Biodegradation of iron oxide nanoparticles in the organism

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The long term outcome of nanoparticles (NPs) in the organism is one of the most important concerns raised by the development of nanotechnology and nanomedicine. Very few is known on the way taken by cells to process and degrade NPs over time. In this context, iron oxide superparamagnetic NPs benefit from a privileged status, because they have shown minor toxicity, allowing their clinical use for MRI diagnosis. It is generally assumed that the specialized metabolism which regulates iron in the organism can also handle iron oxide NPs. However the biotransformation of iron oxide NPs is still not elucidated. Here we describe both *in vivo* and *in vitro* approaches that provide new insights on the fate of nanomagnets in the organism.

In vivo approach [1] - We used the multifunctionality of electron microscopy (*i.e.*, imaging, diffraction, EDX chemical mapping) to follow the biodistribution and the structural evolution of superparamagnetic iron oxide injected to mice within a 90-days period (Fig. 1). These TEM investigations were complemented by the magnetic follow-up of the injected NPs. Ferromagnetic resonance and SQUID magnetization measurements are used to quantify iron oxide NPs and follow the evolution of their magnetic properties. NPs were found into macrophage cells of both liver and spleen. At the subcellular scale, large clusters of NPs are seen sequestered into few intracellular lysosomes (Fig. 1a to 1e). We evidence the biotransformation of superparamagnetic maghemite NPs into poorly-magnetic iron species with a weak crystallinity (Fig. 1e and 1f) over a period of three months. While the original structure and magnetic order of the particle were degraded, “fingerprint like” circular arrays (Fig. 1g) and large clusters of monodisperse NPs (Fig. 1h) were observed in the vicinity of the NPs. Such features, characteristic of iron storage proteins (most likely ferritine), are observed here for the first time coexisting with exogenously-injected NPs. It suggests an ongoing transformation process, by which surplus iron imported by exogenous maghemite NPs could be stored into endogenous storage proteins, as early proposed [2]. To conclude, the original multi-scale method developed in this study (*i.e. in vivo* magnetic and structural follow-up), opens up new ways to understand the biotransformation of promising magnetic materials for biomedical applications.

In vitro approach - If *in vivo* experiments are indispensable to understand the long term outcome of NPs in the organism, *in vitro* methods allow following the degradation of the same NPs over time with better imaging conditions. Here, we have exploited the performances of Cs-corrected electron microscopy to study at the atomic level, the degradation mechanisms of iron oxide nanocubes (NCs) that were degraded on a TEM grid by a solution mimicking the intracellular environment (Acid pH + Chelating agent). As illustrated on figure 2, the full degradation of the NCs occurs in a few hours, but this degradation time varies with the size and morphology of the NC assembly. Isolated NCs are usually more sensitive than large cluster of NCs. Figure 2 clearly shows that the structure of the NCs is progressively attacked and the corners are always firstly degraded. In fact, the key parameter of the mechanisms and kinetic of NCs degradation is the polymer coating. Indeed, Cs-corrected imaging revealed that the corners of the NCs are frequently not well covered by the polymer and are then directly exposed to the degradation medium.

References

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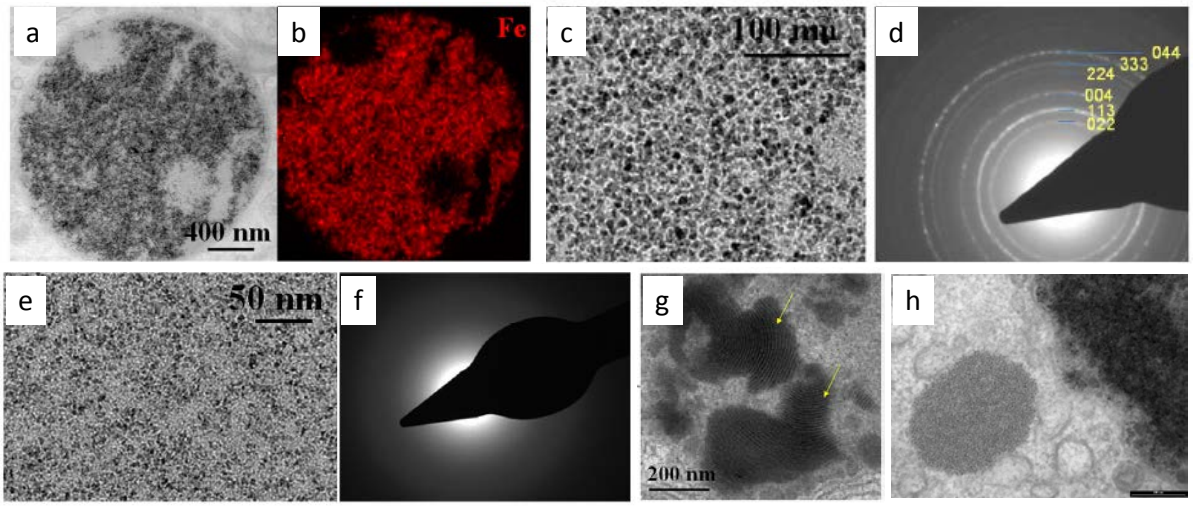

Figure 1. *In vivo biodegradation. NPs observed in the spleen of mice, encapsulated in lysosomes of macrophages. 1 day after injection: (a) STEM images, (b) EDX Fe mapping, (c) TEM image, (d) electron diffraction (maghemite structure). 30 days after injection: (e) TEM image, (d) electron diffraction. Iron storage proteins (f) "fingerprint like" circular arrays, (g) large clusters of monodisperse NPs (ferritine).*

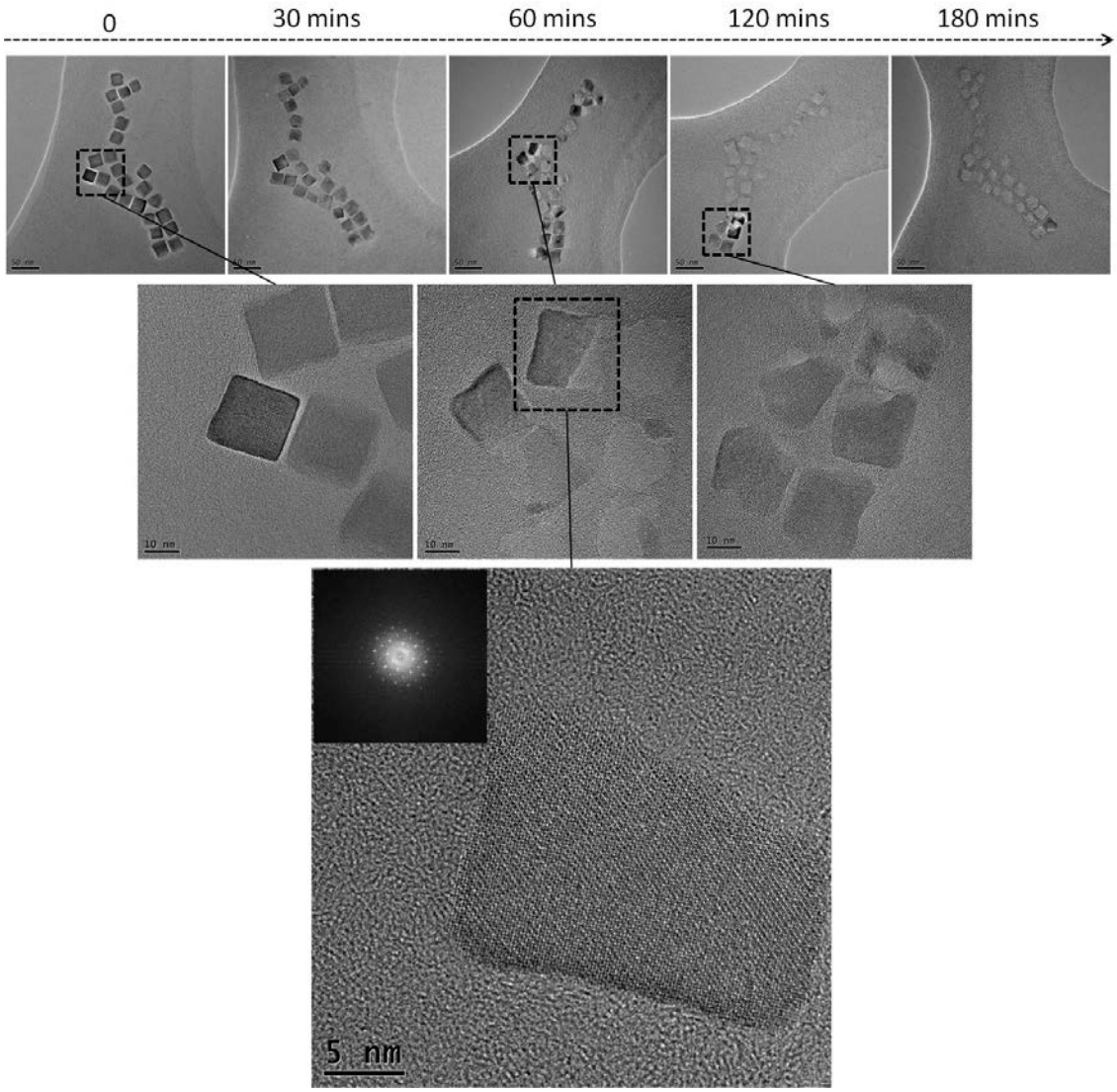

Figure 2. *In vitro biotransformation. Degradation mechanisms of iron oxide nanocubes (NCs) degraded on a TEM grid by a solution mimicking the intracellular environment (Acid PH + Chelating agent)*