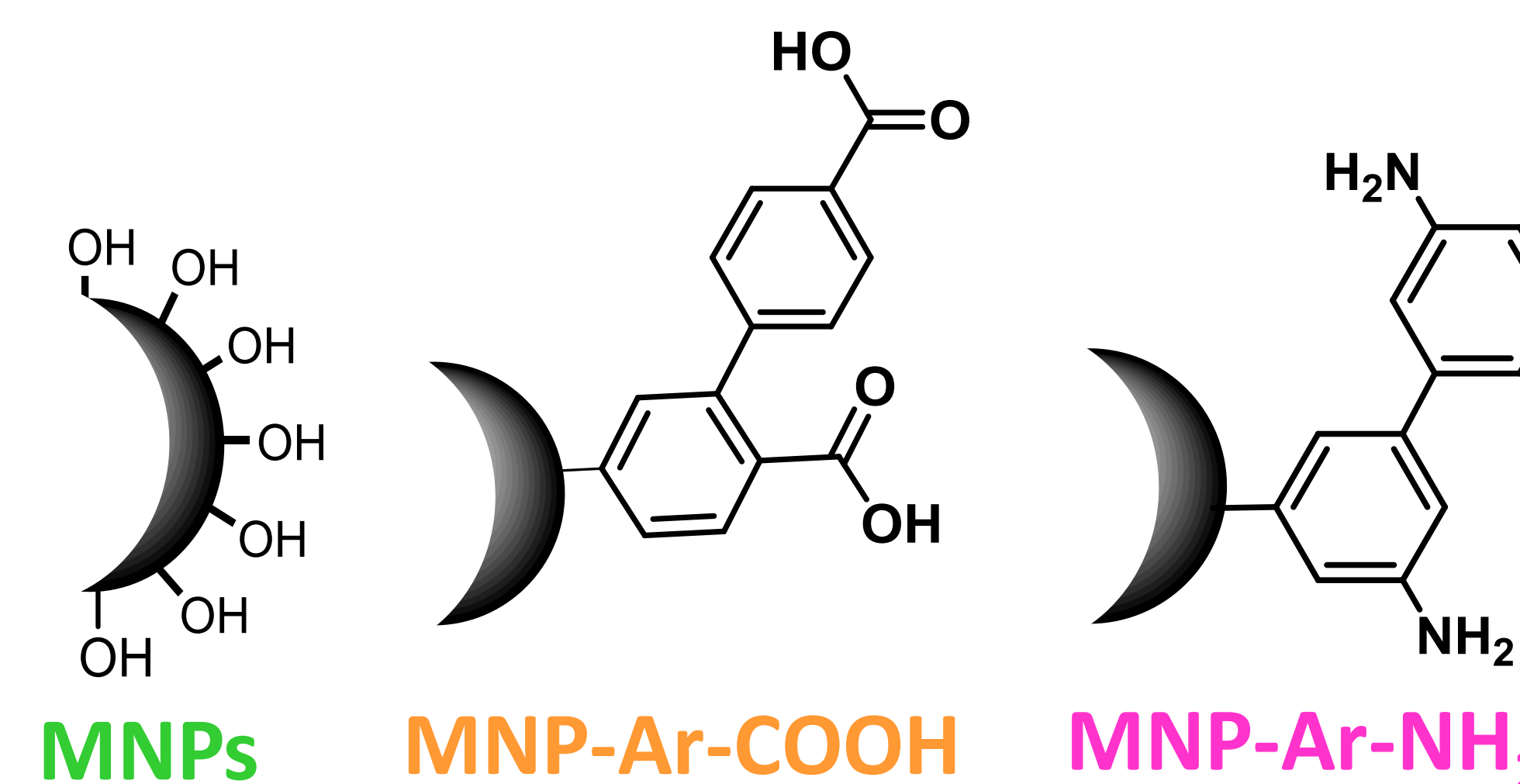


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## Abstract

The biotechnological application of nanoparticles (NPs) of magnetite, requires analyzing how their physicochemical properties influence their cellular internalization and the toxic effects that they can produce. For this, the study of viability and cell uptake was necessary. We synthesize pristine magnetite nanoparticles (MNPs) and functionalized with X (MNP-Ar-X), being X amino or carboxylic acid groups. We also characterize their physicochemical properties and compare the effects based on their surface modification. For the cytotoxicity assay we used the Alamar Blue reagent and to analyze cell uptake we performed an immunofluorescence assay with HeLa LC3B-GFP cells, which allows us to monitor whether the autophagy pathway is activated.



## Nanoparticles properties

Nanoparticles	Size (nm)	Surface coverage (%)
MNPs	11 ± 0,6	-
MNP-Ar-NH <sub>2</sub>	8 ± 1	3,3
MNP-Ar-COOH	6,6 ± 0,7	6,2

## Results

### Superficial charge

❖ Zeta potential of the NPs dispersed in ethanol/water 50% v/v

Nanoparticles	ζ (mV)
MNPs	-15 ± 1
MNP-Ar-COOH	-39 ± 1
MNP-Ar-NH <sub>2</sub>	-13 ± 6

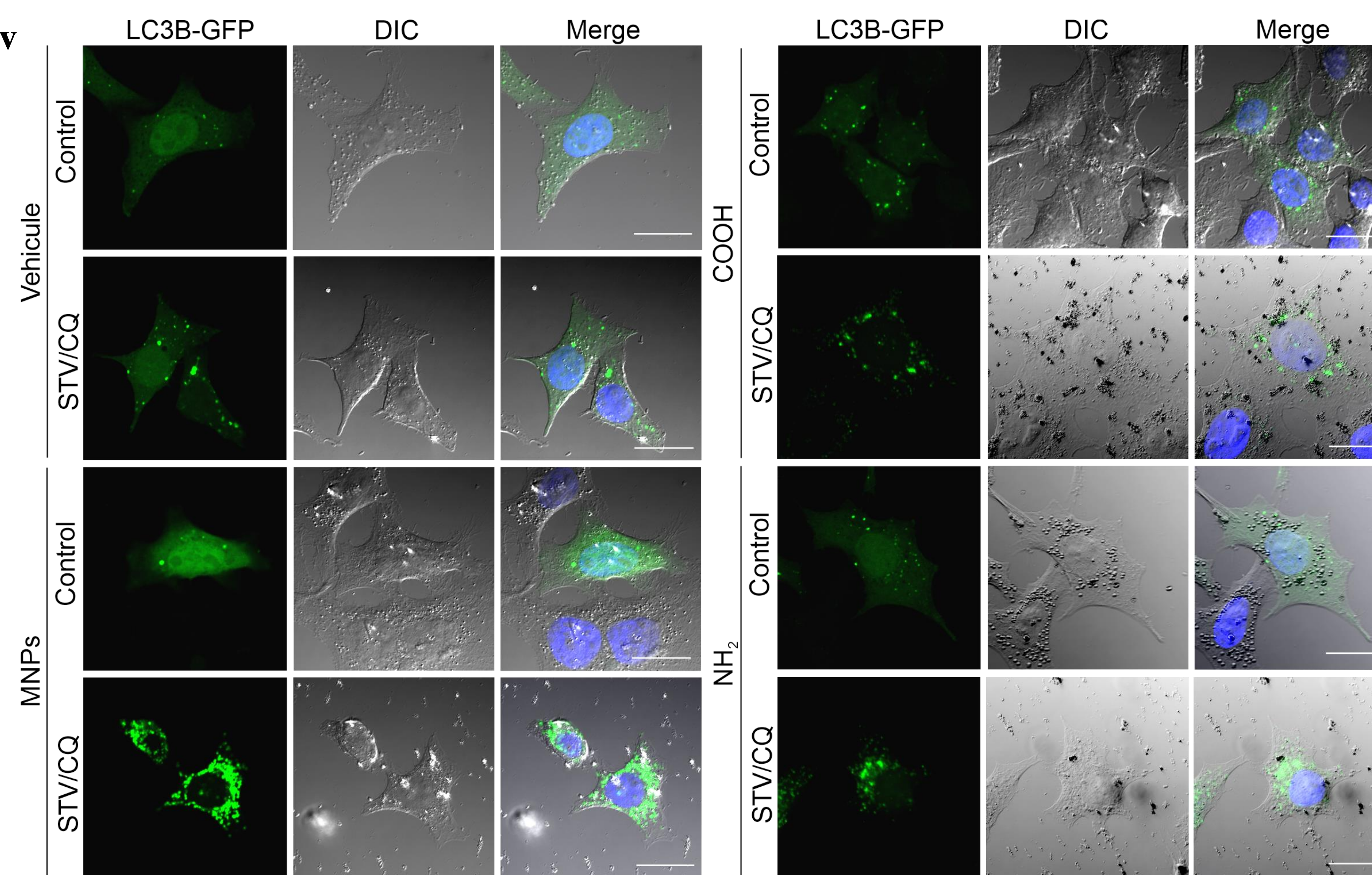
❖ Independently of the surface modification, the particles shows a negative value of ζ at fisiologic pH.

❖ Zeta potential of dilutions of the NPs dispersion in DMEM\* + 10% FBS\*\*

Nanoparticles	Dilution	ζ (mV)	Size (nm)
MNPs	1/100	-2,4 ± 2	25 ± 3
	1/5	-2,4 ± 2	136 ± 24
MNP-Ar-COOH	1/100	-2,7 ± 1	25 ± 7
	1/5	-2 ± 1	112 ± 61
MNP-Ar-NH <sub>2</sub>	1/100	-0,2 ± 1	25 ± 11
	1/5	-2,6 ± 2	90 ± 20

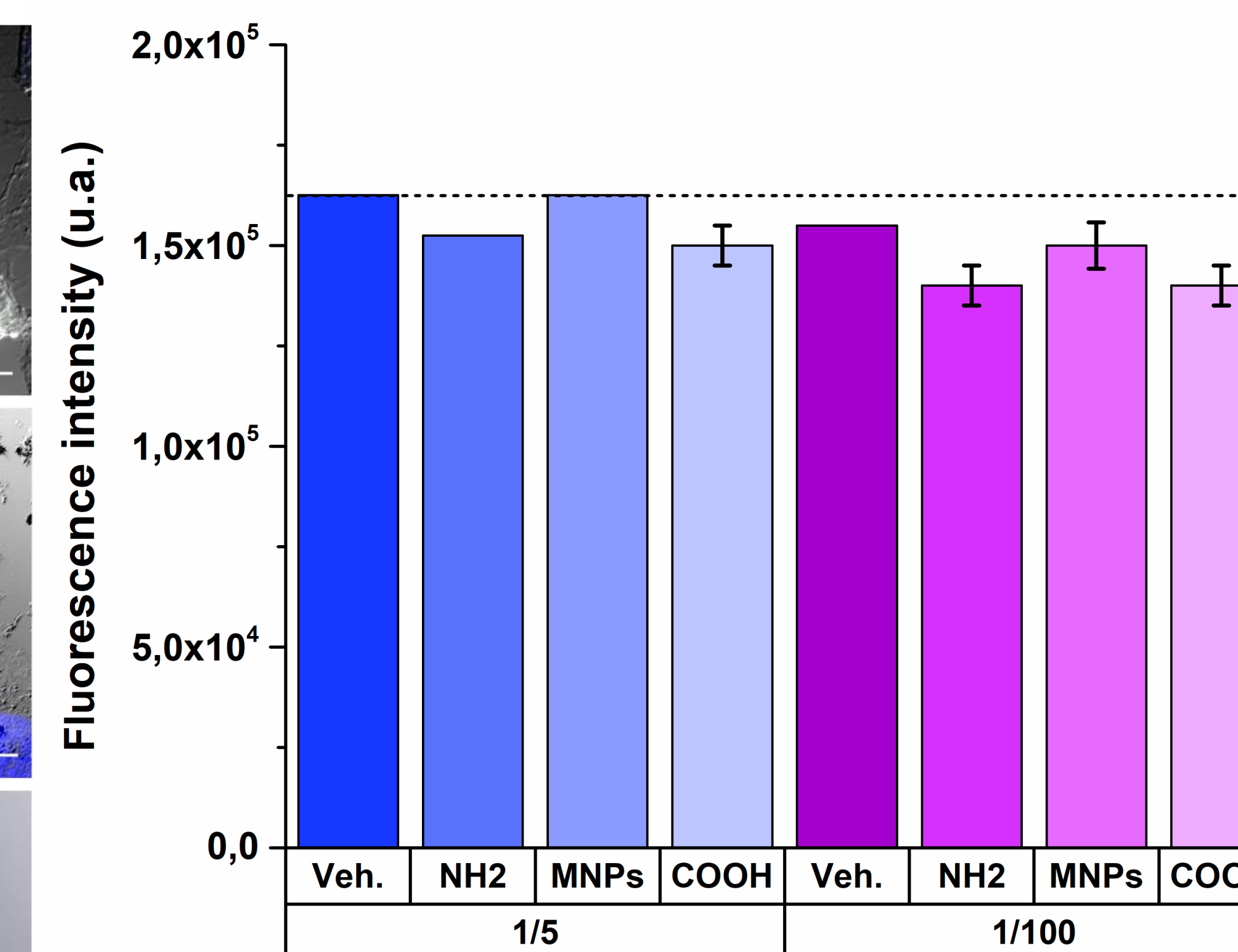
\* Dulbecco's Modified Eagle Medium, \*\* Fetal Bovine Serum

### Immunofluorescence Assay



\* STV: Starvin, \*\* CQ: Cloroquine 20uM

### Cytotoxicity Assay



❖ No significant differences are observed in the viability of cells incubated with MNPs, MNP-Ar-COOH and MNP-Ar-NH<sub>2</sub> under the conditions analyzed.

❖ The presence of proteins in the medium modify the charge exposed due to the formation of a protein corona. The decrease of ζ results in the aggregation of the particles. The size of the aggregates depends on the dilution.

❖ The results obtained showed a differential internalization of the nanoparticles, being the MNPs that showing the higher response. This is observed due to a greater increase in LC3B (+) vacuoles which in turn correlates with the activation of autophagy to promote NP clearance.

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