Biorthogonal chemistry for the functionalization of superparamagnetic nanoparticles: cross olefin metathesis.

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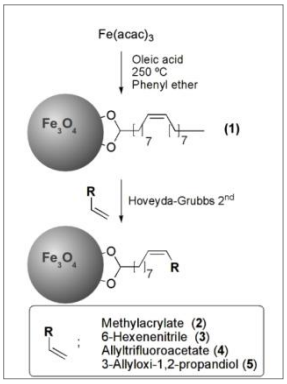
The use of magnetic nanoparticles in biomedical applications has witnessed an exponential growth last years. Iron oxide nanoparticles (NP), particularly, have gained a dominant role because of their physicochemical properties and low toxicity. Due to their superparamagnetic behavior these particles are of paramount importance in imaging techniques like Magnetic Resonance Imaging (MRI) and Magnetic Particle Imaging (MPI).

In order to provide stability and targeting these NPs require specific coating. The association of one or more biologically relevant molecules at the interface of a NP defines a NP-bioconjugate, which combines the unique physicochemical properties of NP materials with biological activity such as selective binding. To date, researchers have largely relied upon the traditional chemistries associated with protein labeling for the preparation of NP-bioconjugates. However, the range of bioconjugation techniques used with NPs has lagged behind the multitude of biological applications proposed. Although traditional bioconjugate chemistries have been adequate for proof-of-concept studies, the optimization of NP-bioconjugates for real applications (e.g., clinical) will require much greater control than these chemistries can offer. Rather, clean, efficient, and bioorthogonal conjugation reactions are required to eliminate undesirable side reactions, minimize nonspecific NP-bioconjugate activity, improve reproducibility in production, and maximize efficacy.[1-3] Within this group of bioorthogonal chemistry, olefin metathesis offers many of these features thanks to the new family of catalysts, especially Hoveyda-Grubbs 2nd generation. The metathesis mechanism reorganizes the carbon atoms of two C=C bonds, generating two new ones in the presence of a catalyst. This kind of reaction allows access from the easily prepared olefins to those that are cumbersome to obtain, being an efficient and stereoselective synthesis of the more substitute olefins in mild conditions. All of these advantages make the metathesis of alkenes one of the most powerful tools in synthetic chemistry, but as far as we know, it has not been applied for the functionalization of iron oxide superparamagnetic.

Here we present our results in the functionalization of superparamagnetic iron oxide nanoparticles with four different terminal olefins through metathesis reaction.[4] First, we synthesized iron oxide nanoparticles by the decomposition of organic precursor obtaining hydrophobic Fe_3O_4 NPs, with oleic acid as surfactant. The olefin metathesis was made between the double bond in oleic acid structure and four different molecules with a terminal double bond; methyl acrylate, 6-hexenetirile, allyltrifluoroacetate and 3-allyloxi-1,2-propandiol, in presence of catalytic amounts (4%mol) of Hoveyda-Grubbs second generation catalyst. These new NPs were fully characterized by TEM, VSM, MS and FTIR, showing the success of the reaction and quite good values for the hydrodynamic size and PDI as can be seen in figure 1.

After the metathesis the ester bond in **2** was hydrolyzed rendering water stable sample **6** due to the presence of the terminal carboxylic acid, with a Z average of 28 ± 10 nm (PDI 0.30 ± 0.07, N=3). These NPs were fully characterized. The physicochemical properties of the inorganic core were studied by TEM and VSM, which demonstrated the superparamagnetic behavior of the sample.[4]



Olefin	NP size (nm)	PDI	Solvent
2	9.1 ± 0.7	0.24 ± 0.04	$CHCl_3$
3	10.6 ± 0.6	0.21 ± 0.16	$CHCl_3$
4	9.8 ± 3.5	0.25 ± 0.05	$CHCl_3$
5	8.7 ± 1.0	0.33 ± 0.31	DMSO

Figure 1.- General metathesis synthesis and summary of the averaged sizes

The presence of the acid was probed through the FTIR spectrum and the ζ potential profile, which exhibit their stability in physiological conditions, with a value of -37 ± 5 mV at pH 7, and the typical profile for NPs stabilized by a carboxylic acid.

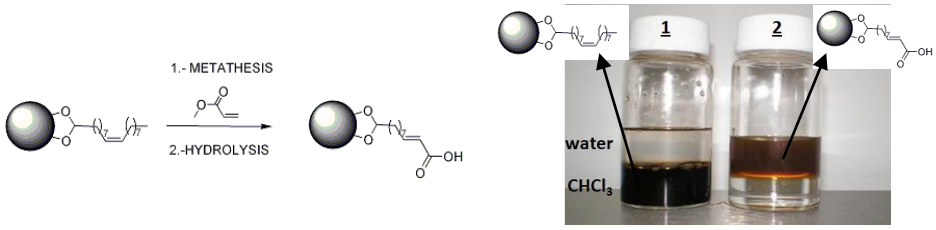


Figure 2.- Hydrolysis of NPs (2) with methyl acrylate, generating hydrophilic NPs.

For biomedical applications the nanoparticles must show high stability in solutions with high ionic strength. To this end metathesis is especially well suited as it allows such modifications in one single step from the hydrophobic particles if the right olefins are used. For this reason once demonstrated the possibility of using the metathesis in USPIO the second stage was the direct bioconjugation with hydrophilic polymers bearing a terminal olefin. On this regard we will focus in the results obtained with Polyethylene glycol (PEG) and different proteins from the extracellular matrix. First, the biopolymers were modified to show a terminal olefin through a substitution reaction. The metathesis was applied as shown before over the sample **1**, rendering hydrophilic NPs. These NPs were fully characterized by FTIR, MS, VSM and TEM, showing the success of the reaction keeping the superparamagnetic behavior of the NPs, which allow their possible use as MRI contrast agent.

In this work we demonstrate, for the first time, the use of the cross olefin metathesis reaction for bioorthogonal functionalization of iron oxide nanoparticles with different ligands, allowing the incorporation of different functional groups and biomolecules. Using appropriate catalyst and reaction conditions it is possible to modify the structure of the surfactant without self-metathesis, as demonstrated with the hydrodynamic size, TEM images and FT-IR spectra reported here. This simplifies the synthesis of hydrophobic and hydrophilic nanoparticles with applications in different fields.

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