Electrostatic co-assembly of magnetic nanoparticles and fluorescent nanospheres: a versatile approach toward bimodal nanorods

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The elaboration of multimodal nanoparticles stimulates tremendous interest owing to their numerous potentialities in many applicative fields like optoelectronics,[1] photonics[2] and especially bioimaging.[3-5] The concomitant association of various properties (optical, electrochemical, magnetic) allows for the use of complementary stimuli in order to probe the interactions between the nanoparticles and their surroundings.[6,7] Nanoparticles (NPs) have thus become highly praised tools to image cells and tissues with a large contrast compatible with the dimensions of biological materials and the existence of quantum confinement effects induced by the reduced dimensions. In this context, the combination of magnetism and emissive properties such as fluorescence appears particularly attractive for non-invasive investigations[6-10], cell sorting or drug vectorization.[11] Therefore, combining both fluorescence and magnetism requires the delicate construction of hybrid assemblies.[12-14] Most of the magnetic nanoparticles are made of metallic oxides or alloys, e.g. γ-Fe₂O₃, Fe₃O₄, FePt, while the target fluorescent entities are often organic dyes or quantum dots (QDs).[15]

The most common route to combine both partners in a single system was based on their covalent association through targeted surface functionalization. However this required tedious and long synthetic steps, especially for QDs to circumvent issues of cytotoxicity, photooxidation and instability in aqueous media. A second route involves the incorporation of magnetic and emissive nanoparticles into the same polymeric or silica scaffolds.[16-18] which results in an enhanced photostability of the organic or inorganic dyes.[17-19] Yet the physical dilution of the active units led to a decrease in the material response efficiency and loss of possible cooperativity. A third alternative approach would consist in establishing cohesive electrostatic interactions between the components through a binding agent. This approach appears particularly attractive for two reasons: first its versatility and second the high density of active components present in the final material. In this context, we have recently demonstrated that 7 nm maghemite (γ-Fe₂O₃) nanoparticles could successfully be assembled through electrostatic interactions promoted by a cationic copolymer and γ-Fe₂O₃ nanoparticles coated by poly(acrylate) units. Indeed, when dialysis of the reaction mixture down to low ionic strength (10⁻³ mol L⁻¹) was performed under a static magnetic field, the components got reproducibly associated into 1 to 50 µm long rods where the superparamagnetic properties of the elementary 7 nm units remained unchanged.[20, 21] We thus decided to take advantage of this assembling process to incorporate fluorescent dyes during the association step. This apparently simple strategy needs however to address numerous issues. The dyes must emit in the red, far from the strong absorption region of the magnetic nanoparticles to avoid reabsorption effects. They must present a large density of fluorophores to produce highly light-sensitive systems. Finally they must possess negative charges on their surface in order to interact with the “gluing” cationic copolymer. It

Figure 1 : Left scale : normalized absorbance spectra of nanospheres (blue), nanoparticles (green), and magnetic fluorescent rods (red). Right scale : normalized emission spectra of nanospheres (blue) and magnetic fluorescent rods (red). Note that both nanospheres and magnetic fluorescent rods emission spectra are superimposed.
We retained superparamagnetic maghemite nanoparticles (γ-Fe₂O₃) which have thoroughly been studied during the last decade with respect to some fundamental properties: i) the control of the size distribution[23] and ii) the colloidal stability and the modification of the surface chemistry of the particles.[24] The ferrofluid dispersion, obtained from the alkaline coprecipitation of iron(II) and iron(III) salts after the protocol of Massart et al.,[23] was characterized by a log-normal size distribution, with a median diameter 7.1 nm and a polydispersity 0.26.[25] As synthesized, the particles were positively charged, with nitrate counterions adsorbed on their surfaces. The maghemite NPs were subsequently coated by poly(acrylic acid) chains of molecular weight of 2000 g mol⁻¹ (hereafter abbreviated as PAA₂₀k), using the precipitation-redispersion method.[26] The thickness of the PAA₂₀k adlayer (2 - 3 nm) was determined by dynamic light scattering by comparing the bare and coated particles (see Supporting Information, Figure SI-2a). This complexation method allowed the formation of anionically charged NPs, stabilized by electrostatic interactions between pH 6 and 11 and for ionic strength up to 1 mol L⁻¹ (NH₄Cl) (Figure SI-2b).[27]

As for the fluorescent systems, we turned our attention to push-pull triarylamine derivatives that we recently synthesized and could precipitate into nanoparticles, strongly emitting from blue to red in water (see Supporting Information, Figure SI-1). Since the emission reabsorption by the γ-Fe₂O₃ particles, absorbing in the visible, must be circumvented, we selected the red-emitting derivative 4-di(4′-tert-butylbiphenyl-4-yl)amino-4′-dicyanovinylenzene FVIN whose fluorescence spectrum peaked at 632 nm in the solid state far from the γ-Fe₂O₃ absorption range.[22] The fabrication of the corresponding fluorescent particles was done by the rapid mixing of a small amount of FVIN stock solution (c = 0.05 wt. %) dissolved in acetone with a large excess of water. For the nanospheres to be assembled by the “gluing” cationic polymers, a 1 wt. % solution of PAA₂₀k (instead of deionized water) was used in the reprecipitation process. The same polymer was employed as that for the coating of the maghemite nanoparticles for obvious compatibility reasons. Remarkably spherical and monodisperse colloids, dubbed nanospheres in the following were obtained, as evidenced by transmission electron and optical microscopy (see Supporting Information, Figure SI-3). Determination of hydrodynamic diameters gave Dₜ = 180 nm. As expected, uncoated particles exhibited slightly reduced Dₜ equal to 150 nm. These results agreed remarkably well with the size measurements obtained by transmission electron microscopy (TEM). The electrophoretic mobilities (μₑ) of the PAA₂₀k-coated nanospheres were found to be μₑ = -5.7×10⁻⁴ cm² V⁻¹ s⁻¹, which shows that the system was strongly anionically charged. Compared to the value for uncoated nanospheres (μₑ = -3.2×10⁻⁴ cm² V⁻¹ s⁻¹), the...
increase in $\mu E$ was assigned to the adsorption of the PAA$_{20k}$ at the nanosphere-solvent interface (Figure SI-2b). The colloidal stability of the coated nanospheres in aqueous solution could be proven for a period longer than 2 months at room temperature.

The UV-visible absorption spectra of PAA$_{20k}$-coated nanospheres exhibited two bands in the blue region and an additional band in the visible region centered at 468 nm (Figure 1). Based on previous experimental and computational studies (time-dependent density functional theory), this latter band has been ascribed to a charge transfer transition from the amino group to the dicyanovinylene unit form ing a radiative excited state. Excitation in the UV or in the visible led to the same broad emission centered at 632 nm, revealing a large Stokes shift of 164 nm. These values were insensitive to the physico-chemical conditions of the dispersions (temperature, pH) and identical to those obtained for uncoated nanospheres. The emission spectrum of the aqueous dispersions of PAA$_{20k}$-coated nanospheres presented a weak spectral overlap with the absorption band of the $\gamma$-Fe$_2$O$_3$ dispersions, validating the choice made of both partners on a spectral basis.

The combination of the fluorescent and magnetic particles was performed after the same protocol as that previously used for getting giant magnetic particles was performed after the same protocol as that previously used for getting giant magnetic particles was performed after the same protocol as that previously used for getting giant magnetic particles was performed after the same protocol as that previously used for getting giant magnetic particles was performed after the same protocol as that previously used for getting giant magnetic particles was performed after the same protocol as that previously used for getting giant magnetic particles was performed after the same protocol as that previously used for getting giant magnetic particles was performed after the same protocol as that previously used for getting giant magnetic particles was performed after the same protocol as that previously used for getting giant magnetic particles was performed after the same protocol as that previously used for getting giant magnetic particles was performed after the same protocol as that previously used for getting giant magnetic particles was performed after the same protocol as that previously used for getting giant magnetic particles was performed after the same protocol as that previously used for getting giant magnetic particles was performed after the same protocol as that previous experimental and computational studies (time-dependent density functional theory), this latter band has been ascribed to a charge transfer transition from the amino group to the dicyanovinylene unit forming a radiative excited state. Excitation in the UV or in the visible led to the same broad emission centered at 632 nm, revealing a large Stokes shift of 164 nm. These values were insensitive to the physico-chemical conditions of the dispersions (temperature, pH) and identical to those obtained for uncoated nanospheres. The emission spectrum of the aqueous dispersions of PAA$_{20k}$-coated nanospheres presented a weak spectral overlap with the absorption band of the $\gamma$-Fe$_2$O$_3$ dispersions, validating the choice made of both partners on a spectral basis.

A movie of two rods subjected to a rotating magnetic field (0.63 Hz) has been recorded and it is shown in Supporting Information. In Figure 4, two snapshots from times 0 – 0.8 s and 1.6 - 2.4 s corresponding each to a 180° turn of the field depict the rotation of the rods, indicating that the rods are magnetically active. By analogy with previous work, we assume that these rods have inherited the superparamagnetic properties from their constituting maghemite nanoparticles. During the rotation, the excitation at 465 nm was switched on at 0.4 s. Its intensity was progressively increased, while the white light used for transmission imaging was dimmed. Bright areas within the bottom rotating rod appeared under illumination at 465 nm, proving the incorporation of the fluorescent nanospheres within the magnetic structures.

Steady-state fluorescent experiments were performed on water suspensions of magneto-fluorescent nanorods. Excitation at 465 nm, at the red edge of the maghemite absorption band and close to the FVIN absorption maximum produced an emission spectrum which superimposed that of FVIN nanospheres dispersed in deionized water or PAA$_{20k}$-coated. This overtly evidenced a lack of significant electronic interactions between the organic material and its inorganic surrounding, and proved our concept of non-covalent self-assembling to get multifunctional architectures with orthogonal properties.
To gain insight into the microscopic structure of the rods, TEM imaging was performed. One clearly observed cylindrical structures with a high aspect ratio, where the 7 nm-nanoparticles form a tight and homogeneous ensemble. The rod displayed in Figure 4a has a diameter of 200 nm for a length of 2.25 mm. The volume fraction occupied by the nanoparticles was assessed by small-angle neutron scattering to be 25 % of the whole.[30] This corresponds to $5 \times 10^4$ nanoparticles per micrometer for rods with a diameter of 200 nm.

**Figure 4**: TEM images of a magnetic rod (a) and magnetic fluorescent rods (b, c, and d) with different magnifications ($\times 3000$ for a and b, $\times 16000$ for c and $\times 32000$ for d). The arrows indicate the fluorescent nanospheres embedded in the rod.

TEM imaging of magneto-fluorescent rods evidenced the same elongated structures where this time circa 200 nm sized-globules were embedded. This dimension matched the hydrodynamic diameter of the fluorescent FVIN nanospheres found at $D_h = 180$ nm. In figures 4b-d, the globules and the metallic nanoparticles forming the rods exhibit a marked difference in the electronic contrast, indicating that the former should be made of organic matter. This reinforces our assumption of FVIN nanospheres incorporated into maghemite nanorods. Figures 4c and 4d show moreover a partial deformation of the nanospheres along the axial direction of the rod. This elongation might be due to strong mechanical constraints arising during the assembling process, as well as to the relative softness of the nanospheres. As already observed by fluorescence microscopy, the nanospheres were randomly distributed in the bulk of the rods, with a preferential localization in the central (instead of apical) part of the rods. These data show that the magnetic field driven assembly of the $\gamma$-Fe$_3$O$_3$ nanoparticles were not impeded by the presence $\sim 20$ times larger organic colloids. The understanding of the growth process in the presence of large entities is under current progress.

In conclusion, highly anisotropic rods containing anionic magnetic nanoparticles and organic fluorescent nanospheres were built using an electrostatic co-assembling strategy in the presence of cationic polyelectrolytes. TEM and optical microscopy showed typical lengths between 1 and 50 mm, and mean diameters around 200 nm. The hybrid elongated architectures maintain the superparamagnetic properties of the $\gamma$-Fe$_3$O$_3$ constituents, as evidenced by their in-phase response to an external rotating magnetic field. Fluorescence could be produced by the embedded nanospheres which kept emitting in the red region far from the strong absorption band of iron oxide. The approach reported here offers an easy and versatile way of elaborating multimodal nanomaterials. In terms of applications, these magneto-fluorescent rods can serve as micrometric actuators and sensors for microfluidics and micro rheology, as well as addressable agents for bioimaging.

**Experimental Section**

Experimental details on the nanoparticle synthesis and the optical, structural and magnetic characterizations of the nanorods and their constituents are given in the supporting information.[21, 24, 25, 33-35]

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TOC figure

Electrostatic co-assembly of magnetic nanoparticles and fluorescent nanospheres: a versatile approach toward bimodal nanorods
Supporting Information

Electrostatic co-assembly of magnetic nanoparticles and fluorescent nanospheres: a versatile approach toward bimodal nanorods
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Outline

SI-1 – Formula and emission spectra of fluorescent molecules
SI-2 – Characterization of particles and nanospheres
  SI-2.1 – Dynamic light scattering and electrophoresis
  SI-2.2 – Optical microscopy and TEM
SI-3 – Characterization of the magneto-fluorescent nanorods
SI-4 – Movies
  Movie#1 : Brownian nanorods as seen by transmission and emission optical microscopy. No magnetic field was applied.
  Movie#2 : Magneto-fluorescent nanorods submitted to a rotating magnetic field oriented by a magnetic of amplitude 10 mT and frequency 0.63 Hz.

SI-1 – Formula and emission spectra of fluorescent molecules

The molecule formula is given in the figure SI-1. Note that the emission wavelength depends strongly on the final end group bounded on the molecule. We selected the 4-di(4’-tert-butylbiphenyl-4-yl)amino-4’-dicyanovinyl benzene FVIN molecule for its emission wavelength at 632 nm.

![Figure SI-1](image)

Figure SI-1 : a) Structure of push-pull triarylamine molecules emitting in the solid state from blue (compound 1) to red (compound 5) when changing the strength of the electron-withdrawing group. b) Normalized emission spectra ($\lambda_{exc} = 343 \text{ nm}$) of 200 nm nanospheres made from compounds 1 - 5.

SI-2 – Characterization of particles and nanospheres

SI-2.1 – Dynamic light scattering and electrophoresis

The hydrodynamic diameter distributions for the colloids and polymers were obtained using a Zetasizer Nano ZS (Malvern Instrument) operating at the scattering angle 174°. The collective diffusion coefficient $D$ was determined from the second-order autocorrelation function of the scattered light. From the value of the coefficient, the hydrodynamic diameter of the colloids was calculated according to the Stokes-Einstein relation, $D_H = k_B T / (3 \pi \eta S D)$, where $k_B$ is the Boltzmann constant, $T$ the temperature (T = 298 K) and $\eta_S$ the solvent viscosity ($\eta_S = 0.89 \times 10^{-3}$ Pa·s for water).

Figure SI-1a shows the intensity distributions as function of $D_H$ for the PAA$_{3K}$-coated magnetic particles and fluorescent nanospheres. The data for the uncoated nanospheres were included for comparison. The average $D_H$ values for the three dispersions are listed in Table SI-I. Note that the $D_H$ for PAA$_{3K}$-$\gamma$-Fe$_2$O$_3$ was 5 nm above that the uncoated particles (i.e. 14 nm). It should be mentioned here that the hydrodynamic sizes appear larger than those determined by TEM. The reasons for that are well-known : i) the particles are slightly anisotropic (aspect ratio 1.2); ii) when particles are distributed, as this is the case here, light scattering is sensitive to the largest particles of the distribution.

Measurements of the electrophoretic mobility $\mu_E$ were carried out on the same three dispersions on the same instrument. Using laser Doppler velocimetry, the technique is based on the Phase Analysis Light Scattering (PALS) method. The electrophoretic light scattering intensities have been plotted as a function of the mobility in Figure SI-1b, whereas the $m_E$-values at the peak maximum are given in the main manuscript and in Table SI-I.

![Table SI-1](image)

Table SI-1 : Hydrodynamic diameter ($D_H$), zeta potential (z) et electrophoretic mobility ($\mu_E$) for coated particles and nanospheres investigated in this work.

SI-2.2 – Optical microscopy and TEM

Phase-contrast and fluorescence images were acquired on an IX71 inverted microscope (Olympus) equipped with a 60X objective. We used a Photometrics Cascade camera (Roper Scientific) and Metaview software (Universal Imaging Inc.) for the
image acquisition. Further treatment was achieved using the ImageJ software (http://rsb.info.nih.gov/ij/).

Figure SI-2: a) Distribution of hydrodynamic diameters $D_H$ obtained by dynamic light scattering for dispersions of PAA-coated nanoparticles and nanospheres. The data for uncoated nanospheres are shown for comparison. b) Electrophoretic data for the same three colloids.

An aqueous dispersion of nanospheres was investigated in emission at the wavelength 465 nm, that is close to the absorption maximum of the FVIN fluorophore. The nanospheres in Figure SI-3 appeared as yellow-orange dots in a red dark background. The field of view of Figure SI-3 covers the range 45 $\times$ 50 mm$^2$. An accurate description of the nanosphere microstructure was published recently.[22] We recall here in the inset a TEM image of the fluorescent colloids obtained by the precipitation technique. The diameter of the sphere is 120 nm. For the TEM experiments, we acknowledge the technical assistance by Aude Michel from the Laboratoire de Physico-chimie des Electrolytes, Colloïdes et Sciences Analytiques for an electron beam microdiffraction experiment with a Jeol-100 CX transmission electron microscope at the SIARE facility of University Pierre et Marie Curie (Paris 6).

Figure SI-3: Emission image of a nanosphere dispersion obtained by optical microscopy ($l_{exc} = 514$ nm). Inset: TEM image of one fluorescent colloid (bar : 50 nm).

SI-3 – Characterization of the magneto-fluorescent nanorods

Figure SI-4 displays the length distribution of the magneto-fluorescent nanorods. The data were those of Figure 4. The statistics was acquired on 123 specimens, yielding a distribution centered around 10 $\mu$m. The data were fitted by a log-normal distribution function with median length $L_{rod} = 9.97 \pm 0.5 \mu$m, and a polydispersity $s = 0.40$. These data are reported in the main text.

Figure SI-4: Length distribution of nanorods observed in Figure 4. The continuous line was derived from best fit calculation using a log-normal distribution.