Dose and Cell Division Drive Intracellular Nanoparticle Redistribution Revealed by Correlative Synchrotron Imaging

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Silica nanoparticles (SiNPs) have emerged as promising nanoplatforms for disease diagnosis and treatment [1]. Understanding nanoparticle–cell interactions is essential for advancing nanomedicine, yet most studies rely on static observations and lack volumetric information under near-native conditions. Protein corona formation plays a key role in modulating nanoparticle behavior [2] and reducing cytotoxicity [3]. Here, we establish a synchrotron-based correlative X-ray microscopy framework to investigate how nanoparticle concentration and successive cell-division cycles govern the intracellular fate of SiNPs in macrophages. Fluorescent SiNPs internalized by RAW 264.7 macrophages were analyzed using a multimodal workflow combining cryo-soft X-ray tomography (cryo-SXT), cryogenic structured illumination microscopy (cryo-SIM), coherent X-ray ptychography, and confocal fluorescence microscopy. Experiments were conducted at the B24 beamline of Diamond Light Source, Mistral beamline of Alba Synchrotron, and the Cateretê beamline of Sirius. Correlative cryo-SXT and cryo-SIM reveal a concentration-dependent redistribution of nanoparticle-containing vesicles toward the perinuclear region while maintaining vesicular confinement. At higher concentrations, nanoparticles approach the nuclear region via vesicles associated with nuclear-envelope invaginations, an effect not observed at lower concentrations (Figure 1a). Successive cell divisions redistribute the intracellular nanoparticle load and promote stable perinuclear clustering, indicating a long-term sequestration pathway (Figure 1b). Confocal fluorescence microscopy supports these findings, showing concentration-dependent increases in ATTO-633 signal intensity, associated with SiNPs, and progressive clustering over time. After two doubling times, fluorescence is predominantly localized in the perinuclear region, suggesting accumulation mediated by late endocytic processes or vesicle maturation, as corroborated by our recent published work [4]. Coherent X-ray ptychography further reveals nanoscale deformations of the nuclear envelope associated with perinuclear vesicle accumulation. These results establish correlative multimodal synchrotron imaging as a powerful multiscale platform for resolving the dynamic intracellular fate of nanoparticles.

Keywords:
Correlative X-ray Microscopy; Intracellular Trafficking; Nano–Bio Interactions; Silica Nanoparticles; Macrophages

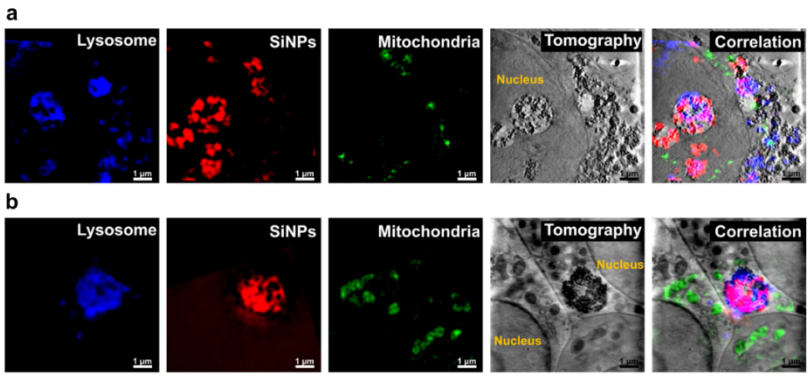

Figure 1. Correlative cryo-SXT/cryo-SIM: (a) 1 h; (b) 32 h (second cell division) at 0.3 mg/mL SiNPs in RAW 264.7 cells.

References:

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Acknowledgements:
This research was supported by FAPESP-2023/00103-6; 2021/12071-6; 2024/00989-7 and Brazilian Society for Microscopy and Microanalysis (SBMM).