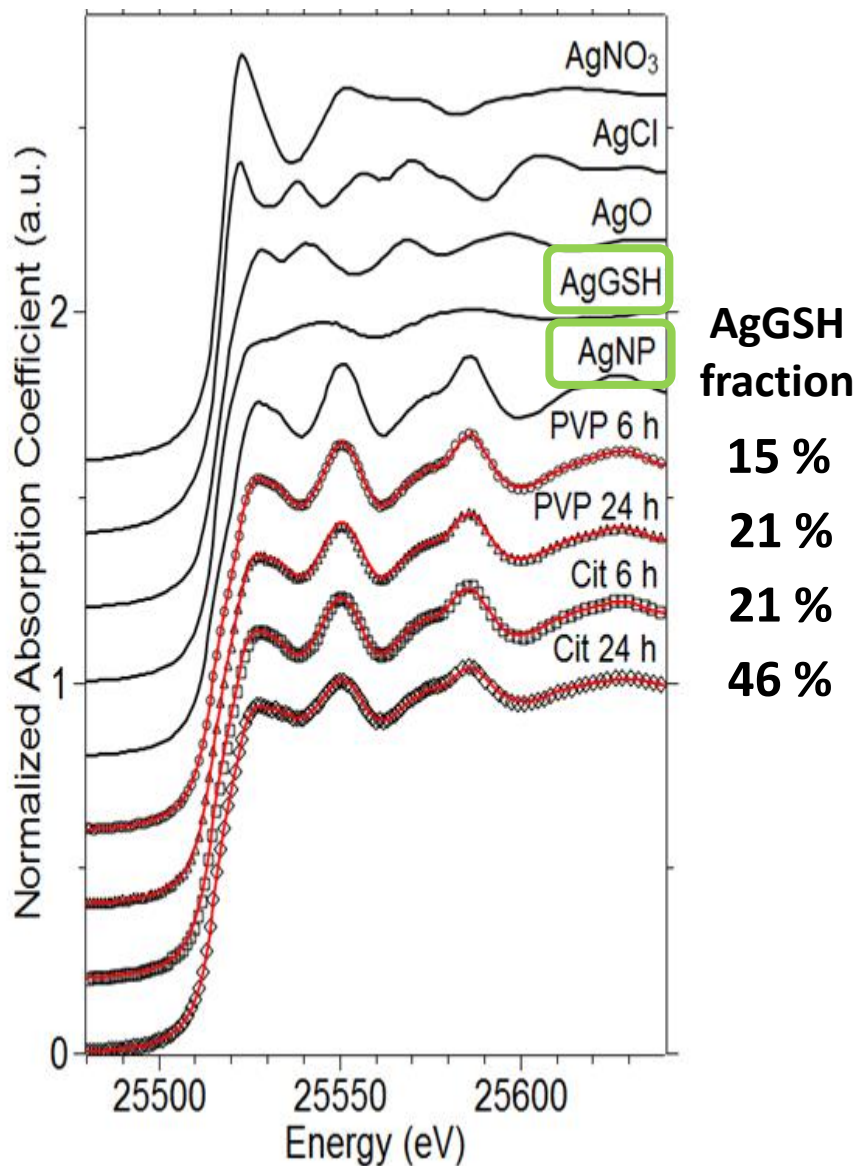


- Characterize Ag(I) coordination sphere (geometry and bond lengths)



@ FAME-BM30B
ESRF

Measuring dissolution rates *in cellulo*



Experimental protocol

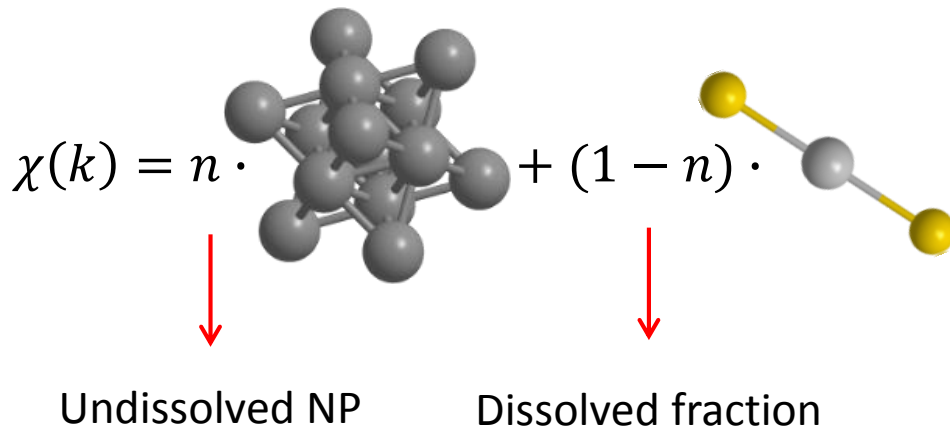
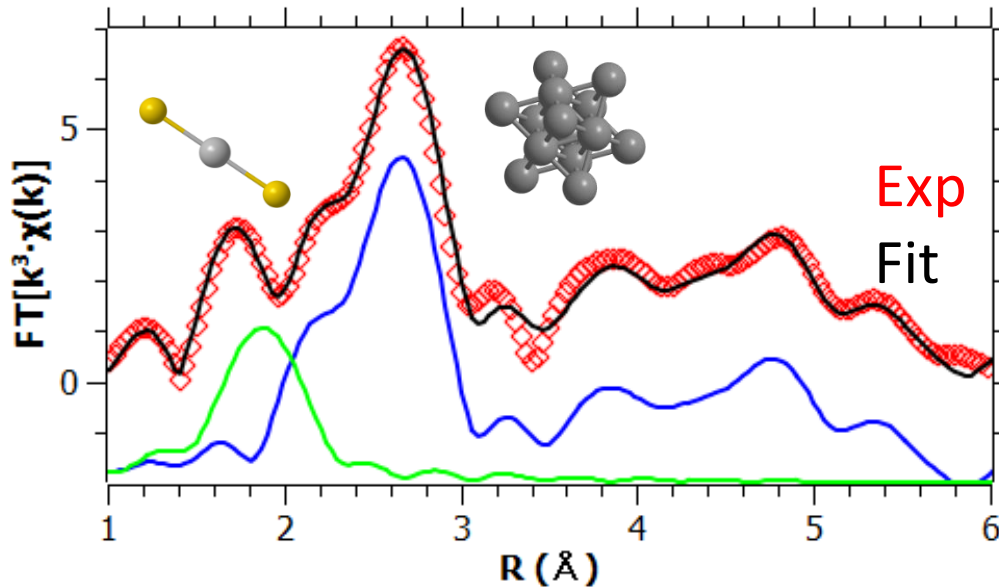
- Expose cell culture to AgNP
- Wash and centrifuge
- Recover **cell pellet** ($\sim 10^7$ cells)
- Resuspend, pour a drop on XAS sample holder
- **Freeze in liquid N₂**
- Acquire XAS spectra

Linear combination fitting of XANES



- Dissolution is faster for citrate- than for PVP-coated AgNPs
- Ag-S species are formed

Disclosing Ag(I) coordination *in cellulo*



Use of a model
Measure Ag-S distance *in cellulo*

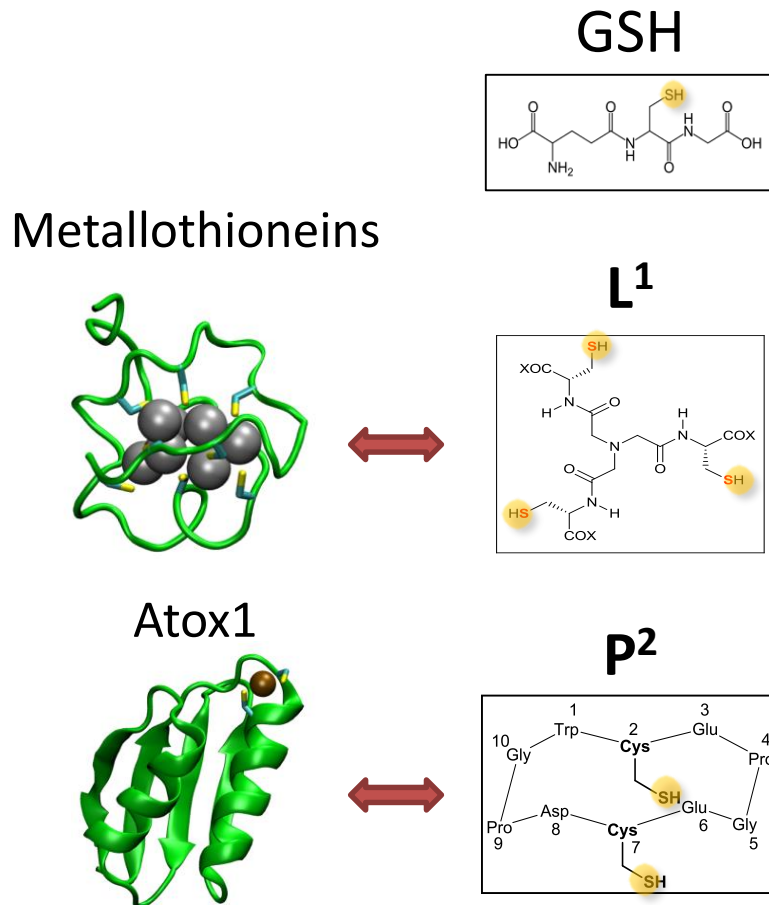


**Circumvent the problem
of separation**

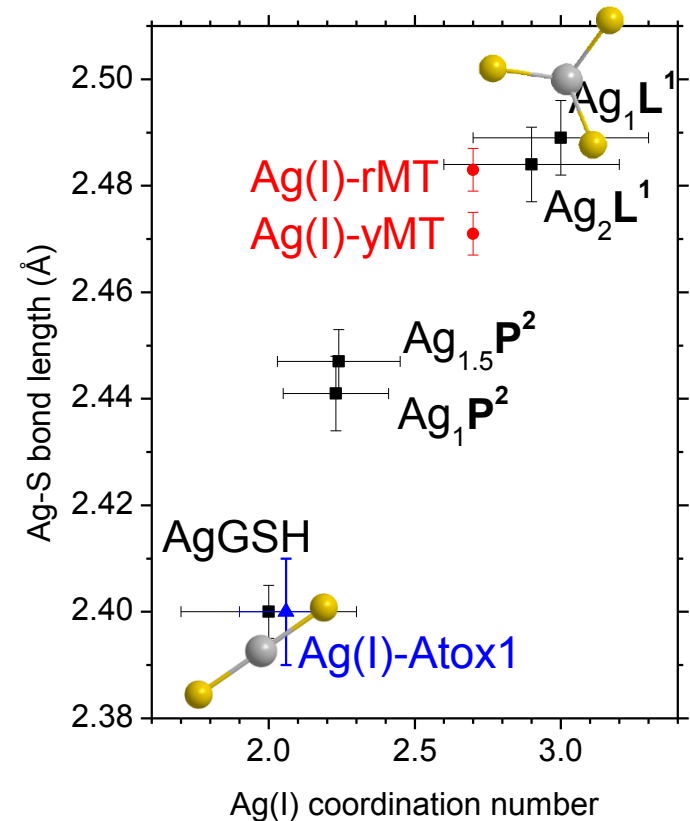
Veronesi et al., *Nanoscale* **2015**, 7, 7323.

Ag(I) binding to biological Cu(I) sites

- XAS study of Ag(I) complexation in biological Cu(I)-thiolate sites.
- Solution, pH 7.4

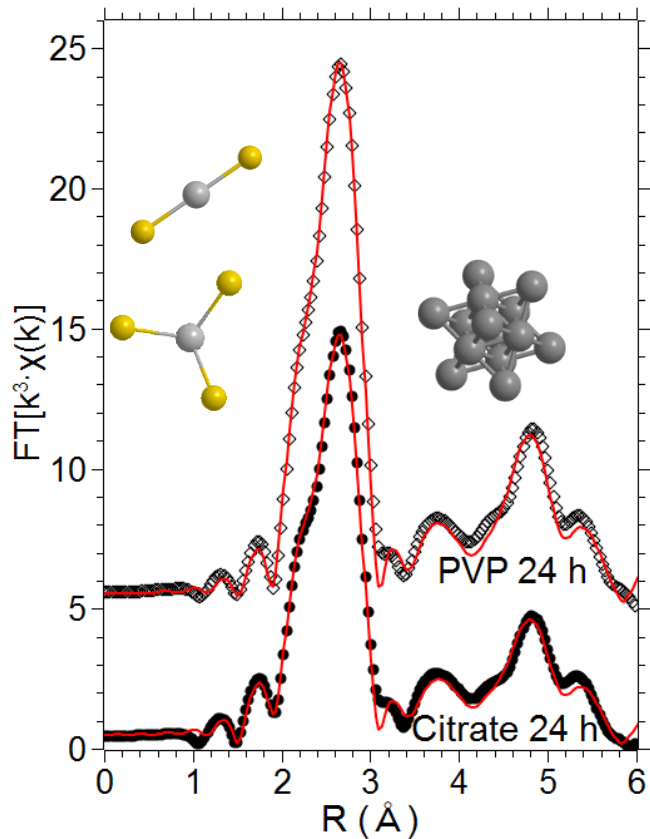


Correlation between coordination number and Ag-S bond length

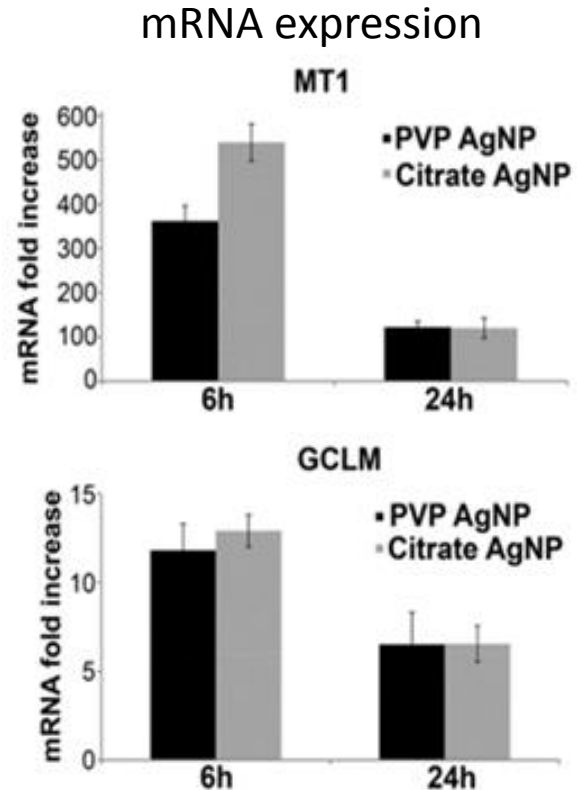


Veronesi et al. *Inorg. Chem.* **2015**, 54, 11688.

Disclosing Ag(I) species formed *in cellulo*



$\text{Ag-S} = 2.45 \text{ \AA}$ → Mixed $\text{AgS}_2/\text{AgS}_3$

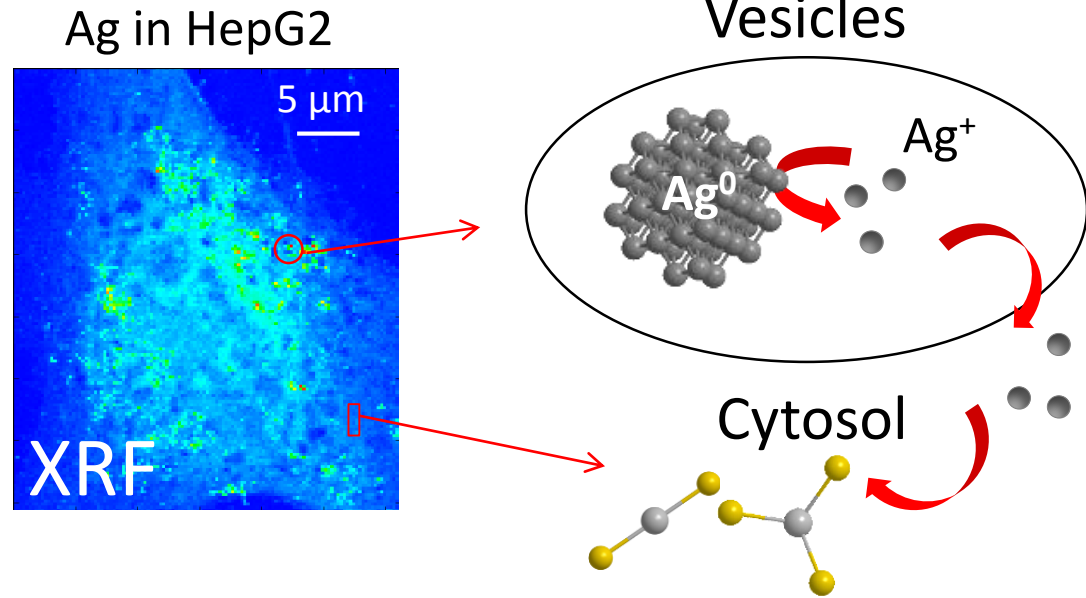


Overexpression of metallothionein 1 (MT1) and glutamate-cysteine ligase (GCLM)

Ag(I) binds to
GSH and Metallothionein

Conclusions

- Visualization and quantification of Ag released from AgNPs in hepatocytes
- Influence of the coating on intracellular dissolution
- Ag(I) binding in Cu(I) sites → perturbation of metal homeostasis



Veronesi et al, *Nanoscale* **2016**, 8, 17012

Synergistic use of advanced imaging, atomic spectroscopy, and molecular biology



FATE of metallic NPs in cells

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Thank you for your attention!

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