

## Title

# CONTROLLED GENERATION OF REACTIVE OXYGEN SPECIES UNDER ULTRASOUND EXPOSURE OF ZINC OXIDE NANOCRYSTALS

## Authors

Veronica Vighetto<sup>1+</sup>, Andrea Ancona<sup>1+</sup>, Luisa Racca<sup>1</sup>, Tania Limongi<sup>1</sup>, Adriano Troia<sup>2</sup>, Giancarlo Canavese<sup>1</sup>, Valentina Cauda<sup>1\*</sup>

<sup>1</sup>Department of Applied Science and Technology, Politecnico di Torino, C.so Duca degli Abruzzi 24, 10129 Turin, Italy

<sup>2</sup>Ultrasounds & Chemistry Lab, Advanced Metrology for Quality of Life, Istituto Nazionale di Ricerca Metrologica (I.N.Ri.M.) Strada delle Cacce 91, 10135 Turin, Italy

Corresponding author: Prof. Valentina Cauda, Phone: +39 011 090 7389; e-mail address: [valentina.cauda@polito.it](mailto:valentina.cauda@polito.it)

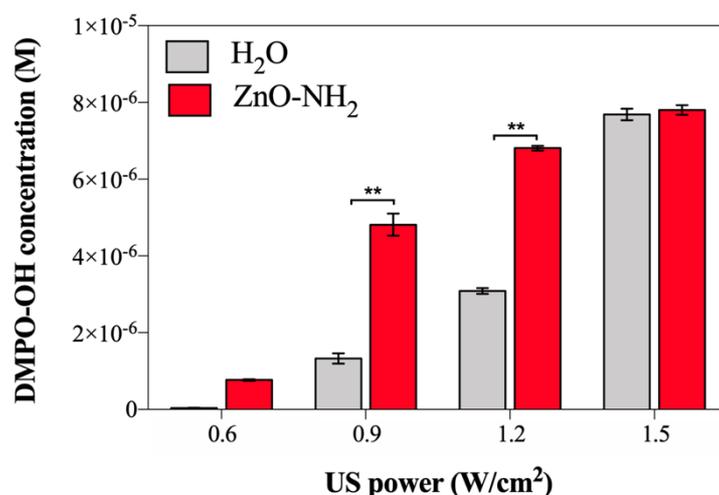
<sup>+</sup> Equally contributing authors

## Abstract

Reactive oxygen species (ROS) are involved in different cell functions and the survival of the cells is related to the ability of maintaining redox homeostasis during all these processes. An instability in this equilibrium results in a variety of possible different diseases. Therefore, controlled generation of ROS can be a promising tool to induce oxidative stress and cell death for cancer therapy applications. Zinc Oxide nanocrystals with a functionalized surface of aminopropyl groups (ZnO-NH<sub>2</sub> NCs) have been proved able to produce ROS in a controlled manner, when stimulated by low frequency ultrasound (US) generated by an already approved medical device (LipoZero G39). The generation of hydroxyl radicals is the result of inertial cavitation under the US exposure.

Passive Cavitation Detection (PCD) and Electron Paramagnetic Resonance (EPR) spectroscopy were used to evaluate the role of acoustic cavitation on the generation of ROS by the ultrasound irradiation of ZnO-NH<sub>2</sub> NCs in water media. Ultrasound B-mode imaging was also applied, proving in respect to pure water, the enhanced ecographic signal generation of the aqueous solution containing ZnO-NH<sub>2</sub> NCs when exposed to pulsed ultrasound.

Furthermore, to evaluate the applicability of ZnO-NH<sub>2</sub> NCs in the biomedical field, the ROS production was studied by interposing different tissue mimicking materials, like phantoms and *ex-vivo* tissues, between the US transducer and the sample well.



**Figure 1** DMPO-OH concentration (M) to evaluate ROS production after 10 min of US irradiation according to different US powers, in presence of ZnO-NH<sub>2</sub> NCs (200 µg/ml). All measurements were conducted in triplicate with 10% DC, 1 MHz frequency, using the LipoZero transducer. 1-way ANOVA was performed to determine statistical significance (\*p<0.05 and \*\*p<0.001).

## Acknowledgments

This work received funding from the European Research Council (ERC) under the European Union's Horizon 2020 research and innovation programme (grant agreement No 678151 – Project Acronym “TROJANANOHORSE” – ERC Starting Grant)

**Presentation session**

Nanomedicine