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SUPPLEMENTARY INFORMATION

A catalase-magnetic switch for cell proliferation

Sofia Municoy, Irene L. Ibañez, Hebe Durán and Martín G. Bellino*

Departamento de Micro y Nanotecnología, Comisión Nacional de Energía Atómica,

Av. Gral Paz 1499 (B1650KNA), San Martín, Buenos Aires, Argentina.

*e-mail: mbellino@cnea.gov.ar

EXPERIMENTAL PROCEDURES

Preparation and characterization of CAT-MNP complex. Magnetite nanoparticles were synthesized by co-precipitation of a mixed solution of ferric chloride ($FeCl_3.6H_2O$, 0.1 M in H_2O) and ferrous chloride ($FeCl_2.4H_2O$, 0.2 M in HCl) through dropwise addition of NaOH (0.15 M) under N_2 atmosphere with vigorous agitation at room temperature. Nitrogen was blown into all solutions for 15 min prior to reaction. In order to remove the residual ions, the obtained precipitate was centrifuged and washed several times with deionized water.

CAT-MNP complexes were prepared as follows: a solution of sonicated magnetite nanoparticles (0.0045 mg/ml in H_2O) were mixed with a solution of catalase (0.45 mg/ml in H_2O), sterilized by filtration and shaked for 10 min at 4°C. Glutaraldyhyde 0.008% was added into the suspension and stirred for 24h, at room temperature in darkness. After cross-linking, magnetic CAT-MNP complexes were separated using a magnet, washed for three times and stored in water at 4 °C.

Optical microscopy images were taken with an Olympus BX51 epifluorescence microscope. Images were serially captured by a CCD camera (Olympus DP70). Transmission electron microscopy (TEM) images were obtained using a Phillips EM-301 electron microscope. The crystalline phases and the average crystallite size were evaluated by XRD using a Philips PW 3710 diffractometer with CuKa radiation and a graphite monochromator.

Cell culture and treatments. The A375 [S1] human melanoma cell line was used. [S2] Cells were grown as previously described, [S2] at 37°C in a 5% CO_2 humidified atmosphere and they were regularly tested to be mycoplasma-free.

Thus, cells were incubated with complete culture medium containing CAT-MNP complexes (catalase activity: 600 U/ml and MNPs 50 μg/ml) for periods of 1, 24 or 48 h. In order to remove CAT-MNP complexes, a 0.35 T magnetic field was applied for 30 min (by placing NdFeB magnets at 0.5 cm from cell culture) or medium was replaced by fresh one. Control cells were left untreated.

Detection of ROS levels by DCFH assay. After 1 h of treatment or after removal of the CAT-MNP complexes by the application of a magnetic field for 30 min, cells were incubated with 2′,7′-dichlorodihydrofluorescein diacetate (DCFH-DA, Molecular Probes) 10 μM for 15 min and the levels of dichlorofluorescein (DCF) oxidized by ROS in cells were analyzed by epifluorescence microscopy.[S3] For each treatment condition, green fluorescent DCF and light microscopy cell images were serially captured.

The mean fluorescence of each cell was quantified by using the NIH Image J software. Three independent experiments were performed with triplicates per condition.

Cell growth analysis. The 3-(4,5-dimethylthiazol-2-y1)-2,5-diphenyltetrazolium bromide (MTT, Sigma) growth assay was performed after treatments on cell cultures as previously described.[S3] Three independent experiments were performed with triplicates per condition.

Statistical analysis. Data are presented as mean ± SD. Significant changes were assessed using two-tailed Student's t-test to compare two sets of data and one-way analysis of variance to compare three or more sets of data followed by Tukey's multiple comparisons test to determine significant differences between group means. P-values less than 0.05 were considered significant for all tests.

[S1] D. Chen, W. Xu, E. Bales, C. Colmenares, M. Conacci-Sorrell, S. Ishii, E. Stavnezer, J. Campisi, D.E. Fisher, A. Ben-Zeev, E. E. Medrano, *Cancer Res.* 2003, **63**, 6626.

[S2] I.L. Ibañez, C. Bracalente, C. Notcovich, I. Tropper, B.L. Molinari, L.L. Policastro, H. Durán, *Plos One* 2012, **7**, art. no. e44502.

[S3] I.L. Ibañez, L.L. Policastro, I. Tropper, C. Bracalente, M.A. Palmieri, P.A. Rojas, B.L. Molinari, H. Durán, *Cancer Letters* 2011, **305**, 58.

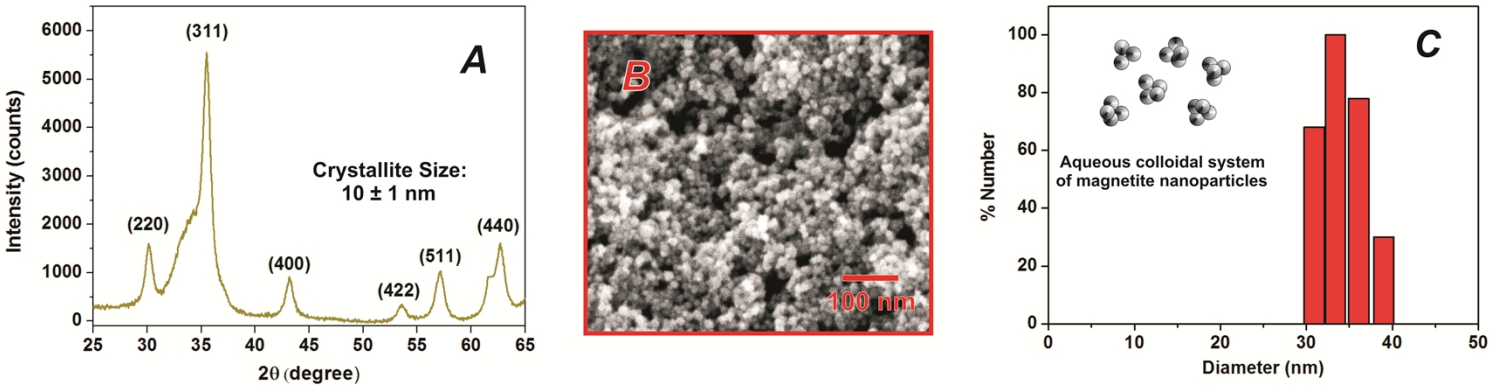

Figure S1**:** *Magnetite nanoparticle characterization:* a) X-ray diffraction (XRD) spectra for the synthesized iron oxide nanoparticles. Approximate crystalline size of nanoparticles was determined using the Scherer equation. (average size = 10 nm). b) FE-SEM image of magnetite nanoparticles. The average nanoparticle size observed from FE-SEM images is in excellent agreement with the values determined by XRD. c) Dynamic Light Scattering (DLS) measurement of MNP colloidal dispersion showing the distribution of sizes of nanoparticle clusters (average size = 35 nm). Dilute suspensions of particles in water were used for the measurements. Schematic in-set represents the formation of nanoclusters in solution from magnetite nanoparticles.

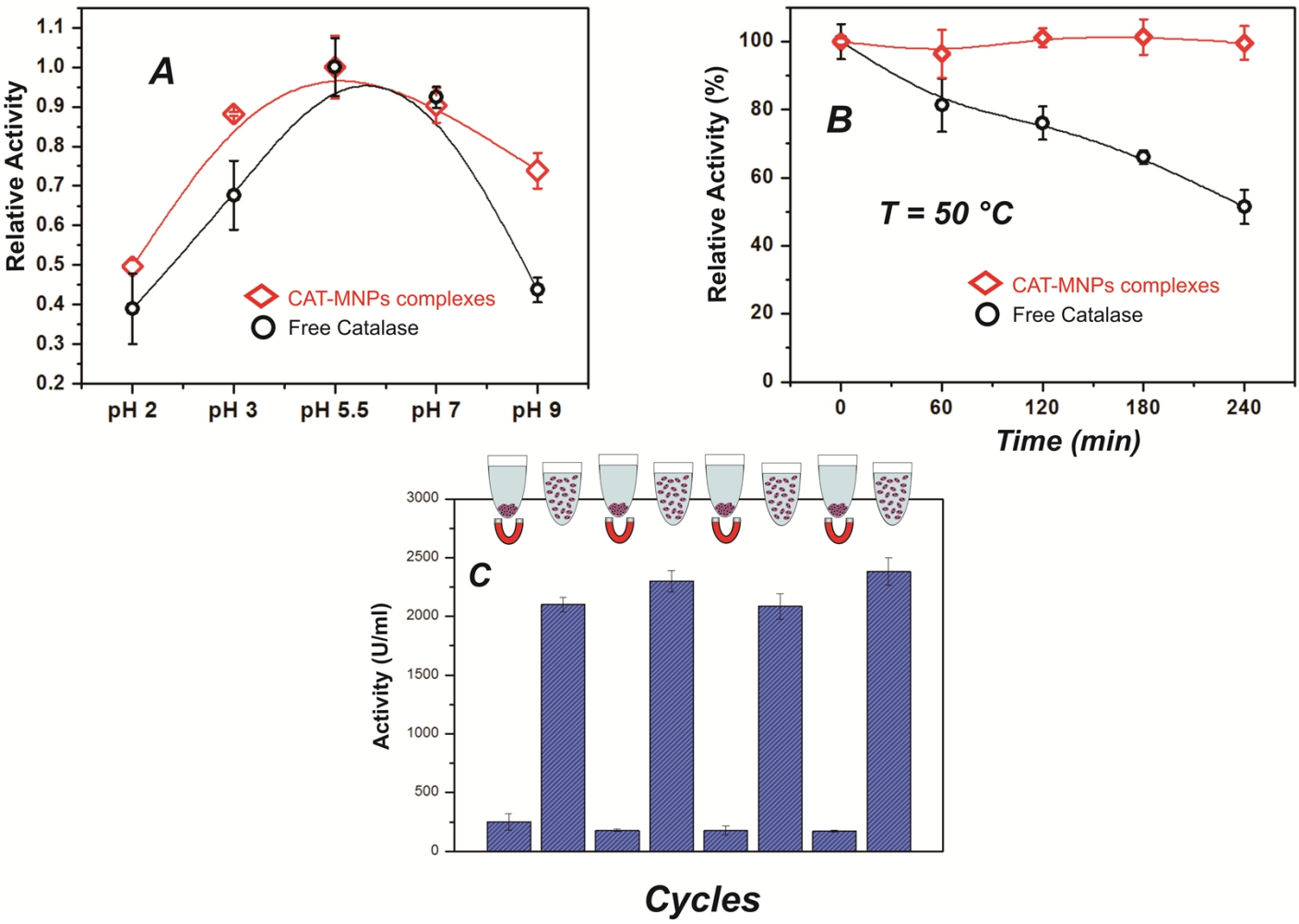

***Figure S2*:** (A) Influence of pH on activity and (B) thermal stability of CAT-MNP complexes and free enzyme. (C) Activity of the CAT-MNP complexes on magnetic recycled use. The presence and disappearance of catalase activity in the aqueous medium can be appreciated in this figure in absence or presence of the magnetic field, respectively. In all cases, the enzymatic activity was determined spectrophotometrically by measuring the decomposition of H_2O_2.[S4] Briefly, 5µL of the aqueous suspension containing the synthesized hybrid catalyst or free enzyme was added to 1 mL of a 15 mM H_2O_2 solution. Then, the absorbance was read at 240 nm in a Hewlett-Packard 8453 spectrophotometer.

[S4] H. E. Aebi, et al. *Method Enzymol*. 1984, **105**, 121.

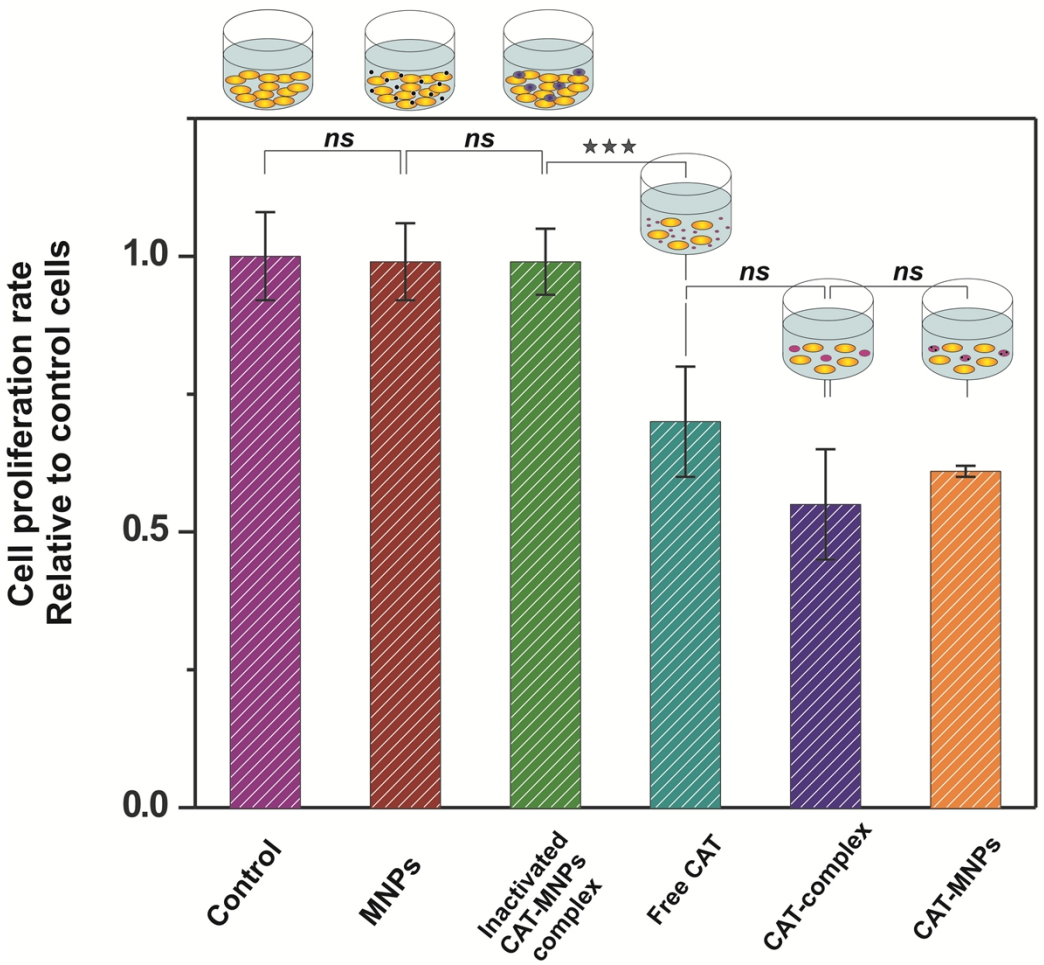

***Figure S3*:** Cell proliferation rate, analyzed by the MTT assay, of A375 cells treated for 24 h with MNPs (50 µg/ml), heat-inactivated CAT-MNPs complex (catalase was inactivated by incubation at 100 °C for 5 min before complex synthesis), free catalase (600 U/ml), CAT-complex (catalase activity: 600 U/ml; CAT-complex was synthesized in the absence of the MNPs) or CAT-MNPs complexes (catalase activity: 600 U/ml and MNPs: 50 µg/ml). Untreated cells were used as control. Data are expressed as mean ± SD. ***p<0.001, *ns*: non- significant.