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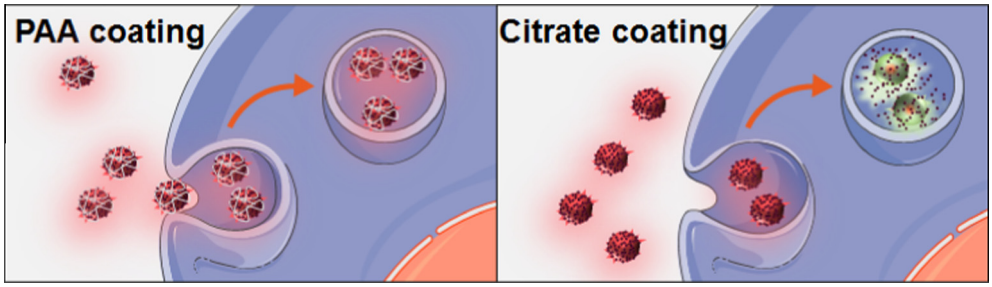
Tuning the architectural integrity of high-performance magneto-fluorescent core-shell nanoassemblies in cancer cells

Adrien Faucon [a], Houda Benhelli-Mokrani [b], Fabrice Fleury [b], Laurence Dubreil [c], Philippe Hulin [d], Steven Nedellec [d], Tristan Doussineau [e], Rodolphe Antoine [e], Tomas Orlando [f], Alessandro Lascialfari [f,g], Jérôme Fresnais [h], Lénaïc Lartigue [a], Eléna Ishow [a,*]

[a] CEISAM–UMR CNRS 6230, Université de Nantes, 2 rue de la Houssinière, 44322 Nantes, France
[b] UFIP–UMR CNRS 6204, Université de Nantes, 2 rue de la Houssinière, 44322 Nantes, France
[c] Pan Ther-UMR 703, INRA-ONIRIS, Atlanpole-Chanterie, 44307 Nantes, France
[d] INSERM UMS 016-UMS CNRS 3556, 8 quai Moncousu, 44007 Nantes, France
[e] Institut Lumière Matière–UMR CNRS 5306, Université de Lyon, 69622 Villeurbanne cedex, France
[f] Department of Physics, Università di Pavia, via Bassi, 27100 Pavia, Italy
[g] Department of Physics, Università degli Studi di Milano and INSTM, via Celoria 16, 20133 Milano, Italy
[h] Sorbonne Universités, UPMC Univ. Paris 06, CNRS, Laboratoire PHENIX, 4 place Jussieu, 75005 Paris, France

GRAPHICAL ABSTRACT



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ABSTRACT

High-density nanoarchitectures, endowed with simultaneous fluorescence and contrast properties for MRI and TEM imaging, have been obtained using a simple self-assembling strategy based on supramolecular interactions between non-doped fluorescent organic nanoparticles (FON) and superparamagnetic nanoparticles. In this way, a high-payload core-shell structure *FON@mag* has been obtained, protecting the hydrophobic fluorophores from the surroundings as well as from emission quenching by the shell of magnetic nanoparticles. Compared to isolated nanoparticles, maghemite nanoparticles self-assembled as an external shell create large inhomogeneous magnetic field, which causes enhanced transverse relaxivity and exacerbated MRI contrast. The magnetic load of the resulting nanoassemblies is evaluated using magnetic sedimentation and more originally electrospray mass spectrometry. The role of the stabilizing agents (citrate versus polyacrylate anions) revealed to be crucial regarding the cohesion of the resulting high-performance magneto-fluorescent nanoassemblies, which questions their use after cell internalization as nanocarriers or imaging agents for reliable correlative light and electron microcopy.

* Corresponding author at: CEISAM-UMR CNRS 6230, Université de Nantes, 2 rue de la Houssinière, 44322 Nantes cedex, France.
E-mail address: elena.ishow@univ-nantes.fr (E. Ishow).

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1. Introduction

Photo- and magneto-active nanostructures have recently appeared as promising diagnostic agents and therapeutic carriers owing to their complementary stimulation mode that can easily be controlled by the intensity and energy of non-invasive electromagnetic fields [1–3]. Therefore, the construction of magneto-emissive nano-objects comprising superparamagnetic and fluorescent units has been boosted by the large variety, the high performances and the easy functionalization of superparamagnetic and fluorophores currently available [4,5]. These attractive features are however counterbalanced by the increasing degree of complexity that tends to make the synthesis and purification steps rather cumbersome [6]. The search for versatile and efficient nanoarchitectures has thus led to the recent adoption of supramolecular chemistry approaches, offering multiple advantages over the classical route based on covalent linkages [7–9]. Hydrophobic, coordination and electrostatic interactions provide straightforward access to high payload architectures, displaying larger performances than those of the corresponding individual units [10–12]. Moreover, they offer reversible association, which can be advantageously exploited for drug delivery upon pH [13,14], temperature or lipophilicity modifications. Finally, non-covalent nanoassemblies undergo more rapid dissociation than covalent edifices like silica matrices, allowing for higher rates of biodegradation, faster diffusion in diseased tissues, and more complete clearance once the target has been reached.

The main strategy to self-assemble functional emissive and magnetic units mostly relies on the compaction of dyes and magnetic nanoparticles into polymersomes [3,15–17], liposomes [18,19], gelatin nanoparticles [20,21], or lipid nanocapsules [22]. It usually implies the use of amphiphilic block copolymers or molecules that get destabilized upon addition of a "bad" solvent, and self-assemble to minimize their potential energy. Encapsulation of the emissive and magnetic components within the organic matrix thus occurs at the same time. In this way, the emissive components are protected from the environment and their accumulation is expected to provide bright nano-objects. Regarding the magnetic properties, the packing of a high number of magnetic nanoparticles creates additive effects [15,18,23]. Hence, magnetic extraction occurs faster, and increased contrast for magnetic resonance imaging (MRI) can be observed, under proper limiting conditions on diffusion processes and molecular re-orientation. In this manner, self-assembled functional units within a single nanostructure enable strong reduction of the injected doses and associated toxicity for *in vivo* investigations.

Although this one-step fabrication strategy appears particularly attractive given its simplicity, several major issues have been noticed. First, strong decrease in the emission signal due to photoinduced oxidation or excitation absorption by the inorganic magnetic units has been observed [4,16,24] while surface magnetic frustration due to spin canting has been reported. Overall, the associated response is considerably impaired. Secondly, the self-assembled nanostructures display only a limited amount of active units due to the physical dilution of the encapsulated active components that are randomly dispersed. Thirdly, non-controlled release of the active units and dissembling of the whole structure can happen in the biological medium, making diagnosis inefficient and conducting to false cross-correlation studies between MRI and fluorescence microscopy imaging. Surprisingly, the latter issue has rarely been considered despite its utmost relevance in the conduction of *in cellulo* and *in vivo* experiments.

In this context, we have recently developed and reported the fabrication of reverse core-shell magneto-fluorescent suprapartricles [25]. They were based on a non-doped core made of self-assembled fluorophores forming fluorescent organic nanospheres (FONs), and coated with a shell of magnetic iron oxide nanoparticles. Such disruptive architectures, dubbed *FON@mag*, were obtained in one step following the direct precipitation of a concentrated solution of dyes into an acidic ferrofluid suspension. Owing to the strongly hydrophobic character of the fluorophores and the presence of iron chelating substituents like carboxylic and phosphonic acids, organic nuclei formed first and got stabilized by the anchoring of iron oxide nanoparticles around their surface. The resulting "raspberry-like" architectures comprised a dense emissive core made of about 10^5 non-covalently associated fluorophores, surrounded by a high density of around 10^4 superparamagnetic iron oxide nanoparticles. In this way, the emission signal of embedded dyes was found insensitive to the pH or ionic strength of the medium, providing brightness under one or two-photon excitation as high as those of quantum dots [26,27]. The tight association of magnetic units generated additive relaxivity effects, which allowed us to obtain an MRI contrast equivalent to that measured with solutions of individual iron oxide nanoparticles, ten times more concentrated. In order to prove their applicability for bioimaging, cohesion of the overall self-assembly was highly required. Structural integrity was then ensured by using polyacrylic acid (PAA) as a coating shell, yielding colloidal solutions stable over a broad range of pH (3–12) and ionic strengths (up to 0.5 mmol L^{-1} NaCl). Usually, stabilization of metal oxide nanoparticles in water is indifferently performed with small ions or polyelectrolytes [28], containing carboxylate, phosphonate or sulfonate moieties which provide large association constants toward iron oxide [11,29,30]. Among them, citrate ion is the most commonly utilized stabilizing anion to efficiently chelate the nanoparticle surface and afford electrostatic repulsions at neutral pH.

Curiously, no comparative assessment of the architecture integrity has been done between small ions and polyelectrolytes despite their largely distinct charge density and anchoring multiplicity, which in turn can impact the complexation dynamics and thereby the long term stability of the nanoassemblies in the cell medium. In order to investigate whether polyelectrolytes or small ligands can indifferently be employed for *in cellulo* studies, we report herein on the fabrication of novel *FON@mag* nanoassemblies by varying the chemical nature of the fluorophore's chelating groups (carboxylic versus phosphonic acid) and the stabilizing shell (citrate versus polyelectrolyte). The amount of magnetic material is evaluated using an original method based on mass spectrometry and confirmed by magnetic sedimentation. By performing magnetic measurements (first magnetization and ZFC-FC experiments) as well as steady-state fluorescence investigations, we can show that the stabilizing outer shell exerts no influence on the magnetic and fluorescent properties of the self-assemblies. By contrast, dynamic light scattering (DLS) and *in cellulo* experiments allow us to demonstrate that the nanoarchitecture integrity is mainly ruled by dynamic exchanges at the solution interface. The outer stabilizing shell indeed appears as a versatile chemical tool that can tune the fate of self-assembled functional architectures according to the therapeutic purposes, and advantageously minimize cytotoxicity investigations due to the reduced number of novel constituents.

2. Experimental section

2.1. Synthesis and stabilization of magneto-fluorescent nanoassemblies

(C)-FON@mag@citrate nanoassemblies: A solution of carboxylic acid (C)-fluorophores, dissolved in THF (50 μL, 0.1 wt%) was added under vigorous stirring to a solution of maghemite nanoparticles in