

Supplementary information:

Emerging investigator series: Linking Chemical Transformations of Silver and Silver Nanoparticles in the Extracellular and Intracellular Environment to their Bio-reactivity.

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SUPPLEMENTARY METHODS

Silver species distributions: Silver ion species were calculated in the exposure media Leibovitz's L-15, L-15/ex and Versene using Visual MINTEQ (Visual MINTEQ, ver. 3.1, 2011, KTH, Sweden). The media pH was set to 7.2 and the temperature to 19°C. Simplified speciation calculation for L-15 took into account Ag-cysteine complexes and complexes with the other amino acids by considering the total amino acid concentration as glycine. The constants for Ag-cysteine complexes according to ⁴³(Adams & Kramer, 1999), were added to the original Visual MINTEQ database as previously described⁹.

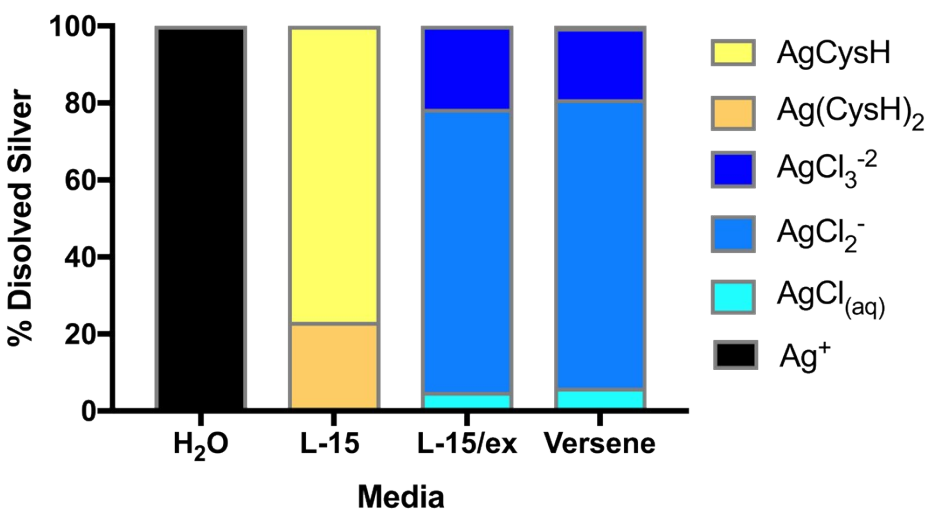


Figure S1: Silver ion species distribution in water, Leibovitz' L15 (L-15), L-15/ex and Versene solution calculated using Visual MINTEQ version 3.1 assuming an initial concentration of silver of 1 μM ; at initial concentration of ionic silver between 0.1 and 100 μM , differences in dissolved silver species distribution were negligible.

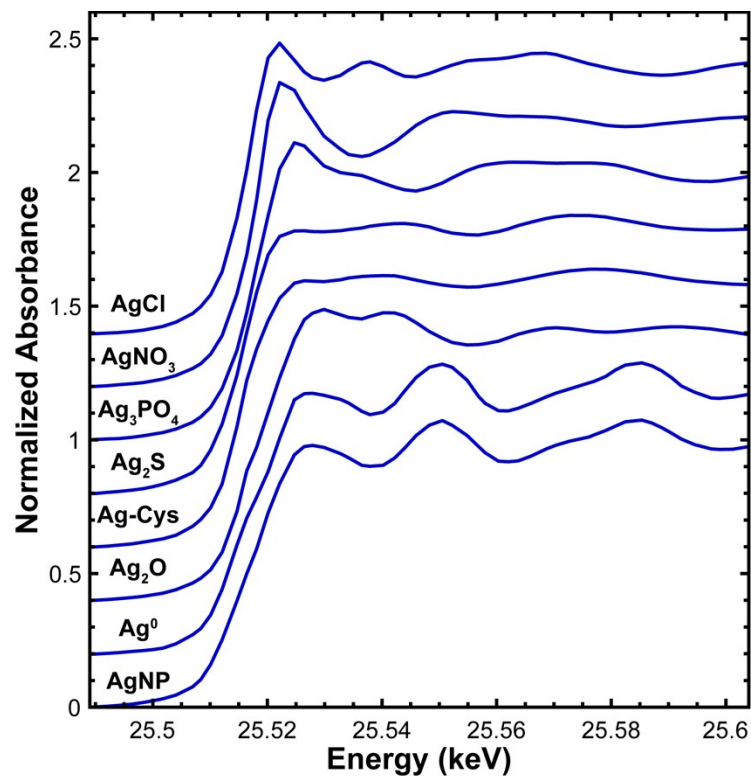


Figure S2: Ag K-edge XANES spectra of silver reference compounds; Ag-cys is silver complexed to cysteine.

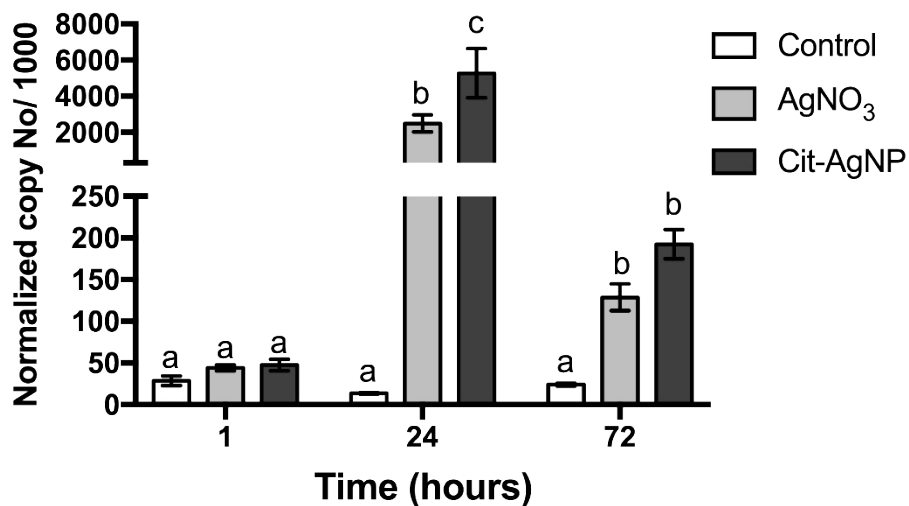


Figure S3: Normalized Metallothionein B (MTb) mRNA expression measured in RTgutGC cells exposed to 1 μ M AgNO₃ or 10 μ M cit-AgNP in L-15/ex medium for 1, 24 and 72 hours. Values

are means \pm SEM (n=3). Statistical difference from respective controls for each time point is indicated by different lettering (p< 0.05, 2-way ANOVA, Tukey's test).

Table S1. Speciation of Ag in RTgutGC determined by XANES spectroscopy.

Exposure Time	Ag K $_{\alpha}$ (cps) ^a	AgCl (mol%)	Ag-Cys (mol%)	AgNP (mol%)	Component Sum	R-factor ($\times 10^4$) ^b	χ^2_v ($\times 10^4$) ^b
<i>AgNP</i>							
1 h	510 \pm 30 ^c	78 \pm 4 ^d	4 \pm 6	18 \pm 4	1.00	8.49	1.31
24 h	1050 \pm 20	0 \pm 2	7 \pm 4	93 \pm 2	1.00	3.11	0.48
72 h	2910 \pm 10	0 \pm 2	5 \pm 3	95 \pm 2	1.00	2.02	0.32
<i>AgNO₃</i>							
1 h	220 \pm 10	88 \pm 6	12 \pm 7	-	1.00	29.21	4.41
24 h	201 \pm 7	100 \pm 7	0 \pm 7	-	0.99	32.36	4.84
72 h	400 \pm 10	6 \pm 4	94 \pm 4	-	1.00	8.29	1.28

^a Ag K $_{\alpha}$ fluorescence counts per second (cps), proportional to concentration.

^b Goodness-of-fit parameters²⁵.

^c Reported uncertainty is the standard deviation of the change in X-ray fluorescence intensity across among the individual scans that were averaged to obtain the reported data.

^d The uncertainty in the percentage of species was determined in the linear-combination fitting procedure and are reported at the 1 σ level (68% confidence interval).