Supplementary Information (SI) for RSC Advances.

Electronic supplementary information (ESI)

Antitumor activity of bimetallic silver/gold nanoparticles against MCF-7 breast cancer cells

Juan J. Martínez-Sanmiguel[1,] Diana Zarate-Triviño[2], Maria Paula García-García[1], José Miguel García-Martín[3] , Álvaro Mayoral[4], Lidia Martínez[5], Yves Huttel[5] and Jorge L. Cholula-Díaz[1,*]*

[1] School of Engineering and Sciences, Tecnologico de Monterrey, Av. Eugenio Garza Sada 2501 Sur, Monterrey 64849, N.L., Mexico.

[2] Facultad de Ciencias Biológicas, Laboratorio de Inmunología y Virología, Monterrey, Universidad Autónoma de Nuevo León, San Nicolas de los Garza 66455, N. L., Mexico.

[3] Instituto de Micro y Nanotecnología (IMN-CNM), CSIC (CEI UAM+CSIC), Isaac Newton 8,

28760 Tres Cantos, Spain

[4] Instituto de Nanociencia y Materiales de Aragón (INMA), CSIC-Universidad de Zaragoza, Pedro Cerbuna, 50009 Zaragoza, Spain.

[5] Instituto de Ciencia de Materiales de Madrid (ICMM), CSIC, Sor Juana Inés de la Cruz 3, 28049 Madrid, Spain.

* Corresponding authors: Juan J. Martínez-Sanmiguel (juanjo_mtz11@hotmail.com); Jorge L. Cholula-Díaz (jorgeluis.cholula@tec.mx).

S1. Effect of H_2O_2 on NAC-pretreated MCF-7 breast cancer cells

To prove a model of inhibition of the oxidant action, the cellular viability of different concentrations of the antioxidant NAC, and the cytotoxic concentration of H_2O_2 were found (Figure S1).

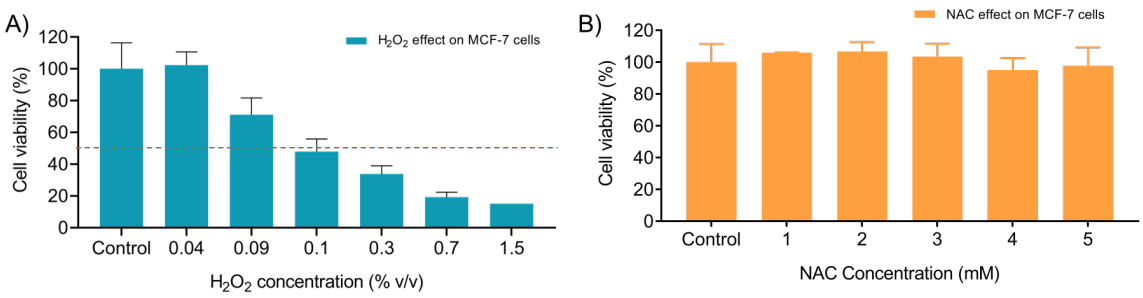

Figure S1. Effect on the cell viability of different concentrations of A) H_2O_2 and B) NAC on MCF-7 human breast cancer cells. Control: cells cultured only with culture medium.

To establish a model of inhibition of the action of oxidant compounds, the cellular viability of different concentrations of the antioxidant NAC was determined in conjunction with the cytotoxic concentration of H_2O_2, finding that the concentration of NAC at 5 mM inhibited the oxidation activity of H_2O_2 to a greater extent, recovering the cell viability from 45% up to 75% (**Figure S2**).

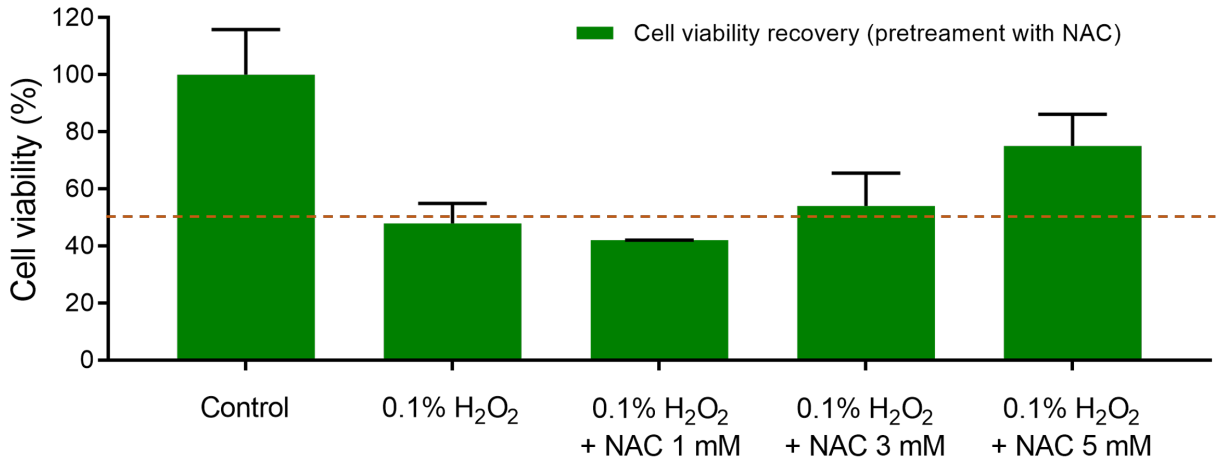

Figure S2. Effect of NAC pre-treatment in co-incubation with H_2O_2 on the cell viability of breast cancer cell line MCF-7. Control: cells cultured only with culture medium.

S2. High-magnification Cs-corrected STEM-HAADF studies

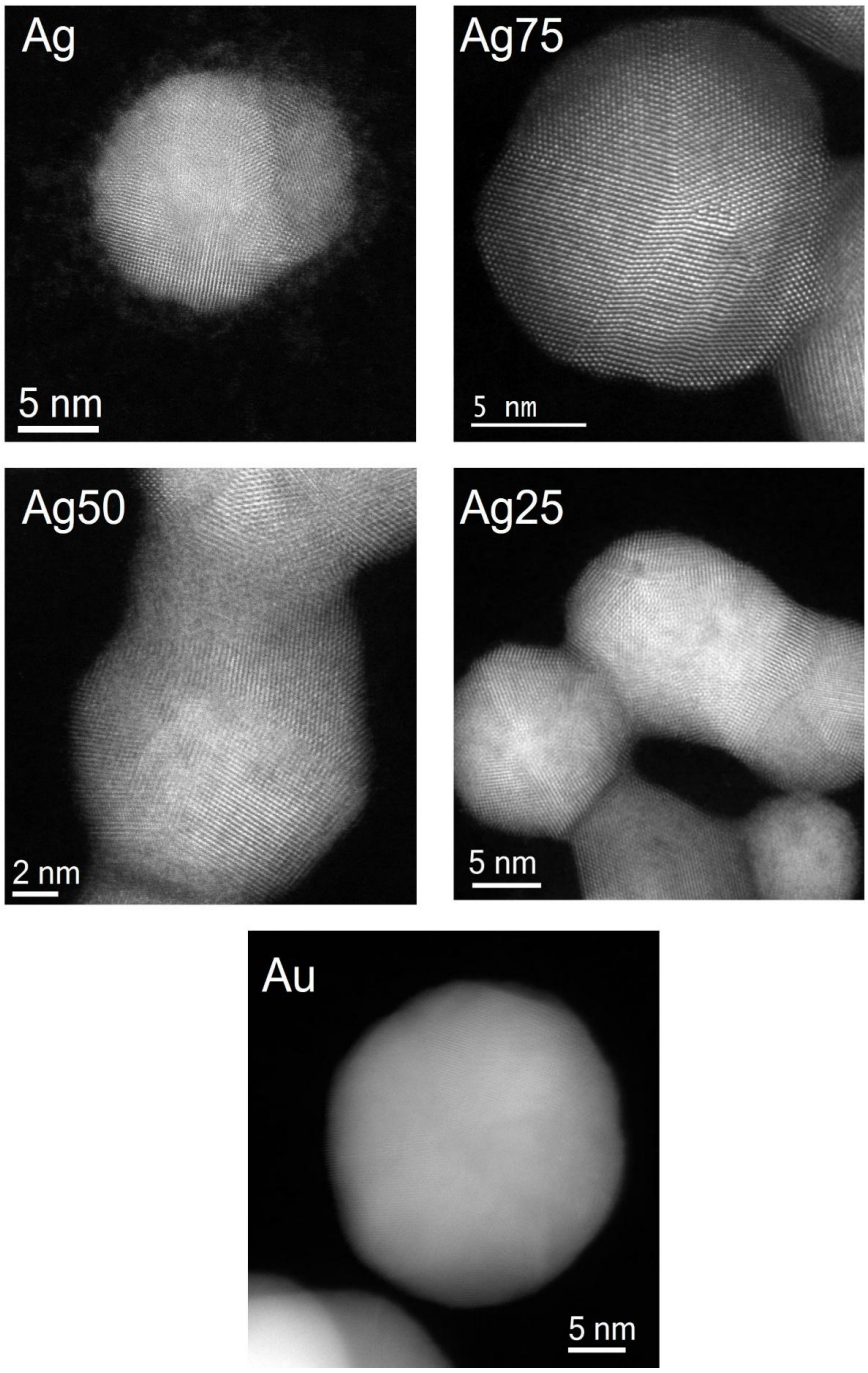

Figure S3. Cs-corrected STEM-HAADF analysis of bimetallic NPs with different Ag:Au ratios showing atomic resolution.

S3. XPS characterization

Table S1. Elemental composition (atomic %, at%) of the Ag/Au NPs based on XPS measurements.

	Composition (at%)						
Nanosystem	Ag	Au	C	Cl	N	O	Si
Ag	0.3	0.0	64.3	0.0	0.8	33.8	0.8
Ag75	8.4	3.0	32.4	2.0	0.7	35.7	17.8
Ag50	7.5	6.4	36.7	3.0	1.4	32.1	12.9
Ag25	10.5	17.8	32.1	4.9	0.0	25.0	9.7
Au	0.0	3.4	27.2	0.0	1.8	40.5	27.1

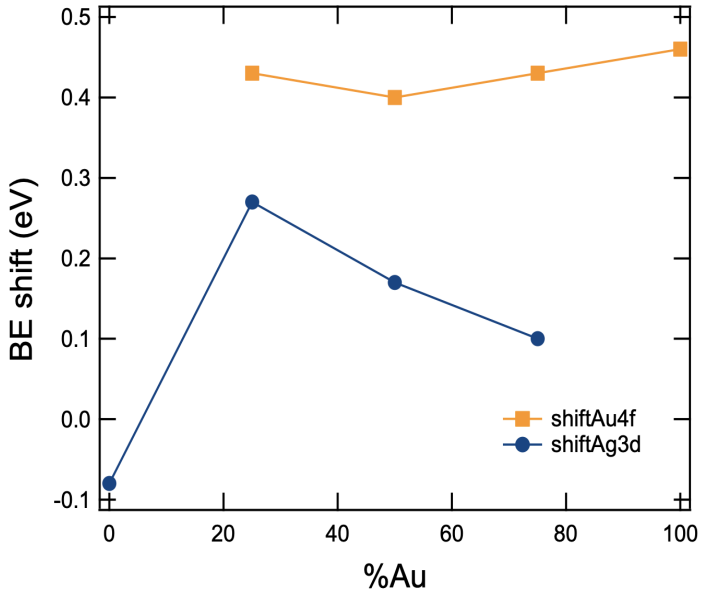

Figure S4. Binding energy shifts observed for the Ag/Au NPs according to the respective XPS core level spectra of Ag 3d and Au 4f with respect to bulk Ag (368.3 eV) and Au (84.0 eV), respectively, as shown in Figure 4 (main manuscript).

Figure S5A presents the analysis of the C 1s core level peak. The spectra have been fitted using four components typical of organic compounds, which correspond to C–C/C–H bonds at 285 ± 0.2 eV; C–O/C–N bonds at 286.3 ± 0.2 eV; C=O bonds at 288.1 ± 0.2 eV; and O–C=O bonds at 289 ± 0.2 eV [37]. The main difference between the NPs relies on the amount of C-C/C-H that was detected. In Au NPs, this component is hardly detectable, while it varies between 20% and 38% for NPs containing Ag. **Figure S5B** displays the analysis of the O 1s core level peak. Initially, six components were used for the fitting. Starting from the lower BE, one of them corresponded to oxidized silver (either as Ag_2O or AgO) in the BE range of 528.4-530 eV. Due to the small amount of silver detected in comparison with the organic compounds as well as the small proportion of oxidized silver this contribution is negligible. The following contributions at 530 ± 0.1 eV and 532.6 ± 0.2 eV are due to C-O-C and C-OH contributions, respectively, from the starch capping. This first signal is below 5% in all samples, while the alcoholic contribution ranges from 10% to 90% in the samples containing Ag. However, the proportion between both components does not match with the proportion of both O-containing functional groups from starch, suggesting an additional hydroxylation of the samples. This fact is confirmed by the presence of an additional component at 533.9 ± 0.1 eV that could be tentatively attributed either to adsorbed H_2O [47] or other organic compounds [36]. Apart from this, the fitting also considered the part of the Si substrate detected as SiO_2 (532.9 ± 0.1 eV). This component was forced to contribute only with the proportion of SiO_2 measured in the wide scans. Finally, there is an additional component at higher BE (534.8 ± 0.2 eV) that represents up to 40% of the O 1s in the case of the Au NP sample. There are different

organic compounds that can have their BE in this range, although we cannot assign them to a particular one.

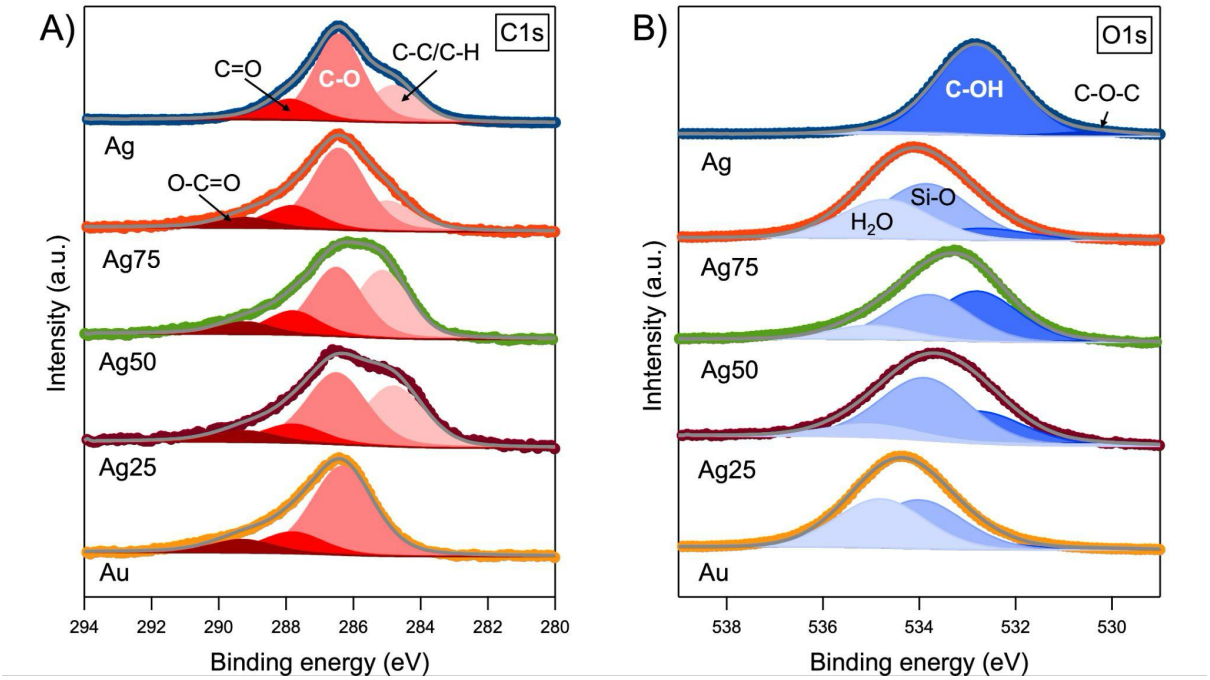

Figure S5. XPS core level spectra of A) C 1s and B) O 1s.

S4. Cytotoxic studies of the Ag/Au NPs upon 48 and 72 h treatment on cancer cells MCF-7

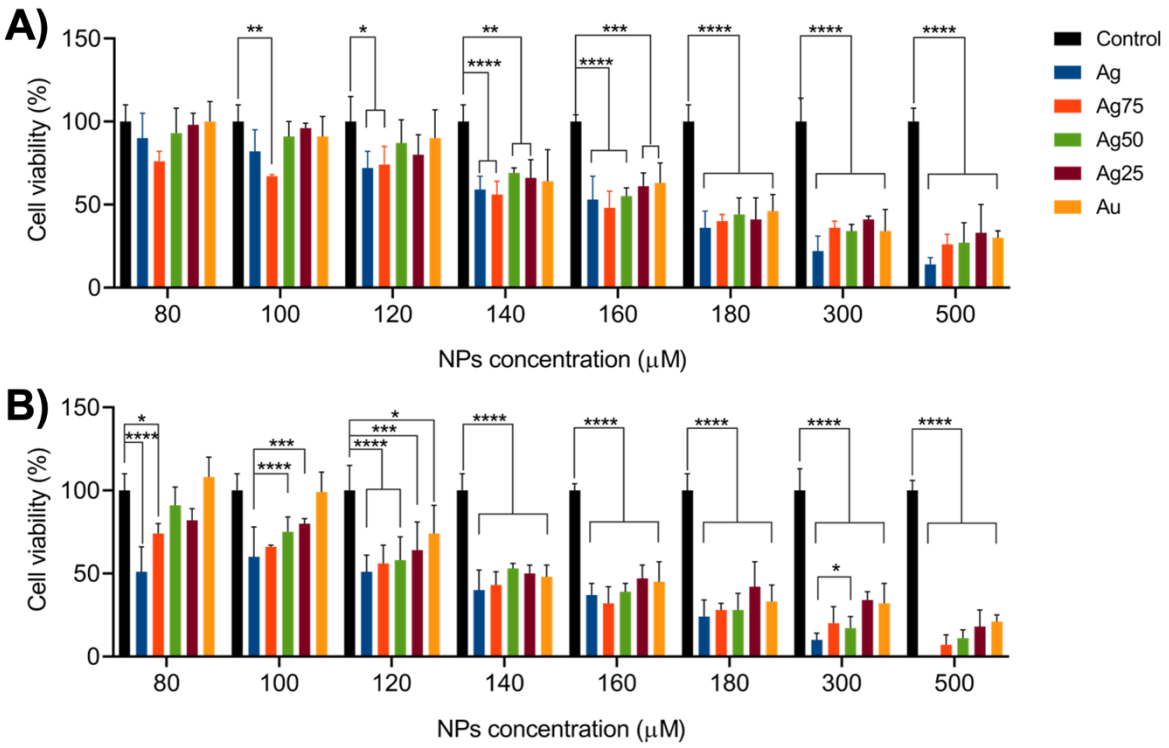

Figure S6. Cytotoxic studies of the Ag/Au NPs on cancer cells MCF-7 at A) 48 h and B) 72 h. *p <0.05 **p <0.01, ***p <0.001 and ****p <0.0001.