Label-free TOF-SIMS imaging of sulfur producing enzymes inside microglia cells following exposure to silver nanowires

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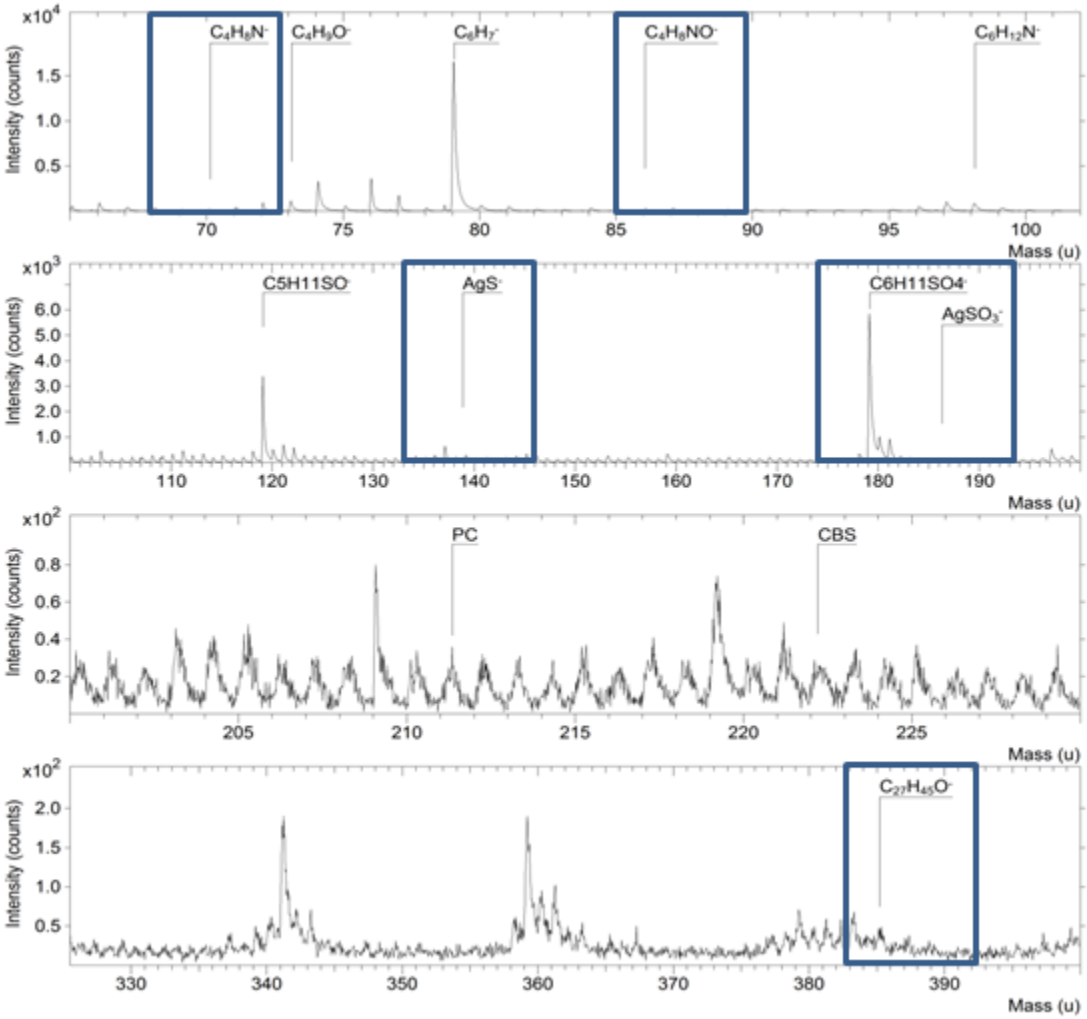


Fig. S1: Secondary-ion spectrum (negative polarity) recorded from AgNWs in microglia cells after sputtering with C60+.

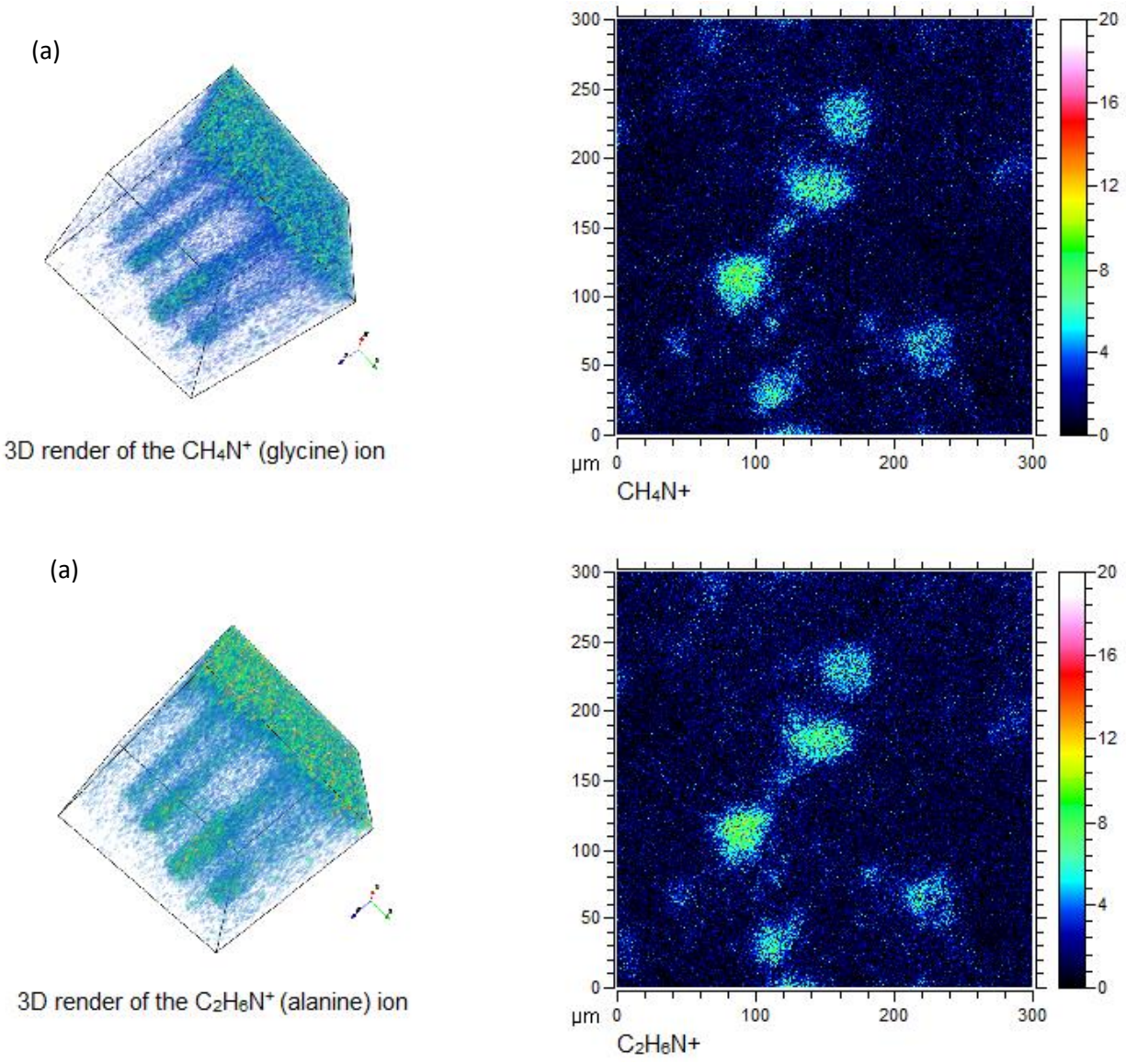


Fig. S2: 3D and 2D ion map of (a) the glycine protein fragment and (b) the alanine protein fragment during the 'sputter clean' with the C60+ ion beam

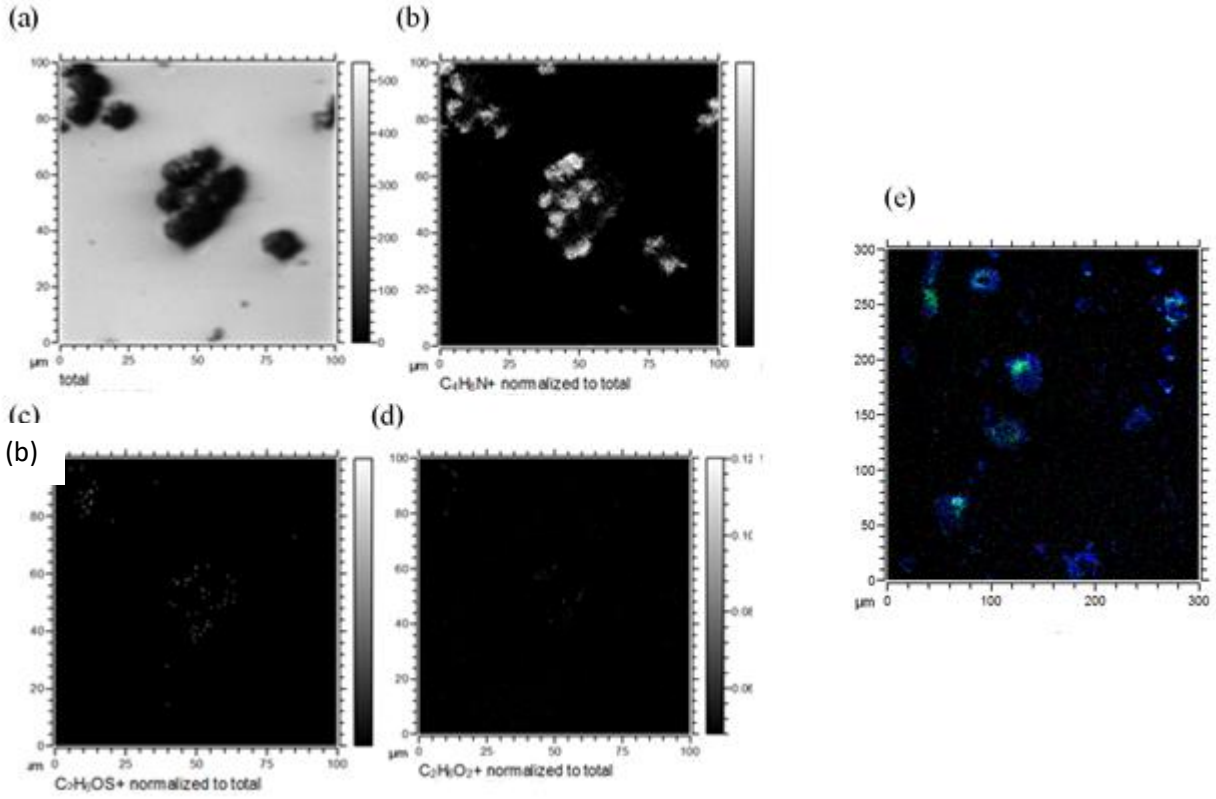


Fig. S3: TOF-SIMS imaging (positive ion mode) of (a) microglia cells (24 h) exposed to the AgNWs, pooled signals of (b) amino acid fragment ions, (c) CSE or MPST enzymes fragment ions, C2H6OS+ and (d) CBS enzyme fragment ions, C2H6O2+. Unlike CBS, the other two H2S producing enzymes (CSE or MPST) do not have metal binding ligands but contain S- groups in their ligands (Tables S2-4). Colour scale bars, with amplitude as number of counts are indicated to the right of each ion image. The amplitude of the colour scale corresponds to the maximum number of counts (mc) and the total number of counts (tc) recorded for the specified m/z (it is the sum of counts in all pixels). Field of view: 100 µm x 100 µm; (e) Overlay images of protein fragments (blue) and cystathionine fragments (green).

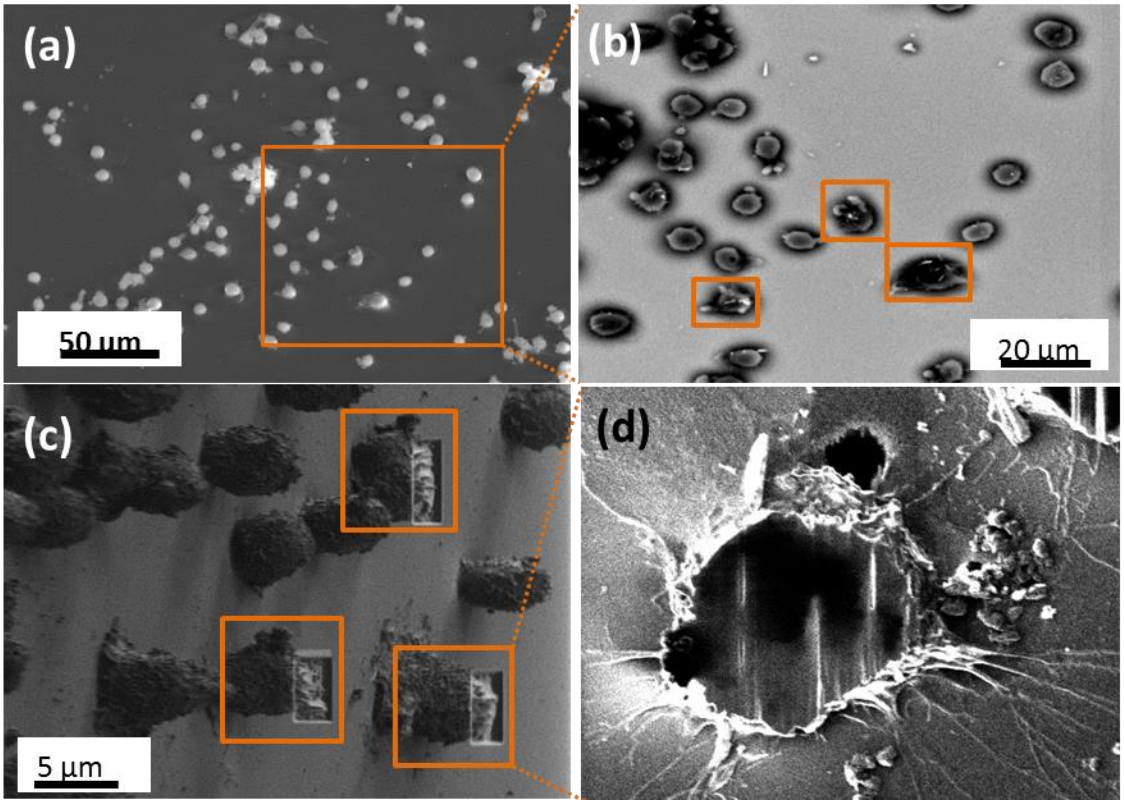


Fig. S4: SEM images of microglial cells exposed to AgNWs before FIB milling at (a) low and (b) high magnification and (c) side view and (d) top view after FIB milling.

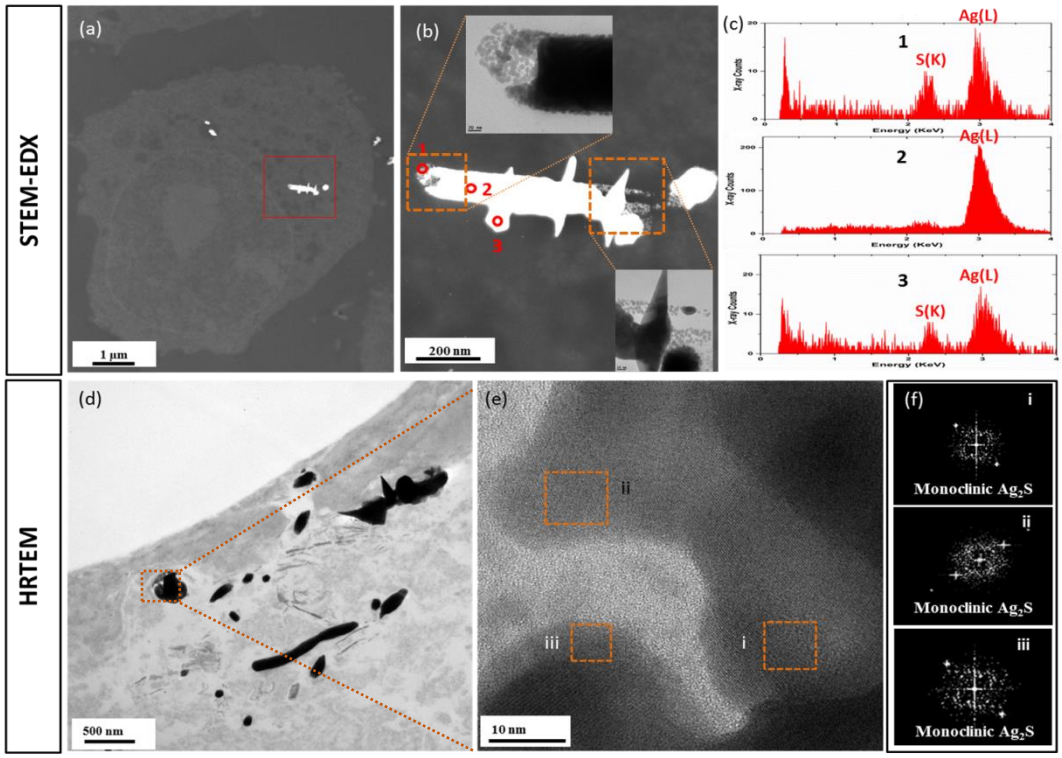
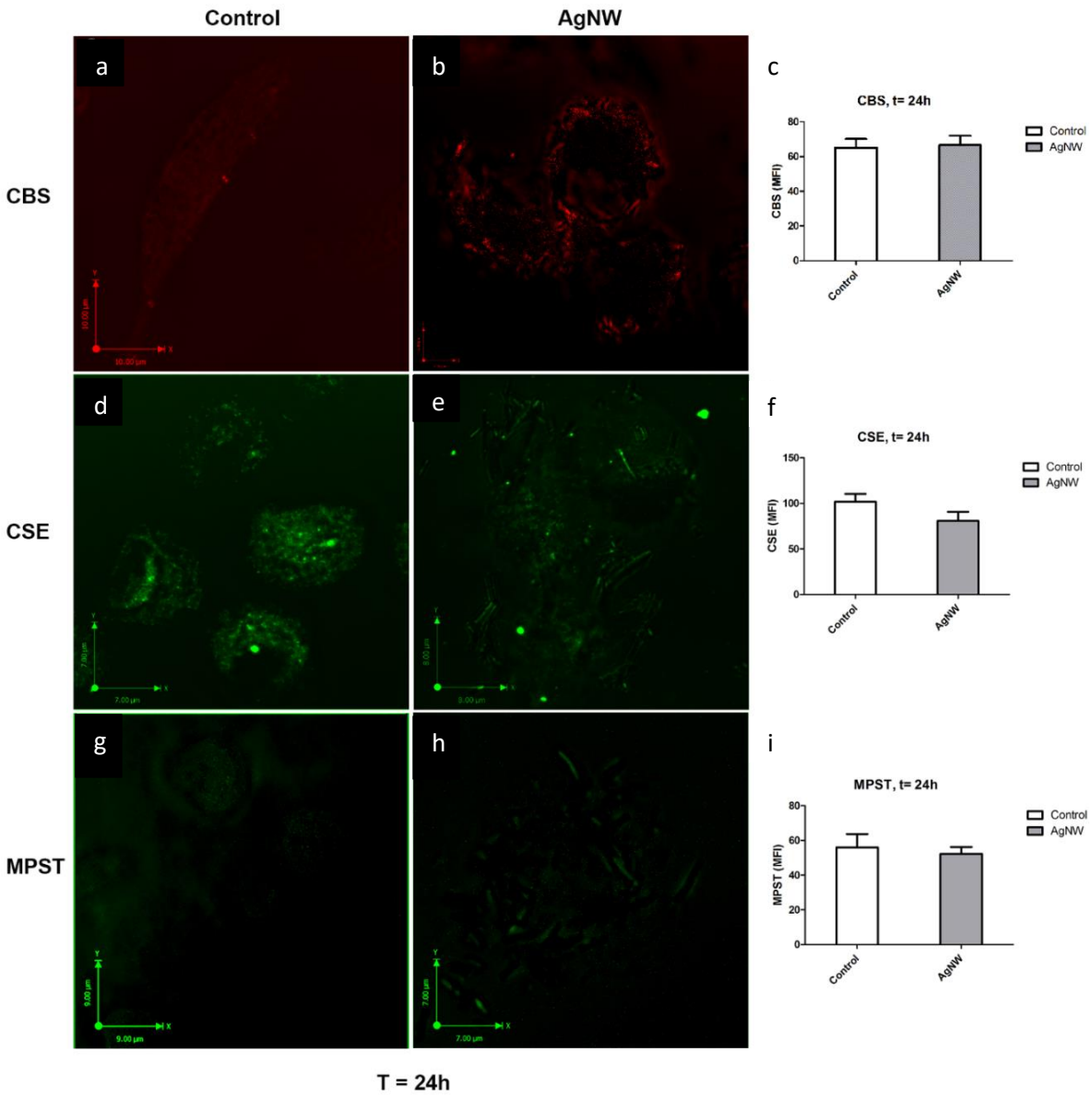
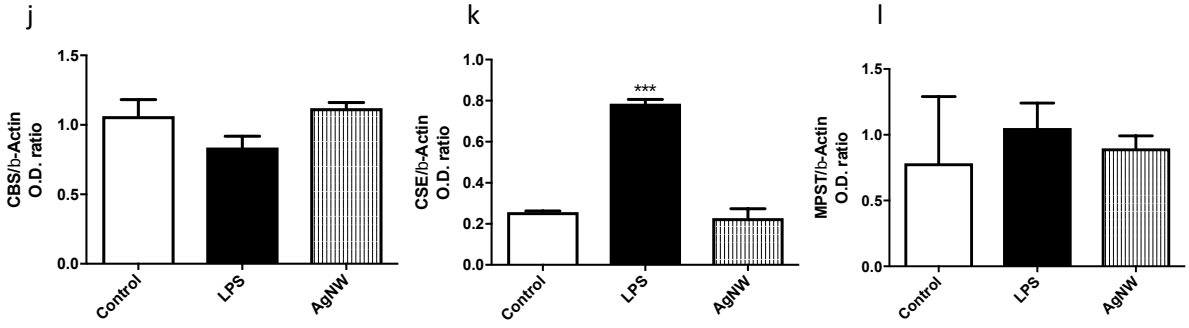


Fig. S5: AgNW uptake by N9 microglia following 1hr pulse AgNW treatment (50 µg/mL) with a 24 hr chase. HAADF-STEM image of AgNWs in microglia cells (a) and a high magnification image (b) of the selected area in (a). STEM-EDX spectra (c) collected from areas 1-3 marked in (b). High pressure frozen, low temperature acetone-treated and epoxy resin-embedded microglia cellular uptake of (d) AgNWs after a 1 h pulse exposure and 24 h chase. (e) HR-TEM image shows ultrafine particles surrounding the surface of the AgNWs. FFT patterns taken from corresponding areas (i - iii) marked in (e), showing that sulfidation occurred at the AgNWs surface and the presence of a large number of Ag2S particles surounding the AgNWs after uptake by microglia. (N: nucleus; C: cytoplasm; E/L: endosome/lysosome; ES: extracellular space).


Control
AgNW
a
b
c
CBS
CBS, t= 24h
CBS (MFI)
Control
AgNW
d
e
f
CSE
CSE, t= 24h
CSE (MFI)
g
h
i
MPST
MPST, t= 24h
MPST (MFI)
T = 24h


j
k
l
CBS/b-Actin O.D. ratio
CSE/b-Actin O.D. ratio
MPST/b-Actin O.D. ratio
***
Control
LPS
AgNW

m

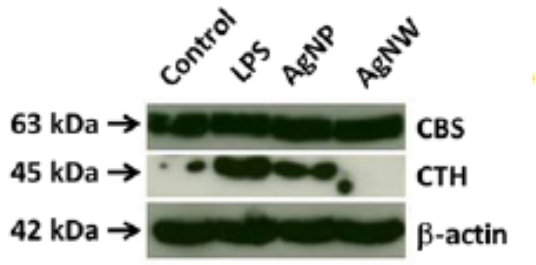


Fig. S6: a) Quantification of CBS (a-c), CSE (d-f) and MPST (g-i) enzymes in N9 cells exposed to AgNWs (50µg/ml) for 24h in by confocal microscopy. (c,f,i) The fluorescence detected in 40 cells was subtracted from the background fluorescence, averaged and compared with the control cells. Modulation of microglia (j) CBS, (k) CSE and (l) MPST expression by AgNWs or LPS treatment using immunostaining Western blots quantification method. N9 microglia were treated with AgNWs (50 µg/ml) or LPS (positive control) for 1 h (pulse) followed 24 h chase period. CBS/CSE/MPST protein was then quantified by immunostaining Western blots from whole cell lysates. Immunostaining for β-actin was used as loading control. (j-l) The enzyme expression was quantified by normalizing the enzymes' optical density against the β-actin optical density. *** indicate $p < 0.005$ vs. control, as assessed by a one-way ANOVA with a Tukey's post-hoc test. (m) Original Western blots used to plot (j-l) with comparison to the spherical silver nanoparticles used in our previous study that do show increased expression of CSE (here labelled as CTH) [6].

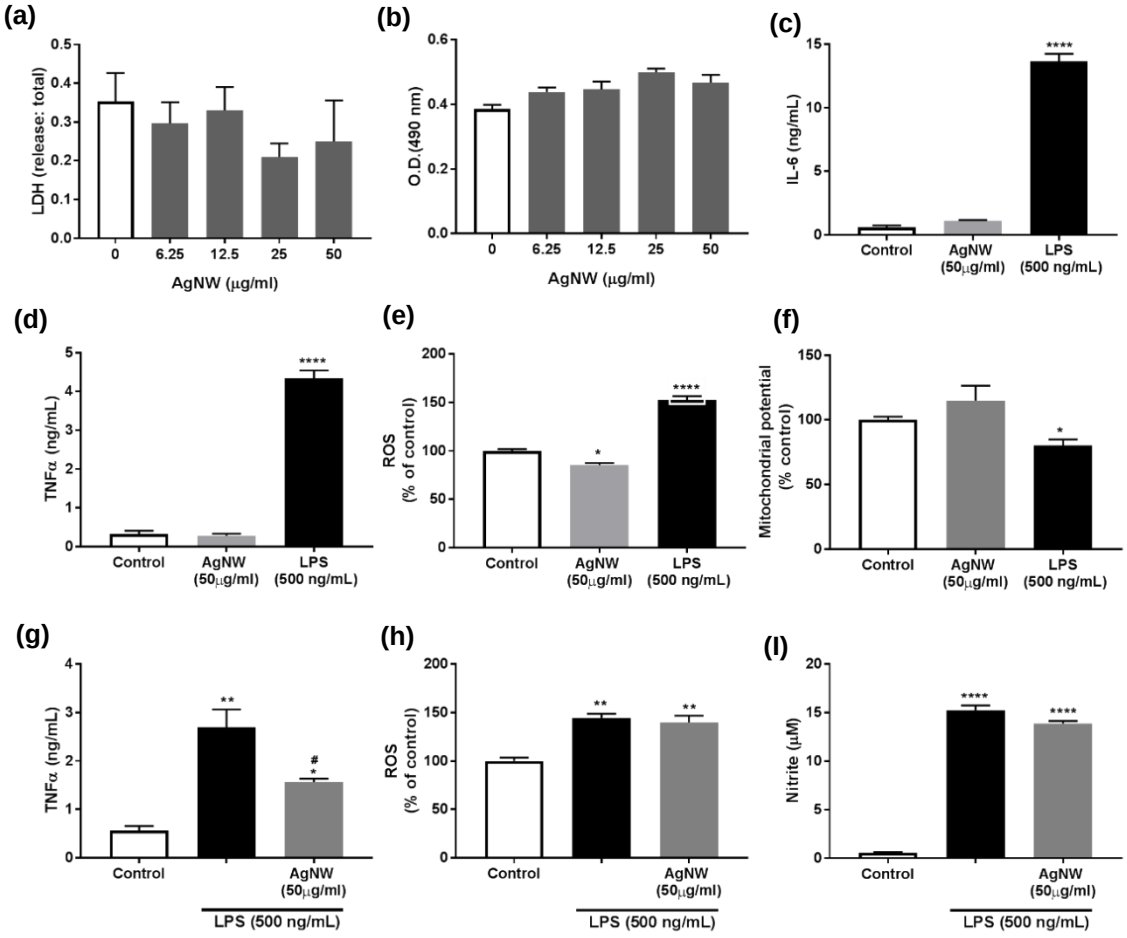


Fig. S7: Modulation of microglial reactivity and cell viability by AgNW treatment. N9 microglial cells were treated with LPS (500 ng/mL) with or without AgNWs for a 1 hr pulse period followed by a 24 h chase period. Cell viability was assessed through (a) LDH release assay, (b) MTS assay and (c) IL-6. Microglial inflammation was assessed through quantification of (d,g) TNFα release, (e,h) ROS production, (f) mitochondrial potential, and (I) nitrite production. Data is presented as mean ± SEM of three independent experiments; *, **, **** indicate $p < 0.05$, 0.01, 0.001, respectively, vs. control; # indicate $p < 0.05$ vs. LPS treatment as assessed by a one-way ANOVA with Tukey's post-hoc test.

Table S1: Summary of the physicochemical characteristics of AgNWs.

Material	**Silver Nanowires (AgNWs)**
Average Size	72 nm (36 - 108 nm) in diameter
	1.5 μm (0.1 - 3.1μm) in length
Capping Agent	PVP (poly(vinyl pyrrolidone))
	average molecular weight $M_w \approx 360k$
Surface Charge	- 14.8 ± 0.1 mV

Table S2: List of ligands which are found in the CBS enzyme structures (source: European Protein Data Bank)

Cystathionine-β-synthase (CBS) (Enzyme Commission number, EC 4.2.1.22)

LIGAND	**FORMULA**	**SYSTEMATIC NAME**
PLP	$C_8 H_{10} N O_6 P$	PYRIDOXAL-5'-PHOSPHATE
HEM	$C_{34} H_{32} Fe N_4 O_4$	PROTOPORPHYRIN IX CONTAINING FE
NA	Na	SODIUM ION
MPD	$C_6 H_{14} O_2$	(4S)-2-METHYL-2, 4-PENTANEDIOL
ACT	$C_2 H_3 O_2$	ACETATE ION
PE4	$C_{16} H_{34} O_8$	2-(2-[2-(2-(2-[2-(2-ETHOXY-ETHOXY)-ETHOXY]-ETHOXY-ETHOXY)-ETHOXY]-ETHOXY)-ETHANOL
KOU	$C_{11} H_{15} N_2 O_8 P$	(E)-N-((3-hydroxy-2-methyl-5-[(phosphonooxy)methyl]pyridin-4-yl)methylidene)-L-serine
SEP	$C_3 H_8 N O_6 P$	PHOSPHOSERINE
P1T	$C_{11} H_{15} N_2 O_7 P$	2-[((3-HYDROXY-2-METHYL-5-[(PHOSPHONOOXY)METHYL]PYRIDIN-4-YL)METHYL)AMINO]ACRYLIC ACID
EDO	$C_2 H_6 O_2$	1,2-ETHANEDIOL

OAS	C_5 H_9 N O_4	O-ACETYLSERINE

Table S3: List of ligands which are found in the CSE enzyme structures (source: European Protein Data Bank).

Cystathionine ϒ-lyase (CSE) (EC 4.4.1.1)

LIGAND	**FORMULA**	**SYSTEMATIC_NAME**
GOL	C_3 H_8 O_3	GLYCEROL
PLP	C_8 H_{10} N O_6 P	PYRIDOXAL-5'-PHOSPHATE
0JO	C_{11} H_{13} N_2 O_7 P	2-{[(E)-{3-hydroxy-2-methyl-5-[(phosphonooxy)methyl]pyridin-4-yl}methylidene]amino}prop-2-enoic acid
SO4	O_4 S	SULFATE ION
SER	C_3 H_7 N O_3	SERINE
PYR	C_3 H_4 O_3	PYRUVIC ACID
NAK	C_3 H_5 N O_2	AMINO-ACRYLATE
KOU	C_{11} H_{15} N_2 O_8 P	(E)-N-({3-hydroxy-2-methyl-5-[(phosphonooxy)methyl]pyridin-4-yl}methylidene)-L-serine
2AG	C_5 H_9 N O_2	(2S)-2-aminopent-4-enoic acid
NO3	N O_3	NITRATE ION
PEG	C_4 H_{10} O_3	DI(HYDROXYETHYL)ETHER
BCT	C H O_3	BICARBONATE ION
BME	C_2 H_6 O S	BETA-MERCAPTOETHANOL
CO3	C O_3	CARBONATE ION

Table S4: List of ligands which are found in the MPST enzyme structures (source: European Protein Data Bank).

3-mercaptopyruvate sulfurtransferase (MPST) (EC 2.8.2.1)

LIGAND	**FORMULA**	**SYSTEMATIC_NAME**
GOL	C3 H8 O3	GLYCEROL
SO4	O_4 S	SULFATE ION
PYR	C_3 H_4 O_3	PYRUVIC ACID

Table S5: The crystal structure of silver and various silver compounds based on the Inorganic Crystal Structure Database.

Ag Crystal System (Cubic) - ICSD ref: 01-087-0597					
h	**k**	**l**	**d(Å)**	**2Theta (°)**	**I (%)**
1	1	1	2.359	38.115	100.0
2	0	0	2.043	44.299	45.7
2	2	0	1.445	64.443	22.5
3	1	1	1.232	77.397	22.2

AgCl crystal system (Cubic) - ICSD ref: 00-031-1238					
h	**k**	**l**	**d(Å)**	**2Theta (°)**	**I (%)**
2	0	0	2.774	32.244	100.0
2	2	0	1.962	46.234	50.0
1	1	1	3.203	27.831	50.0

Ag_2O crystal system (Cubic) - ICSD ref: 00-041-1104					
h	**k**	**l**	**d(Å)**	**2Theta (°)**	**I (%)**
1	1	1	2.729	32.791	100.0
2	0	0	2.360	38.067	28.0

Ag_2S crystal system (Monoclinic) - ICSD ref: 00-014-0072					
h	**k**	**l**	**d(Å)**	**2Theta (°)**	**I (%)**
-1	2	1	2.606	34.385	100.0
1	2	1	2.440	36.806	80.0
-1	0	3	2.383	37.719	75.0
-1	1	2	2.836	31.521	70.0
0	2	2	2.583	34.701	70.0
1	1	2	2.456	36.557	70.0
1	1	1	3.080	28.967	60.0
0	1	3	2.421	37.105	60.0
0	3	1	2.213	40.740	45.0
2	0	0	2.083	43.407	45.0
-1	1	1	3.437	25.902	35.0
-2	1	3	1.718	53.278	20.0
0	1	2	3.383	26.323	20.0