# Cell Penetrating Peptide-Superparamagnetic Iron Oxide Nanoparticle Conjugates as Bimodal Imaging Nanoagents

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Molecular imaging has attracted more and more attention in the past decades. There is a strong interest in developing and validating imaging technologies such as fluorescence imaging and magnetic resonance (MR) to image biological target *in vivo*. Exploiting the intrinsic properties of superparamagnetic iron oxide nanoparticles (SPION) could result in obtaining medical breakthroughs in diagnosis and therapy. SPION can be used as magnetic contrast agents in magnetic resonance imaging (MRI) as well as hyperthermia agents, where the magnetic particles are heated selectively by application of an high frequency magnetic field (i.e. in thermal ablation/hyperthermia of tumors) or as magnetic vectors that can be directed by means of a magnetic field gradient towards a certain location, such as in the case of the targeted drug delivery.[1] Cell penetrating peptides have been used to increase cellular uptake of these nanoparticles. As an example HIV-1 Tat protein-derived peptide sequences, in particular GRKKRRQR48-57, have been extensively used as an efficient way of increasing internalization into cells.[2]

In this project we developed a bioorthogonal chemical approach[3] to functionalize SPION with a new class of cell-penetrating peptide, cis-γ-amino-proline derived peptides, previously developed in our group.[4] A schematic representation of the general approach is shown in Figure 1. Commercially available amino-functionalized SPION (SPION-$NH_2$) were partially modified with a reacting group (blue triangle, SPION-RG). Fluorescent labelled cell penetrating peptides, previously modified with a complementary reacting group (yellow triangle), were subsequently conjugated to SPION-RG exploiting bioorthogonal chemical reactions that occurred in buffer media close to physiological conditions. A Tat sequence, modified as described above, was included in the study as positive control for cellular uptake, while acetylated peptides were used as negative controls for proving the occurrence of the ligation. An advantage of this approach is that crude peptides could be used during the conjugation thanks to the high chemoselectivity of the reaction. Purification occurred during dialysis of the final nanoconjugate samples.

This study comprised of a synthetic part, in which the peptide-nanoparticle conjugates were prepared and characterized, followed by biological studies conduced to evaluate their cellular uptake and toxicity. In Figure 2 confocal laser microscopy confirmed internalization into HeLa cells of the SPION-CCP obtained by the conjugation of a cis-γ-amino-proline derived peptide. Currently, we are performing MRI investigations to validate such new nanodevices as novel bimodal contrast agents for optical imaging as well as MRI.

**References**


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**Figure 1**

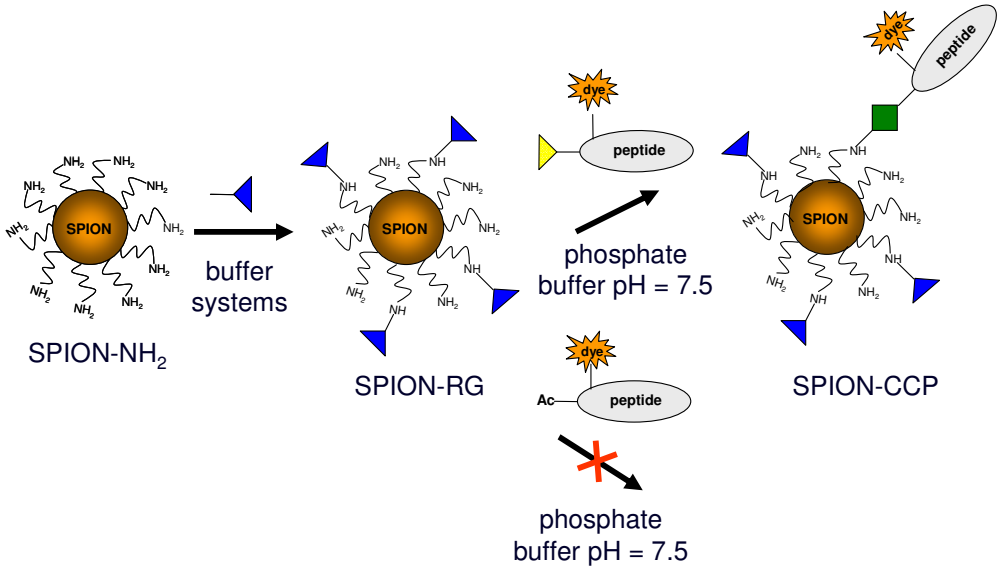


*Figure 1. Schematic representation of the general approach.*

**Figure 2**

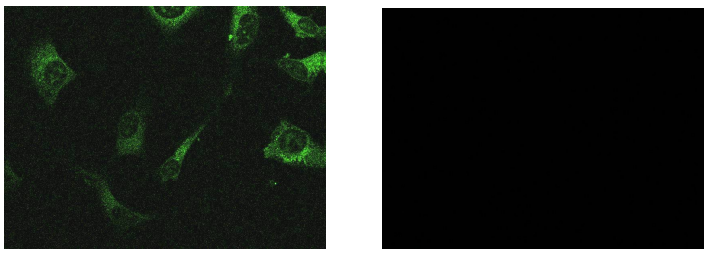

*Figure 2. Confocal laser microscopy images of HeLa cells incubated with 1 μM concentration of SPION-CCP sample (left panel) and negative control with SPION-FG + AcCCP (right panel).*