

Supplementary data for:

Different in vitro exposure regimens of murine primary macrophages to silver nanoparticles induce different fate of nanoparticles and different toxicological and functional consequences

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Supplementary Material and Methods:

RT-qPCR analysis of metallothionein gene expression

Total RNA was isolated from Ag-NP-exposed cells using Nucleospin RNA II kit (Macherey-Nagel, France). Reverse transcription was performed using the Enhanced Avian HS RT-PCR cDNA synthesis kit (Sigma, France) according to the manufacturer's instructions. LuminoCTTaq polymerase (Sigma, France) was used for qPCR, using the following primers: Metallothionein I (MT-I) forward 5'-agctcctgctgcaagaac-3'; MT-I reverse 5'-tcaggcacagcacgtcactt-3'; Metallothionein II (MT-II) forward 5'-acccaactgctcctgtg-3'; MT-II reverse 5'-actgtcggaagcctctttg-3'. Amplification was performed in a Cfx96 thermocycler (Bio-Rad, France) with the following cycling program: 95 °C for 20 s, followed by 40 cycles at 95 °C for 5 s and 60 °C for 20 sec. At the end, PCR products were dissociated by incubating at 95 °C. These assays were run in triplicate and data were comparatively analyzed using Cfx software (Bio-Rad Cfx manager).

Figure S1. Physico-chemical characterization of the Ag-NPs. Ag-NPs were imaged by scanning electron microscopy (SEM) (A, B); their diameter was deduced from measurement of particle diameter on SEM images (C).

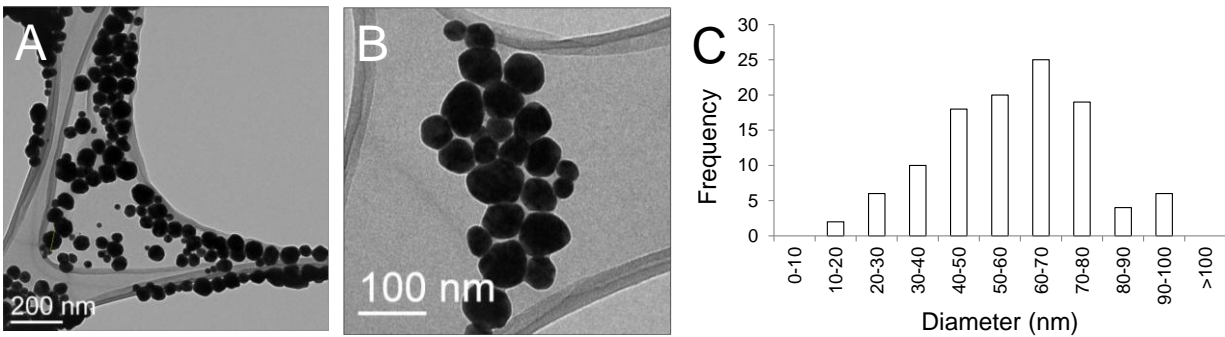


Table S1. Stability of Ag-NPs^a

	Diameter \pm SEM	% polydispersity \pm SEM
Day 0	87.40 \pm 0	22.3 \pm 0
Day 1	84.40 \pm 0.1	22.5 \pm 0
Day 2	69.53 \pm 3.72	26.5 \pm 1.9
Day 3	84.20 \pm 1.70	21.1 \pm 1.6
Day 4	81.13 \pm 1.0	22.6 \pm 0.3

^aNPs were diluted in sterile culture medium at the concentration of 5 μ g/mL and their hydrodynamic diameter, as well as the percentage of polydispersity of the suspension, were measured once a day during 4 days by DLS.

Figure S2. Cytotoxicity of Ag-NPs. Primary macrophages were exposed for 24h to increasing concentrations of nanoparticles and cell viability was checked by the trypan blue exclusion assay. Results are expressed as mean \pm SEM.

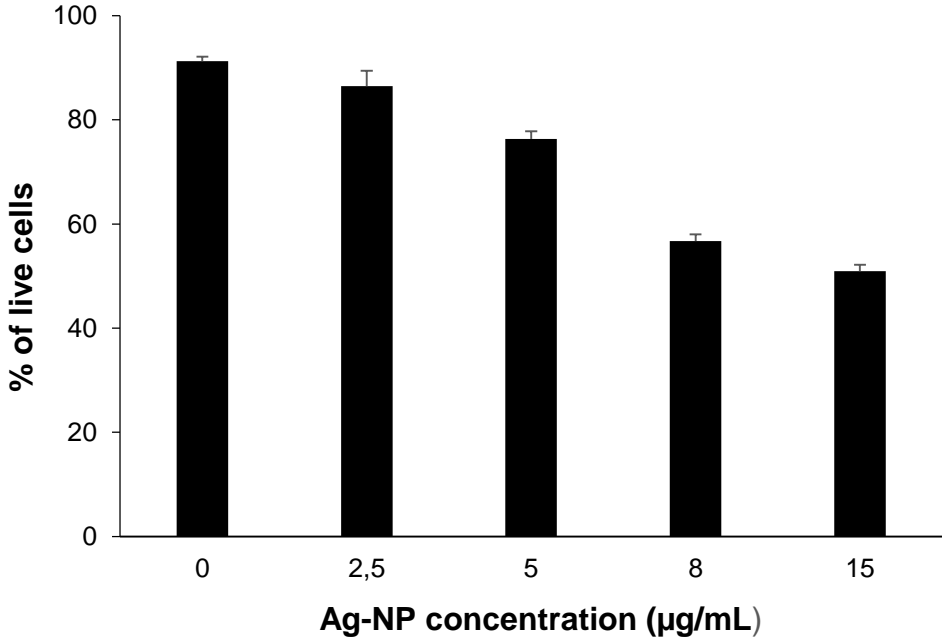


Figure S3. Metallothionein gene expression. mRNA expression of metallothionein I (MT-I) and metallothionein II (MT-II) in exposed cells was analyzed by RT-qPCR and normalized to mRNA expression of MT-I and MT-II in unexposed cells. Results are expressed as means \pm SEM.

