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From bench to cell: a roadmap for assessing the bioorthogonal reactivity of magnetic nanoparticles

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The heat generation by magnetic nanoparticles (MNPs) in the presence of an alternating magnetic field (AMF), known as magnetic hyperthermia (MH), has traditionally been studied for cancer treatment applications focused on the internalization of MNPs in target cells. Herein, we propose an innovative approach to apply localized heating onto living cell membranes for inducing changes of membrane biophysics. Our approach is based on the covalent immobilization of MNPs on the cell membranes via bioorthogonal click chemistry, more specifically the strain-promoted [3+2] azide-alkyne cycloaddition (SPAAC) between azide-labelled cell membranes and strained alkyne-functionalized MNPs.

Understanding nano–bio interactions is non-trivial, as there are many factors that play crucial roles (nanoparticle size, shape, surface coating, protein corona, etc.). MNP immobilization on cell membranes poses several challenges as well, as membranes are very complex and dynamic systems. Herein, we present a roadmap for assessing the bioorthogonal reactivity of MNPs from bench to cell.

Hydrophobic 12 nm iron oxide MNPs were synthesized following a seed-mediated thermal decomposition methodology and transferred to water by coating with an amphiphilic polymer. The MNPs were further functionalized with two different types of passivation molecules: polyethyleneglycol (PEG, 750 Da) and a glucopyranoside derivative (Glc) to increase colloidal stability and biocompatibility. In a second functionalization step, MNPs were decorated with two different strained alkynes with different reactivity towards azides, namely cyclooctyne (CO) and dibenzocyclooctyne (DBCO).

Firstly, the bioorthogonal reactivity was evaluated in MNPs in suspension by using two azide-containing molecules, 3-azido-7-hydroxicoumarin and a polyethyleneglycol of 5000 Da, rendering a quantification of the reaction kinetics and the number of strained alkynes per MNP for each functionalization. Then, in a closer approach to the final goal, the reactivity towards azide-functionalized surfaces was measured with flow cytometry using azide-functionalized microbeads and with quartz crystal microbalance. Finally, the click reaction in the membranes of human breast adenocarcinoma (MCF7) and colorectal carcinoma (HCT116) cells was evaluated by fluorescence and electron microscopy, flow cytometry and elemental mass analysis.

Results have revealed a faster bioorthogonal reaction of the DBCO- functionalized MNPs in water and in cell culture conditions. The passivation molecule does not seem to impact the reactivity of both types of strained alkynes; however, it influences the internalization rate of the MNPs, with a longer cell membrane retention time for PEG-functionalized MNPs.

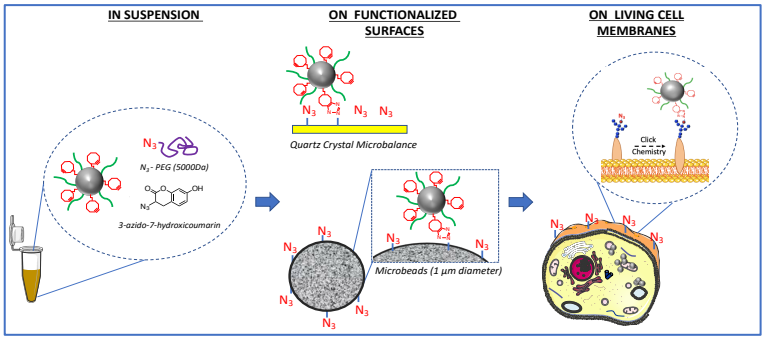

Figure 1. *Roadmap for the characterization of MNPs bioorthogonal reactivity*