Supporting Information

I- Chemicals

QA Synthesis.

Materials for QA Synthesis. Melting point was determined on an electrothermal IA9300 (capillary) apparatus and was not corrected. NMR spectra 200 MHz for ^{1}H or 50 MHz for ^{13}C were recorded on a Bruker Avance 200 instruments using DMSO-d_6 as solvent (Bruker Biospin, Wissembourg, France). Chemical shifts were referenced to the residual DMSO signals at 2.50 ppm (^{1}H NMR) and 39.5 ppm (^{13}C NMR). Infrared spectra was recorded in KBr pellets on a FT vector 22 (ν expressed in cm^{-1}). Electrospray ionization mass spectra (ESI-MS) was obtained on a TSQ 7000 ThermoQuest Finnigam (Les Ulis, France). The sample was analyzed in CH_3CN/H_2O (1/1, v/v, containing 1% HCOOH) in positive mode, at a final concentration of 5 pmol/µL. ESI-MS spectrum was recorded by averaging of 10 spectra. Microanalyses were performed by Analytical Laboratory of the CNRS (Vernaison, France) for the elements indicated and were within 0.4% of the theoretical values unless indicated. 3-Bromopropylammonium bromide (98%) and pyridine (>99%) were purchased from ACROS Organics (Halluin, France) while solvents were obtained from CARLOS ERBA-SDS (Val de Reuil, France). The pyridine, acetonitrile and diethyl ether were dried using common techniques.[1]

SRP functionalization with QAs. N-(3-Dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDC, > 98.0 %), N-hydroxysuccinimide (NHS, > 97.0 %), Gadolinium chloride hexahydrate ([$GdCl_3.6H_2O$], 99 %), Sodium Hydroxide (NaOH, 99.99 %), Hydrochloric Acid (HCl, 36.5-38 %), Sodium Chloride (NaCl, > 99.5 %), Dimethyl Sulfoxide (DMSO, > 99.5 %), Acetonitrile (CH_3CN, > 99.9 %) and Trifluoroacetic Acid (TFA, > 99 %) were purchased from Aldrich® Chemical (France) and used without further purification. The SRP synthesis has been already described elsewhere.[2] The gadolinium oxide core was purchased from Nano-H SAS (Saint-Quentin Fallavier, France) and the DOTAGA (1,4,7,10-tetraazacyclododecane-1-glutaric anhydride-4,7,10-triacetic acid) chelate was purchased from CheMatech (Dijon, France). For the preparation of an aqueous solution of nanoparticles, only milli-Q water (ρ > 18 MΩ.cm) was used.

Radiolabelling. $^{111}InCl_3$ was purchased from Perkin-Elmer (Waltham, USA). The activity was 222 MBq in 250 µL HCl 0.05 N and the specific activity was 15.4 GBq/mg at calibration date. Citrate buffer 100 mM (pH 5.0), used for SRP radiolabelling, was prepared from citric acid monohydrate and NaOH 1N solution. For the preparation of 1000 mL citrate buffer 100 mM (pH 5), 21,014 g of citric acid monohydrate (0.1 mole (M=210.14 g/mol)) were dissolved in 300 mL NaOH 1N. The volume was then adjusted to 1000 mL with milli-Q water. PD-10 Sephadex™ G-25M columns (GE healthcare, Uppsala, Sweden) were used for purification of radiolabelled SRP. Silica gel ITLC-SG paper chromatography (Varian inc., Palo Alto, USA) was used for radiolabelling efficiency controls.

II- Techniques of particle characterization

Relaxivity measurements.

Relaxivity experiments were performed on a Bruker® mq60 NMR Analyzer at 37 °C. Sample were measured at a specific Gd(III) concentration, calculated from ICP-OES. Measurements were plotted as $1/T_1$ *vs* Gd(III) concentration (mM), assuming that the water longitudinal relaxtation rate is negligible with respect to sample longitudinal relaxation rate. The slope of this line provides the molar relaxivity.

Inductively Coupled Plasma – Optical Emission Spectroscopy (ICP-OES) Analysis. The determination of the accurate concentration of gadolinium in SRP samples was performed by ICP-OES with a Varian® 710-ES spectrometer. First, SRPs were pre-dispersed in water (100mM). Small amounts of SRP aqueous solutions were diluted and heated at 80°C for 3 hours in 5 mL of concentrated nitric acid (67% HNO_3 (w/w)). Subsequently, the samples were scattered in 50 mL of water. For the calibration of the ICP-OES, single element standard solution was used and prepared from 1000 ppm Gd-standard from SCP Science® by successive dilutions with an HNO_3 5% (w/w) matrix.

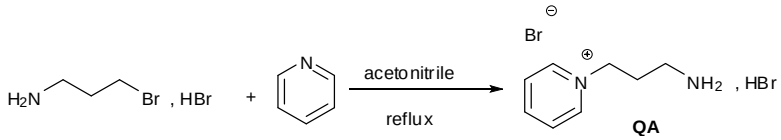
Photon Correlation Spectroscopy (PCS) Size and ζ-Potential Measurements. Hydrodynamic diameters (HD) and ζ-Potentials of our samples were determined with a Zetasizer NanoS PCS (Photon Correlation Spectroscopy, laser He-Ne 633 nm) from Malvern Instruments®. For HD measurements, 200 µL of an aqueous solution of SRPs (10 mM) were pipetted into a disposable micro-cuvette (ZEN0040). Attenuator and position were optimized by the device. Prior to ζ-Potential experiments, the SRPs (10 mM) were diluted in an aqueous solution containing 0.01 M NaCl and adjusted to the desired pH. ζ-Potential measurements were recorded at 25°C with palladium electrodes within a high concentration cell (ZEN1010). The ζ-Potential was automatically calculated from electrophoretic mobility based on the Smoluchowski equation, $\nu = (\varepsilon E/\eta)\zeta$, where ν is the measured electrophoretic velocity, η is the viscosity, ε is the electrical permittivity of the electrolytic solution and E is the electric field.

Mass Spectrometry (MS). Full scan mass experiments were performed using a linear quadrupole ion trap mass spectrometer (LTQ, Thermo Fisher Scientific, San Jose, CA) with enlargement for the high 400-4000 Th range. The nanoparticle solution was electrosprayed at a flow rate of 5 µg/min in positive ion mode. Isotopic distributions of fragment ions were recorded using the zoom scan mode of the LTQ quadrupole ion trap mass spectrometer.

Ultraviolet-visible Absorption spectra. Aqueous solutions of SRPs were analyzed by a UV-vis spectrophotometer (Varian Cary50) in the range of 200 to 800 nm, with a Hellma semi-micro cell, 10 mm light path, 1400 µL, manufactured from Suprasil quartz.

III- Synthesis of QAs and SRP functionalization with QAs

QA Synthesis.



Synthesis of 1-(3-ammoniopropyl)pyridinium dibromide (**QA**). This compound was synthesized with *slight modification*s of the procedure described by Steullet et al.[3] Briefly, to a solution of 3-bromopropylammonium bromide (500 mg, 2.28 mmol) in dry acetonitrile and under argon was added dropwise freshly distilled pyridine (1 mL, 12.4 mmol). The reaction mixture was stirred under reflux for 5 hours. After cooling to room temperature, the precipitated was collected by filtration, washed with anhydrous diethyl ether (10 mL) and dried overnight in a desiccator under reduced pressure to give 1-(3-ammoniopropyl)pyridinium dibromide (**QA**) as a white solid (534 mg, 78%). Mp 172-174 °C (litt.[4] 176.5-177.5 °C). Elemental analysis: Found: C, 32.05; H, 4.95; Br, 53.38; N, 9.39. Calc. for $C_8H_{14}Br_2N_3$: C, 32.24; H, 4.74; Br, 53.62; N, 9.40%. IR (KBr) ν_{max}/cm^{-1} 3550-3300, 3100-2800, 1632, 1489, 1175 cm^{-1}. NMR δ_H (DMSO-d_6) 2.24 (quint, 2H, J = 7.1 Hz), 2.85 (t, 2H, J = 7.1 Hz), 4.76 (t, J = 7.1 Hz), 7.96 (brs, 3H), 8.20 (t, 2H, J = 7 Hz), 8.64 (t, 1H, J = 7.5 Hz), 9.17 (d, 2H, J = 6 Hz). NMR δ_C (DMSO-d_6) 28.6, 35.5, 57.6, 128.3 (2C), 144.8 (2C), 145.8. ESI-SM m/z 137.00 $[M+H]^+$.

SRP functionalization with QAs.

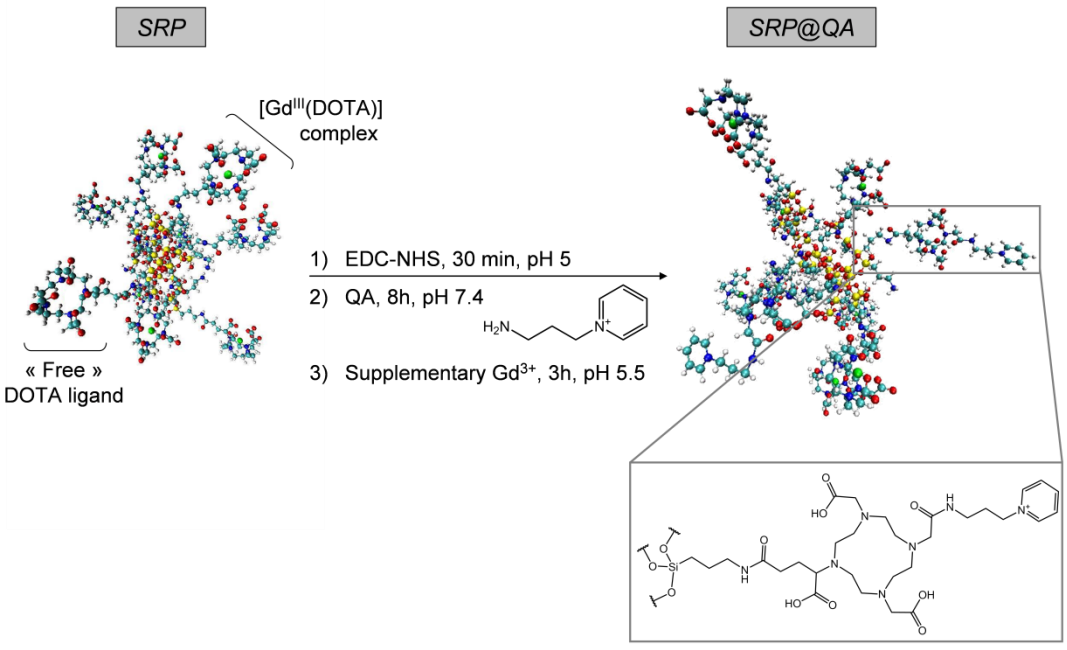


Figure 1. *Grafting protocol of the QA on the SRPs*

Syntheses were performed at room temperature. SRP concentrations are stated in $mol.L^{-1}$ of Gadolinium element.

SRPs were dispersed in water (100mM) for 15 min and treated by EDC and NHS at pH 5 for 30 min (EDC/NHS/Gd molar ratio 3:3:1). The activated SRP solution was divided into two aliquots: one to be grafted with the QA and the other one to be used as a reference. The QA was mixed with one of the two previously activated SRP aliquots at pH 7-7.4 for 8 hours (QA/Gd molar ratio: 2:1). Similarly, the other sample considered as the reference was subjected to the same conditions, *i.e.* previously remaining activated SRP aliquot was stirred at pH 7-7.4 for 8 hours. To achieve higher proton longitudinal relaxivities, additional Gd^{3+} ions were inserted in both SRP aliquots by adding a 100mM Gd^{3+} solution prepared with $GdCl_3.6H_2O$ in water (additional Gd^{3+}/initial Gd molar ratio 0.5:1). The final mixtures were stirred for 3 hours at pH 5-6. Each SRP aliquot was subjected to tangential filtration to a 3000 factor over a 5 kDa membrane to remove any unconjugated product and was freeze-dried for storage, using a Christ Alpha 1-2 lyophilizer. The freeze-dried SRPs are stable for months without alteration.

IV- SRP characterization: validation and quantification of the AQ covalent conjugation to SRPs

Relaxometry

First, the longitudinal proton relaxivities, r_1 ($mM^{-1}.s^{-1}$), of both unfunctionalized SRPs and functionalized SRPs were calculated according to **Eq. 1**[5]:

$$r_1 = \frac{1}{[Gd^{3+}]}\left(\frac{1}{T_{1obs}} - \frac{1}{T_{1H_2O}}\right)$$

where $[Gd^{3+}]$ is the actual gadolinium concentration of the sample (mM) measured by ICP analysis, $T_{1,obs}$ and $T_{1,H2O}$ are the longitudinal relaxation times (ms) in the presence and absence of the SRPs, respectively. Relaxivity values of water protons in the presence of SRPs at 60 MHz are reported equalled 11.2 $mM^{-1}.s^{-1}$ and 12.2 $mM^{-1}.s^{-1}$ at 60 MHz for unfunctionalized SRPs and for functionalized SRPs@QA, respectively. The longitudinal proton relaxivity is a measure of the contrast promoted by MRI positive contrast agents[6]. The longitudinal proton relaxivity depends on various factors including the external field, the electronic properties of the gadolinium ion, the water exchange, the rotational diffusion, the coordination sphere hydratation and the paramagnetic ion to water proton distance[7]. Thus, the covalent conjugation of the QA to SRPs might be responsible for the slight increase of relaxivity compared to unfunctionalized SRPs. Moreover, the longitudinal relaxivities of both unfunctionalized SRPs and SRP@QA were higher than the ones of the common contrast agents (Dotarem® and Magnevist®), which are less than 4 $mM^{-1}.s^{-1}$ at 60 MHz[1]. Then, the relaxivity per element (nanoparticle or molecule) was higher for SRPs than for small organic contrast agents, which means that the SRPs display high contrast for a small volume and result in a better contrast in MRI[2].

Mass Spectrometry.

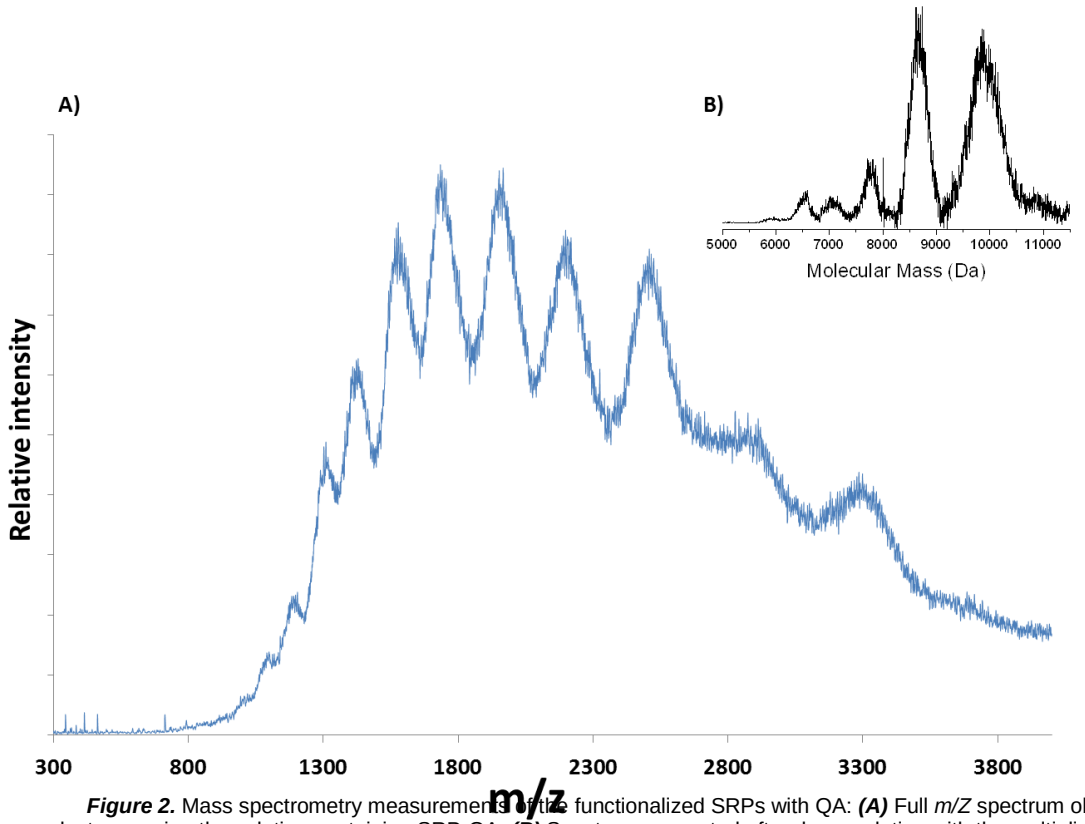


Figure 2. Mass spectrometry measurements of the functionalized SRPs with QA: ***(A)*** Full *m/Z* spectrum obtained after electrospraying the solution containing SRP-QA, ***(B)*** Spectrum generated after deconvolution with the multiplicative correlation algorithm.

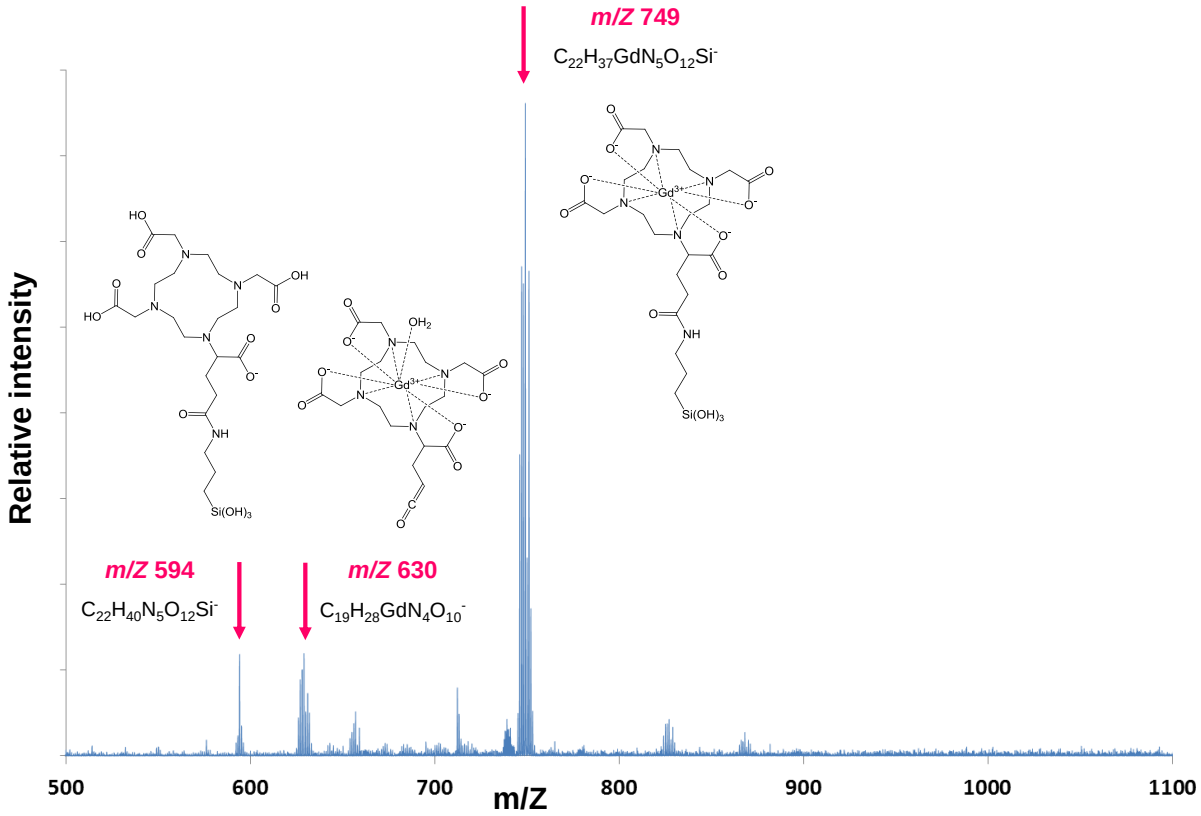


Figure 3. Mass Spectrometry measurements: Low *m/Z* part of the mass ESI spectrum in a negative mode of SRPs@QA (the same fragments were observed for unfunctionalized SRPs in this same negative mode)

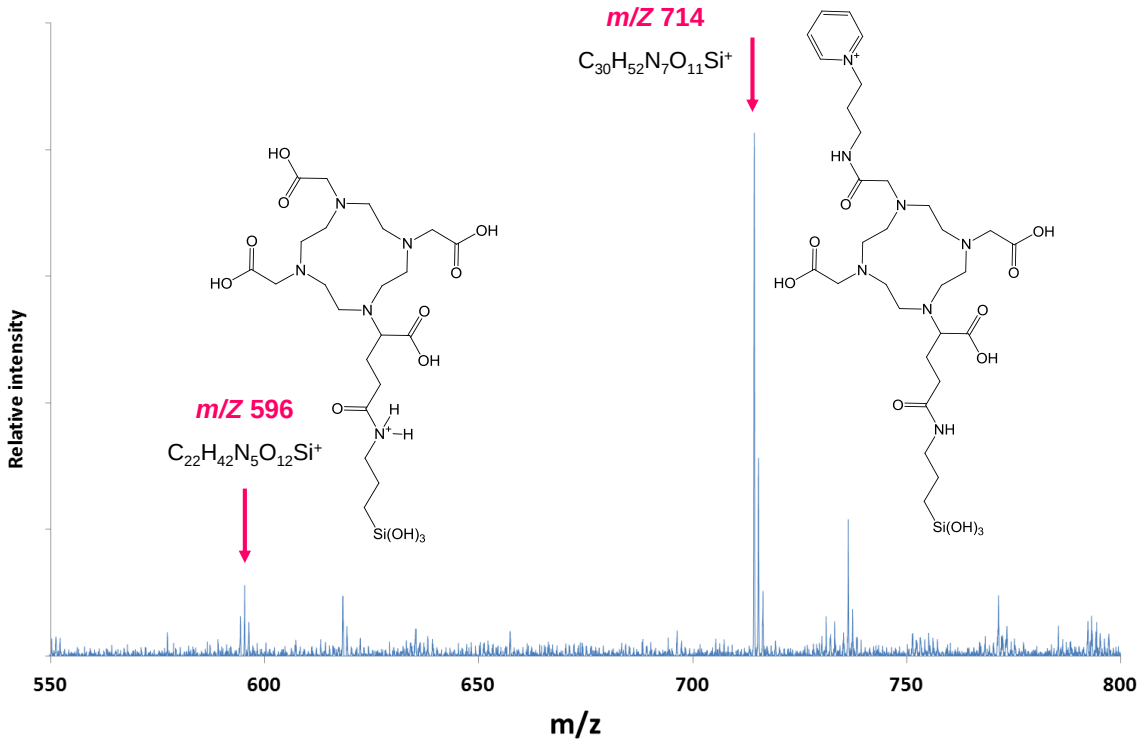


Figure 4. Mass Spectrometry measurements: Low *m/Z* part of the mass ESI spectrum in a positive mode of SRPs@QA. The peak at m/Z = 714 is one of the four possibility for the grafting of the QA on the DOTA (possessing 4 free carboxylic functions) at the surface.

UV-Visible Absorption

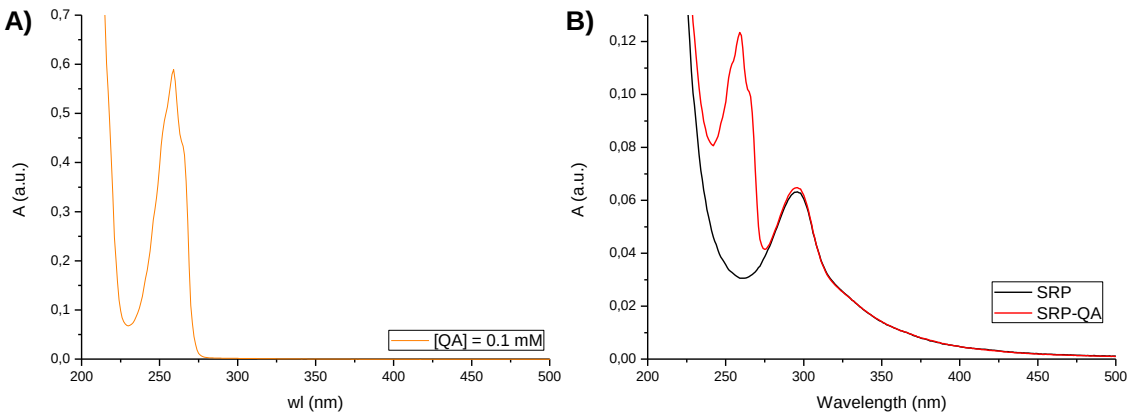


Figure 5. (A) UV-visible absorption spectrum of QA (0.1 mM). ***(B)*** UV-visible absorption spectra of unfunctionalized SRPs and functionalized SRPs with QA.

Elemental Analysis.

Two major hypotheses were made to estimate the SRP chemical compositions: (i) each SRP displays 10 ± 1 DOTA molecules; (ii) the APTES/TEOS molar ratio of the polysiloxane network (1 Si = 0.6 APTES + 0.4 TEOS) remains constant during synthesis and purification steps. Considering these two hypotheses and the molar ratios (*i.e.*, Si/Gd, C/Gd and N/Gd) deduced from weight percentages, an empirical method allowed tracing back to the approximate molecular formula of both SRPs. Since only gadolinium, silicon, carbon and nitrogen weight percentages were measured, it was relevant to include any atom being different from the previously mentioned ones in our calculations. For example, water molecules and any counterion (*e.g.* Na^+, OH^-, etc.) were considered. The number of water molecules is presented on an indicative basis and these H_2O molecules could be replaced by any counterion, thus slightly modifying the global molecular mass. The molecular formulas were deduced with an absolute error below 0.4% of the weight percentages supplied by elemental analyses.

	SRPs			SRPs@QA		
Elemental Analysis - (Weight percent of each element)						
	Exp.	Theo.	Err.	Exp.	Theo.	Err.
%Gd	12.09	12.16	0.07	12.04	11.66	0.38
%Si	7.89	7.94	0.05	9.57	9.27	0.30
%N	8.6	8.57	0.03	8.54	8.87	0.33
%C	31.37	31.32	0.05	31.21	31.59	0.38

Table 1. Elemental analyses given in weight percent of element in the compound. For each sample, first the experimental weight percentages (carried out by the "Service Central d'Analyses de Solaize", CNRS), second the theoretical weight percentages deduced from the estimated molecular formulas and third the absolute error between the experimental and theoretical weight percentages are reported.

V- radiolabelling and biodistribution studies of ^{111}In-SRP@QA and unfunctionalized ^{111}In-SRP

Radiolabelling. SRPs were reconstituted in milli-Q water at a concentration of 100 mM (gadolinium equivalent (M_{Gd}= 157.25 g/mol)). After addition of milli-Q water, the solutions were thoroughly vortexed for 30 minutes before radiolabelling. $^{111}InCl_3$ (100 MBq) was added to 10 µL of a 100 mM solution of SRPs and diluted with 390 µL of citrate buffer 100 mM (pH 5.0) (final volume: 400 µL). The reaction mixture was incubated at 30 °C for 20 minutes. After radiolabelling, free ^{111}In was removed from the solution by chromatography through a PD-10 Sephadex™ G-25M column previously washed with 20 mL 0.9 % saline and eluted with citrate buffer. The eluted fractions (1.0 mL) were collected in test tubes. Radioactivity of the ^{111}In-SRPs before and after column purification was measured with a Capintec CRC-15R dose calibrator (ARIES, Chatillon, France). Generally, the desired ^{111}In-SRP was collected in fractions 2-5. Thin layer chromatography (TLC) of the purified ^{111}In-SRP was run on ITLC-SG sheets developed with citrate buffer and the radio TLC was recorded by a Scanalytic gamma imager ambis® 101 (Rockville, USA). After purification, no free ^{111}In was detected on TLC controls. A TLC stability control was performed before intravenous (i.v.) administration and at 24 hours after radiolabelling in order to confirm that ^{111}In remains associated with the SRPs over 24 hours and that the biodistribution patterns observed are not from free, dissociated ^{111}In. ^{111}In-SRP@QA and unfunctionalized ^{111}In-SRP were obtained with a radiochemical yield of 75 and 78% respectively, with a high radiochemical purity (≥ 99%) and were stable after 24 hours at room temperature with a radiochemical purity remaining higher than 99%.

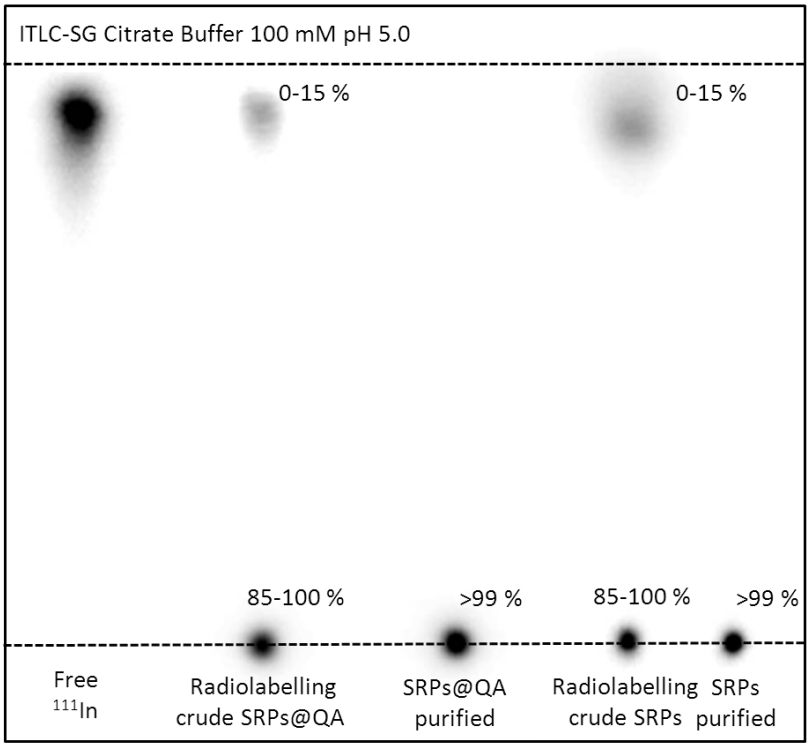


Figure 6. Typical radiochromatograms obtained after instant thin layer chromatography (ITLC) of radiolabelled SRPs and SRPs@QA before and after purification by gel filtration chromatography

Particle accumulation in femorotibial joints: ex vivo planar scintigraphic imaging and radioactivity counting. Both radiolabelled nanoparticles were administered by iv route to animals (10 MBq/animal and 3 to 4 animals/group), that were sacrified at 1 hour and 3 hours pi respectively. Ex vivo planar scintigraphic imaging was performed on posterior paw being removed and positioned over the collimator of a gamma camera dedicated to small animal imaging (GammaImager Biospace; field of view of 10 cm). 10 minutes-duration acquisition was performed with two 15% windows centered on the two peaks of ^{111}In, at 171 keV and 245 keV. After acquisition, posterior paws were dissected, with tibial plateau and muscle being removed, weighted and counted using a gamma counter (Packard). All measured activities were corrected for radioactive decay, expressed as cpm/g of sample and Plateau tibial to muscle ratio was determined.

---

[1] W. L. F. Armarego, D. D. Perrin, in *Purification of laboratory chemicals.* eds. Butterworth-Heinemann; Reed educational and professional Publishing Ltd: Oxford, 4th ed., **1996**.
[2] F. Lux, A. Mignot, P. Mowat, C. Louis, S. Dufort, C. Bernhard, F. Denat, F. Boschetti, C. Brunet, R. Antoine, P. Dugourd, S. Laurent, L. Vander Elst, R. Muller, L. Sancey, V. Josserand, J.-L. Coll, V. Stupar, E. Barbier, C. Rémy, A. Broisat, C. Ghezzi, G. Le Duc, S. Roux, P. Perriat, O. Tillement, *Ange. Chem. Int. Ed.*, **2011**, *123*, 12507.
[3] V. Steullet, D. W. Dixon, *J. Chem. Soc., Perkin Trans. 2*, **1999**, *2*, 1547.
[4] M. Boese, *Chem. Ber.*, **1920**, *53*, 1994.
[5] Y. Li, M. Beija, S. Laurent, L. vander Elst, R.N. Muller, H.T.T. Duong, A.B. Lowe, T.P. Davis, C. Boyer, *Macromolecules*, **2012**, *45*, 4196-4204
[6] R.B. Lauffer, *Chem. Rev.*, **1987**, *87*, 901-927
[7] P. Caravan, *Chem. Soc. Rev.*, **2006**, *35*, 512-523