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Comment

Reply to "Comment on "Free-Radical Formation by the Peroxidase-like Catalytic Activity of MFeO (M = Fe, Ni and Mn) Nanoparticules.""

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Reply to "Comment on "Free-Radical Formation by the Peroxidase-like Catalytic Activity of MFe_2O_4 (M = Fe, Ni and Mn) Nanoparticules.""

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Abstract

Recently we have reported a qualitative, quantitative and reproducible study of the generation of free radicals as a result of the surface catalytic activity of Fe_3O_4, Fe_2O_3, $MnFe_2O_4$ and $NiFe_2O_4$ nanoparticles as a function of the Fe^{2+}/Fe^{3+} oxidation state under different pHs (4.8 and 7.4) and temperatures (25 ºC and 40 ºC) condition. These results were contrasted with those obtained from the in vitro experiments in BV2 cells incubated with dextran-coated magnetic nanoparticles. Based on these results we affirm that our ferrite magnetic nanoparticles catalyze the formation of free radicals and the decomposition of H_2O_2 by a 'peroxidase-like' activity. B. In a comment on this article, Meunier and A. Robert question two points: First they assert that the measured free radicals are not produced by a peroxidase reaction. Also, based on a different normalization method from those reported in our work, they also discuss that the reaction is not catalytic. Here we reply the arguments of the authors about these two points.

Recently we have reported a qualitative, quantitative and reproducible study of the generation of free radicals as a result of the surface catalytic activity of Fe_3O_4, Fe_2O_3, $MnFe_2O_4$ and $NiFe_2O4$ nanoparticles as a function of the Fe^{2+}/Fe^{3+} oxidation state. The study was performed by Electron Paramagnetic Resonance (EPR) Spectroscopy under different pHs (4.8 and 7.4) and temperatures (25 ºC and 40 °C) condition. These results were contrasted with those obtained from the *in vitro* experiments in BV2 cells incubated with dextran-coated magnetic nanoparticles. Based on these results we affirm that ferrite magnetic nanoparticles catalyze the formation of free radicals and

the decomposition of H_2O_2 producing reactive oxygen species (ROS) by a 'peroxidase-like' activity.[1]

In the comment,[2] B. Meunier and A. Robert question two points: First they assert that the measured free radicals are not produced by a peroxidase reaction. Also, based on a different normalization method from those reported in our work, they also discuss that the reaction is not catalytic.

Related to the first point, we agree with the authors that it is not a peroxidase reaction, *senso strictu*, that produced the free radicals observed in our work. It is evident that many conditions of a typical peroxidase reaction could not be present (e.g., no enzymes or haem groups participate in our reactions) or were not used (e.g., a peroxidase substrate). For this reason, we adopted the term 'peroxidase-like' reaction, defined more than 20 years ago[3] and widely used in the scientific literature since then [4]. Within the context of inorganic nanoparticle surface chemistry,[5-10] the term 'peroxidase-like' has been used to describe the ability of inorganic (e.g. iron oxide) nanoparticles to catalyze the typical colorimetric reaction involving hydrogen peroxide (H_2O_2) and chromogenic reagents that are oxidized by the peroxidase enzyme.[4,11] It is well known that the oxidation of substrates by the peroxidase enzyme involves the formation of free radicals,[12-14] as evidence by EPR measurements with DMPO.[15] We used the term 'peroxidase-like' to describe the activity in Fenton-based reactions, whereby iron oxides catalyze the decomposition of hydrogen peroxide (H_2O_2) into reactive oxygen species (ROS), such as the radicals •OH and •OOH.[16]

The authors also hold that the, from the results of our work, no catalytic activity can be attributed to the nanoparticles. Their assertion is based on a somewhat simplified calculation, as for example they did not consider the actual iron distribution in a Fe-containing nanoparticle, that is,

that only ions located at the particle surface can participate of the reaction, and this is a minor fraction of total iron that depends on the particle size. Neither had they considered that the DMPO trapping efficiency varies for every oxygen-centered radical.

For those reasons, in our manuscript we quantified the free radical production by comparing against control samples (i.e., without nanoparticles), and we further reported the absolute concentration in each case. These measurements indicate that the free radical concentration increased by a factor of ~10 in presence of magnetite nanoparticles. Furthermore, we observed that the reaction rate was dependent on the ferrite composition.

We believe that it is not adequate-to normalize the free radical concentration by the total amount of Fe(II) of the nanoparticles in the solution because only the superficial iron ions participate in the reaction. Specifically, assuming that available Fe(II) ions are those located within a surface shell of ≈ 0.8 nm thickness (i.e., the lattice parameter value for spinel), the ratio $\boldsymbol{R} = \frac{\boldsymbol{Volume\ Shell}}{\boldsymbol{Volume\ of\ Nanoparticle}}$ for the particles of our work having sizes between 10-12 nm is R ~ 0.35-0.40, respectively. That is, only ~40% of the Fe(II) ions can participate in the reaction. Indeed, we recently published a work in which we compare the TMB oxidation rate by the ferrite nanoparticles with the free iron ions from metal salts at the same nanoparticle iron concentration, and within our experimental sensitivity, we observed that only nanoparticles were able to oxidize TMB and not free iron ions.[17] This result rules out the effect of iron impurities as suggested by the comment´s authors. Also, the Fenton reaction *is* a catalytic reaction.

A final remark related to the comment about the non-observed catalytic activity at physiological pH: although we reported non-catalytic activity at pH 7.4 (PBS), free radical formation in presence of nanoparticles was clearly observed *in vitro*. The expected intracellular

localization of the dextran-coated nanoparticles once they are incorporated into the cell could be the lysosome (pH 4.8),[18,19] and an important free radical formation was observed at this pH, in our work and others that reported a dual enzymatic-like activity of peroxidase at acidic pH and catalase at neutral pH.[6] Therefore, contrary to the final remark of the comment, these results show that at physiological conditions, free radicals were clearly formed.

The aim of our work is centered in the quantification and the study of the catalytic activity with different particle compositions. The analysis of the free radical production is relevant because the magnetic nanoparticles are experimentally or clinically used in different applications such as magnetic resonance imaging (MRI), ophthalmology, and hyperthermia treatment among others.[20] However, we did not attempt to extrapolate our results to a further interpretation from the point of view of any neurodegenerative pathology. The application of these results in neurodegenerative pathologies in general, and in the Alzheimer disease in particular, is very distant from the scope of our article.[1]

REFERENCES

[1] Moreno Maldonado, A. C.; Winkler, E. L.; Raineri, M.; Toro Córdova, A.; Rodríguez, L. M.; Troiani, H. E.; Mojica Pisciotti, M. L.; Mansilla, M. V.; Tobia, D.; Nadal, M. S.; et al. Free-Radical Formation by the Peroxidase-Like Catalytic Activity of MFe2O4 (M=Fe, Ni, and Mn) Nanoparticles. *J. Phys. Chem. C* **2019**, *123* (33), 20617–20627. https://doi.org/10.1021/acs.jpcc.9b05371.

[2] Meunier, B.; Robert, A. Comment on "Free-Radical Formation by the Peroxidase-like Catalytic Activity of MFe2O4 (M = Fe, Ni and Mn) Nanoparticules". *J. Phys. Chem. C* **2019**.

[3] Mugesh, G., et al., Diferrocenyl Diselenides: Excellent Thiol Peroxidase-Like Antioxidants. *Chem. Commun.* **1998**, 2227-2228. https://doi.org/10.1039/A805941A.

[4] Gao, L.; Fan, K.; Yan, X. Iron Oxide Nanozyme: A Multifunctional Enzyme Mimetic for Biomedical Applications. *Theranostics* **2017**, *7* (13), 3207–3227. https://doi.org/10.7150/thno.19738.

[5] Wei, H.; Wang, E. Fe 3 O 4 Magnetic Nanoparticles as Peroxidase Mimetics and Their Applications in H2O2 and Glucose Detection. *Anal. Chem.* **2008**, *80* (6), 2250–2254. https://doi.org/10.1021/ac702203f.

[6] Chen, Z.; Yin, J.-J.; Zhou, Y.-T.; Zhang, Y.; Song, L.; Song, M.; Hu, S.; Gu, N. Dual Enzyme-like Activities of Iron Oxide Nanoparticles and Their Implication for Diminishing Cytotoxicity. *ACS Nano* **2012**, *6* (5), 4001–4012. https://doi.org/10.1021/nn300291r.

[7] Yang, Y.-C.; Wang, Y.-T.; Tseng, W.-L. Amplified Peroxidase-Like Activity in Iron Oxide Nanoparticles Using Adenosine Monophosphate: Application to Urinary Protein Sensing. *ACS Appl. Mater. Interfaces* **2017**, *9* (11), 10069–10077. https://doi.org/10.1021/acsami.6b15654.

[8] Zhang, X.-Q.; Gong, S.-W.; Zhang, Y.; Yang, T.; Wang, C.-Y.; Gu, N. Prussian Blue Modified Iron Oxide Magnetic Nanoparticles and Their High Peroxidase-like Activity. *J. Mater. Chem.* **2010**, *20* (24), 5110. https://doi.org/10.1039/c0jm00174k.

[9] Yu, F.; Huang, Y.; Cole, A. J.; Yang, V. C. The Artificial Peroxidase Activity of Magnetic Iron Oxide Nanoparticles and Its Application to Glucose Detection. *Biomaterials* **2009**, *30* (27), 4716–4722. https://doi.org/10.1016/j.biomaterials.2009.05.005.

[10] Liu, S.; Lu, F.; Xing, R.; Zhu, J.-J. Structural Effects of Fe3O4 Nanocrystals on Peroxidase-Like Activity. *Chem. - A Eur. J.* **2011**, *17* (2), 620–625. https://doi.org/10.1002/chem.201001789.

[11] Jiang, B.; Duan, D.; Gao, L.; Zhou, M.; Fan, K.; Tang, Y.; Xi, J.; Bi, Y.; Tong, Z.; Gao, G. F.; et al. Standardized Assays for Determining the Catalytic Activity and Kinetics of Peroxidase-like Nanozymes. *Nat. Protoc.* **2018**, *13* (7), 1506–1520. https://doi.org/10.1038/s41596-018-0001-1.

[12] Sadrzadeh, S. M. H.; Graf, E.; Panter, S. S.; Hallaway, P. E.; Eaton, J. W. Hemoglobin. A Biologic Fenton Reagent. *J. Biol. Chem.* **1984**, *259* (23),14354-14356.

[13] Svistunenko, D. A. Reaction of Haem Containing Proteins and Enzymes with Hydroperoxides: The Radical View. *Biochim Biophys Acta.* **2005**, *1707 (1)*, 127-155. https://doi.org/10.1016/j.bbabio.2005.01.004.

[14] Chen, S. X.; Schopfer, P. Hydroxyl-Radical Production in Physiological Reactions. A Novel Function of Peroxidase. *Eur. J. Biochem.* **1999**, *260 (3)*, 726-735. https://doi.org/10.1046/j.1432-

1327.1999.00199.x.

[15] Ai, J.; Zhang, W.; Liao, G.; Xia, H.; Wang, D. NH2Fe3O4@SiO2 Supported Peroxidase Catalyzed H2O2 for Degradation of Endocrine Disrupter from Aqueous Solution: Roles of Active Radicals and NOMs. *Chemosphere* **2017**, *186*, 733–742. https://doi.org/10.1016/j.chemosphere.2017.08.039.

[16] Pereira, M. C.; Oliveira, L. C. A.; Murad, E. Iron Oxide Catalysts: Fenton and Fentonlike Reactions – a Review. *Clay Miner.* **2012**, *47* (3), 285–302. https://doi.org/10.1180/claymin.2012.047.3.01.

[17] Raineri, M.; Winkler, E. L.; Torres, T. E.; Vasquez Mansilla, M.; Nadal, M. S.; Zysler, R. D.; Lima, E. Effects of Biological Buffer Solutions on the Peroxidase-like Catalytic Activity of Fe3 O4 Nanoparticles. *Nanoscale* **2019**, *11*, 18393-18406. https://doi.org/10.1039/C9NR05799D.

[18] Clerc, P.; Jeanjean, P.; Hallalli, N.; Gougeon, M.; Pipy, B.; Carrey, J.; Fourmy, D.; Gigoux, V. Targeted Magnetic Intra-Lysosomal Hyperthermia Produces Lysosomal Reactive Oxygen Species and Causes Caspase-1 Dependent Cell Death. *J. Control. Release* **2018**, *270*, 120–134. https://doi.org/10.1016/j.jconrel.2017.11.050.

[19] Arbab, A. S.; Wilson, L. B.; Ashari, P.; Jordan, E. K.; Lewis, B. K.; Frank, J. A. A Model of Lysosomal Metabolism of Dextran Coated Superparamagnetic Iron Oxide (SPIO) Nanoparticles: Implications for Cellular Magnetic Resonance Imaging. *NMR Biomed.* **2005**, *18* (6), 383–389. https://doi.org/10.1002/nbm.970.

[20] Nguyen TK Thanh (Ed.). *Clinical Applications of Magnetic Nanoparticles. From Fabrication to Clinical Applications.*; CRC Press: Boca Raton, USA, 2018.