Electronically Supplementary Information

SERRS/MRI multimodal contrast agent based on naked Au nanoparticles functionalized with a Gd(III) loaded PEG polymer for tumor imaging and localized hyperthermia

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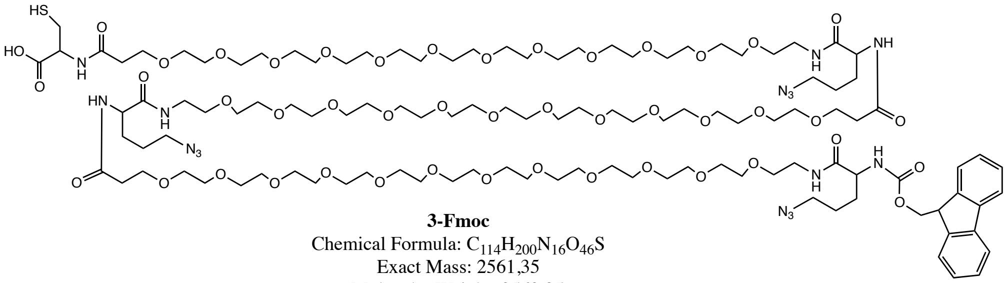
Characterization of (3-Fmoc): HPLC and Mass spectra



3-Fmoc
Chemical Formula: $C_{114}H_{200}N_{16}O_{46}S$
Exact Mass: 2561,35
Molecular Weight: 2562,95

Figure S 1. Molecular formula of the intermediate products (**3-Fmoc).**

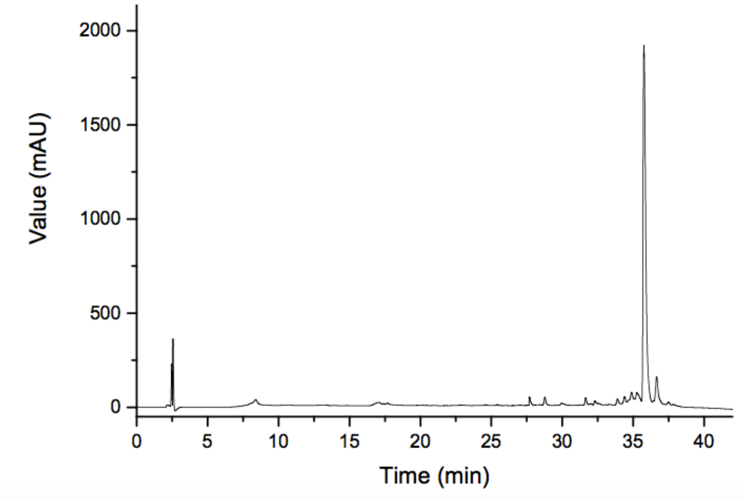


Figure S 2. HPLC chromatogram for (**3-Fmoc)** intermediate.

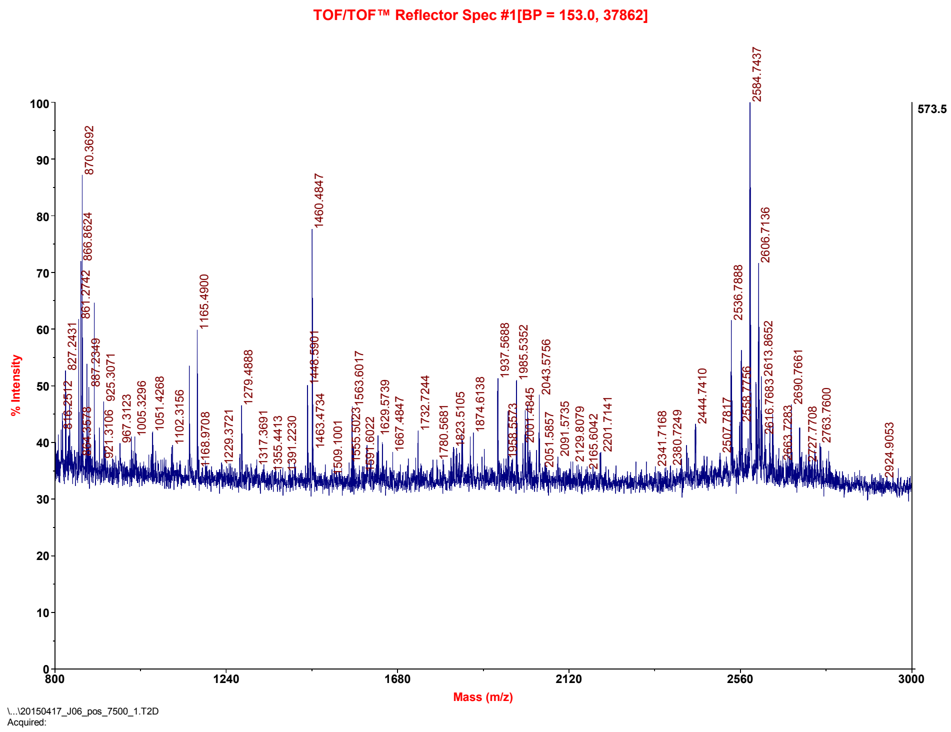
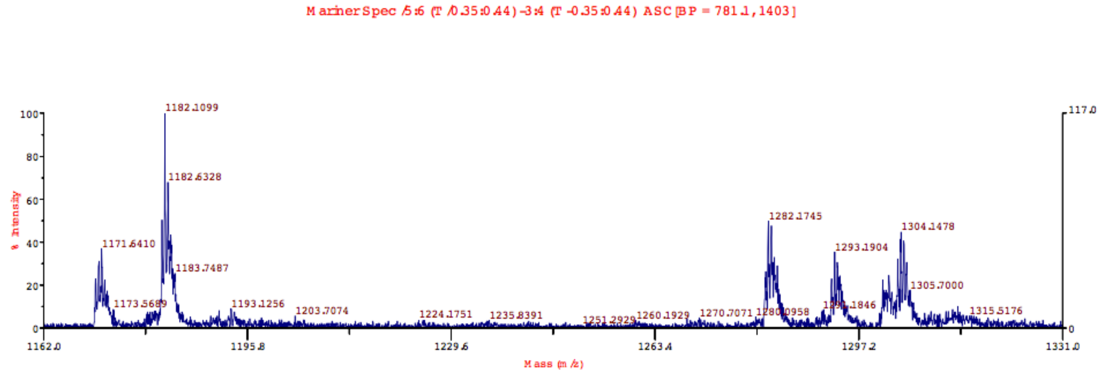


Figure S 3. MALDI-TOF/TOF mass spectra (**3-Fmoc)**, calc. (m/z) 2562.95 Da, found (m/z) 2584.74 Da (molecular ion + Na).



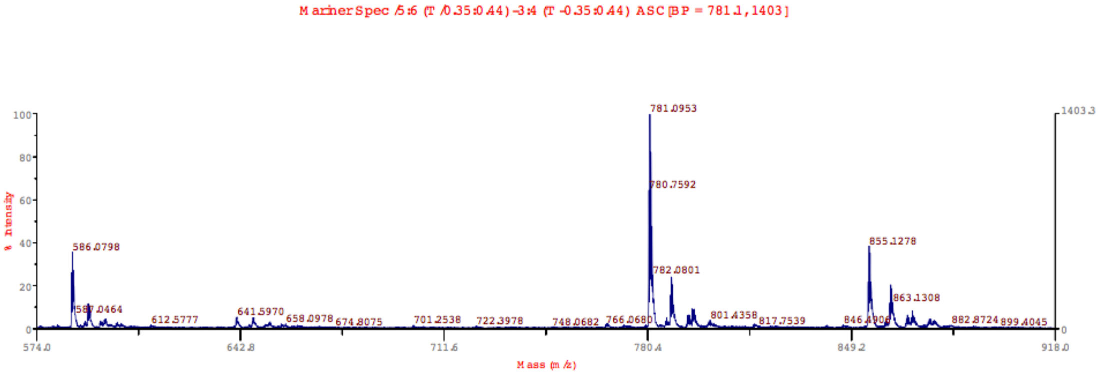
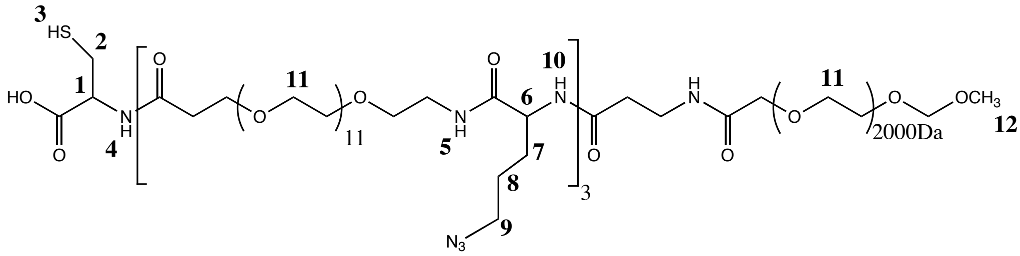


Figure S 4. ESI-TOF mass spectra (**3-Fmoc)**, calc. (m/z) 2562.95 Da, found (m/z) 1282.17 Da ($[M+2H]^{2+}$), 1182.11 Da ($[M\text{-}Fmoc+H+Na]^{2+}$), 1171.64 Da ($[M\text{-}Fmoc+2H]^{2+}$), 855.13 Da ($[M+3H]^{3+}$), 781.09 Da ($[M\text{-}Fmoc+3H]^{3+}$), 586.07 Da ($[M\text{-}Fmoc+4H]^{4+}$).

Characterization of (2): NMR and Mass spectra



Scheme S1. Molecular structure of **2** with numbered carbons for NMR assignment.

Table S1. Carbon and hydrogen assignments from related 1D and 2D spectra reported in Figure S5 - S11

Atom Index	^{1}H-NMR chemical shift (ppm)	^{13}C-NMR chemical shift (ppm)	^{1}H-NMR expected area	^{1}H-NMR experimental area
1	4.74	53.81	1	0.8
2	2.97	26.49	2	1.3
3	1.54	-	1	0.3
4	7.38	-	1	0.8
5	7.10	-	-	-
6	4.39	52.47	3	2.9
7	1.87 1.63	29.60	12	10.2
8	1.58	25.00		
9	3.25	50.98	6	6.1
10	7.04	-	-	-
11	3.58	70.51	304	306.5
12	3.31	58.97	3	3.0 (assigned value)

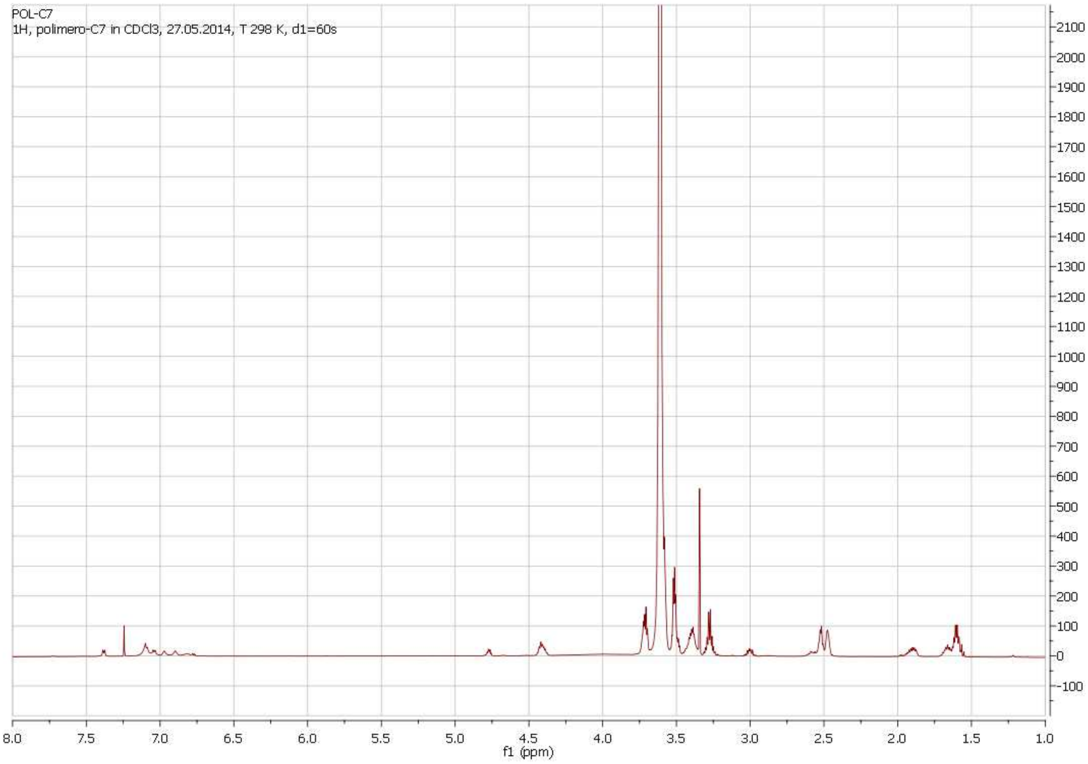


Figure S 5. ^{1}H-NMR spectra of (**2**) in $CDCl_3$ at 298K.

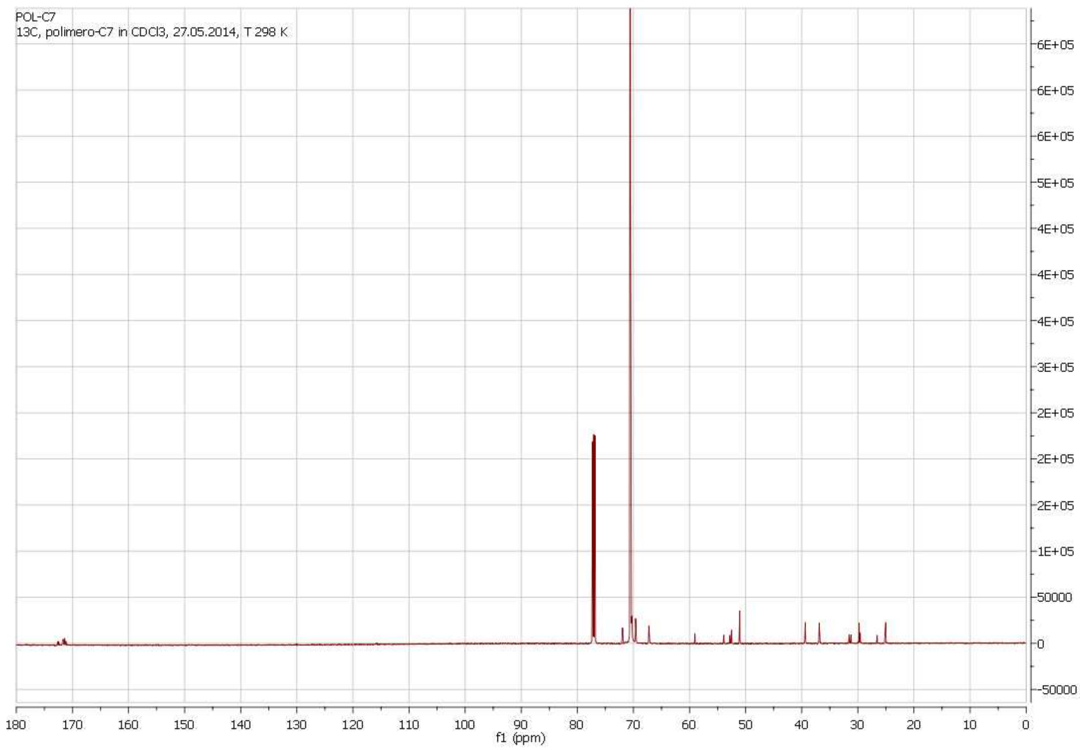


Figure S 6. ^{13}C-NMR spectra of (**2**) in $CDCl_3$ at 298K.

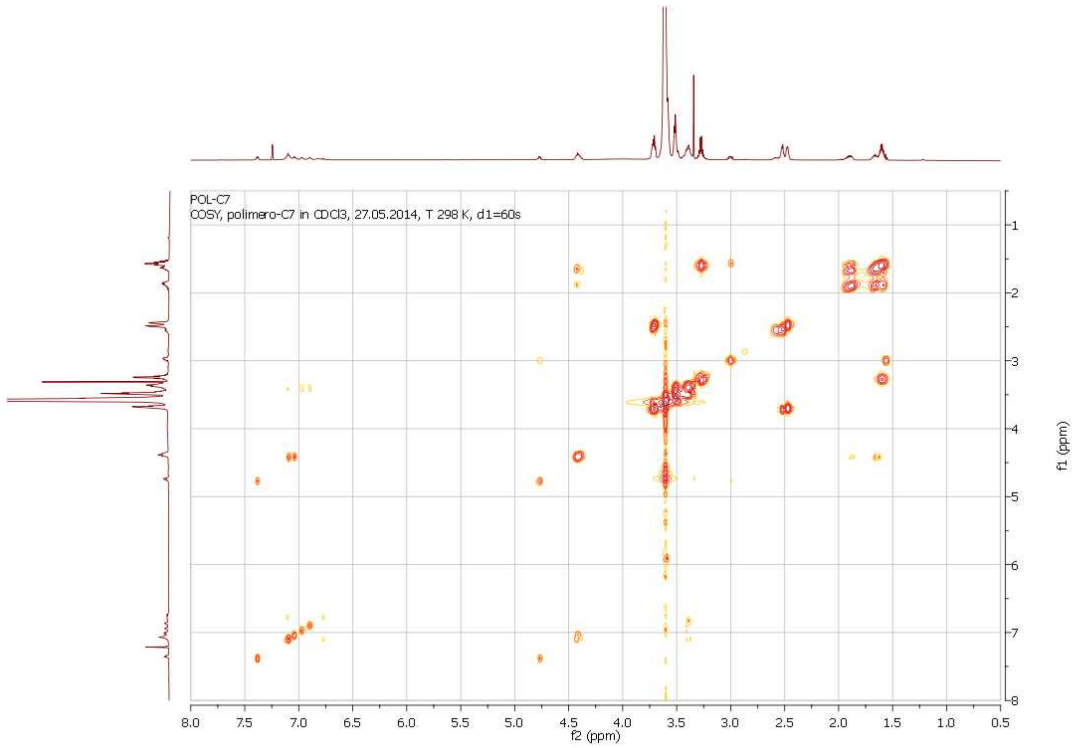


Figure S 7. 1H,1H-COSY spectra of (**2**) in CDCl3 at 298 K.

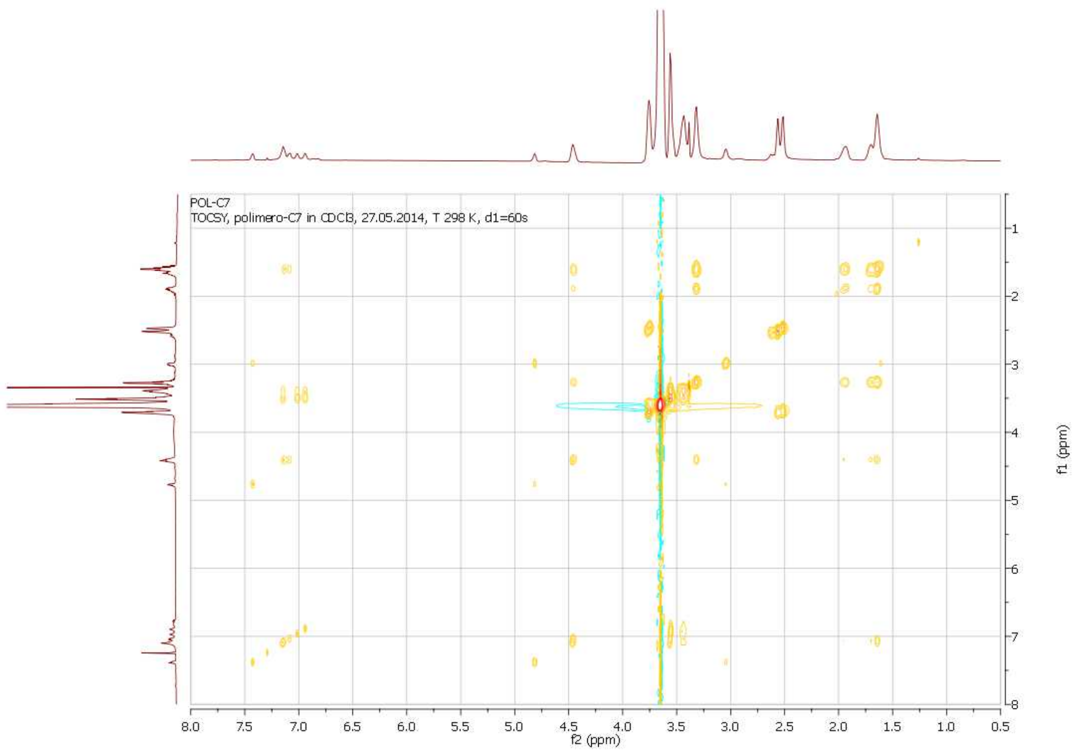


Figure S 8. $^{1}H,^{1}H$-TOCSY spectra of (**2**) in $CDCl_3$ at 298 K.

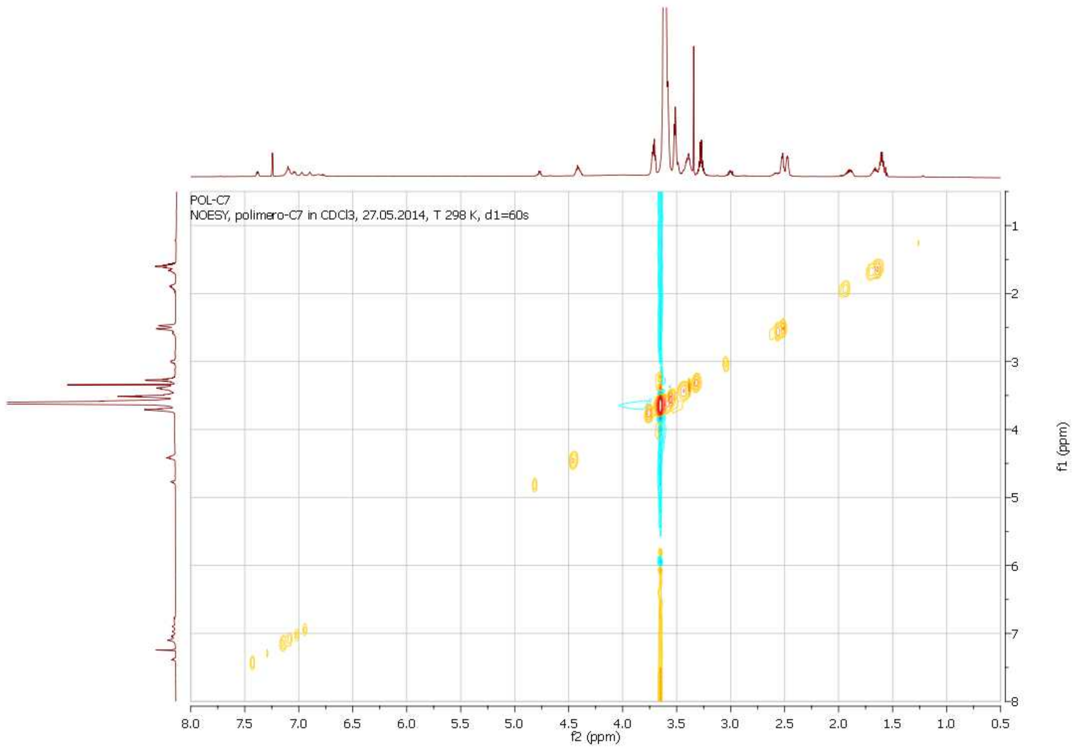


Figure S 9. $^{1}H,^{1}H$-NOESY spectra of (**2**) in $CDCl_3$ at 298 K.

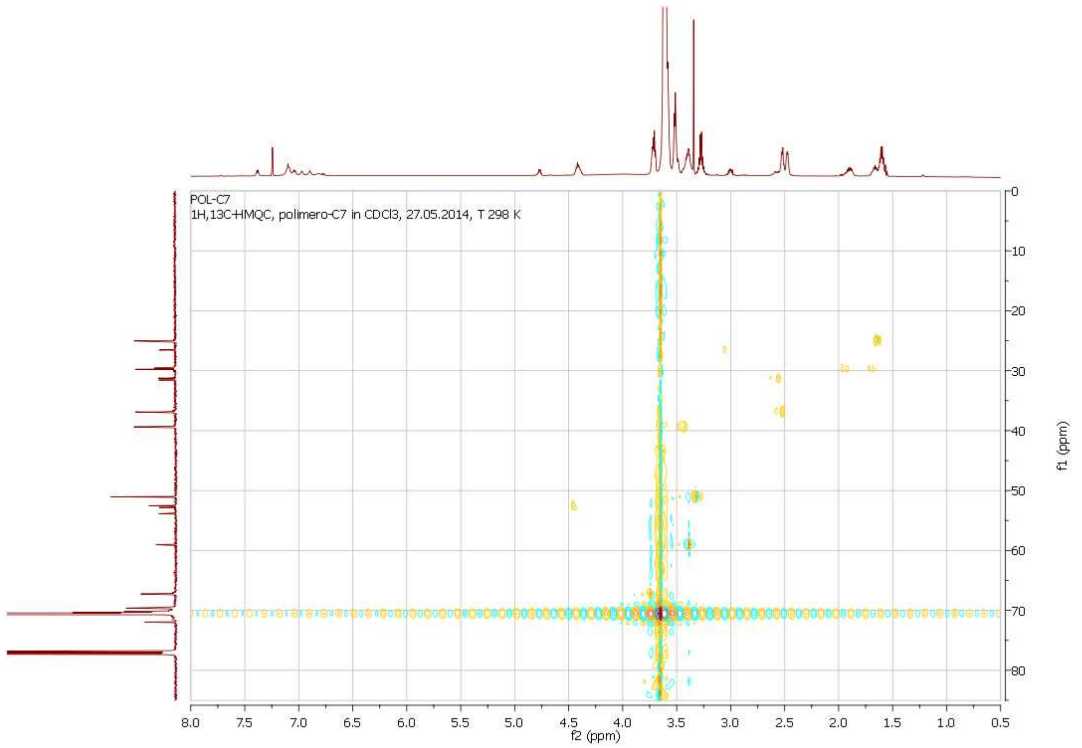


Figure S 10. $^{1}H,^{13}C$-HMQC spectra of (**2**) in $CDCl_3$ at 298 K.

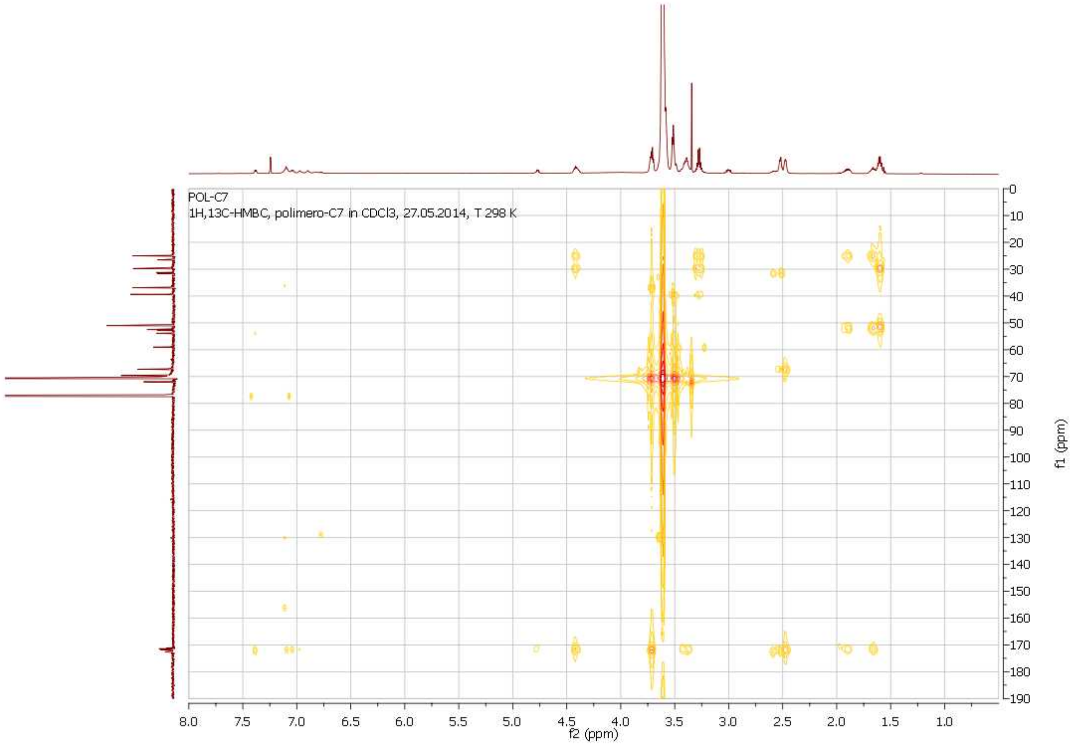


Figure S 11. $^{1}H,^{13}C$-HMBC spectra of (**2**) in $CDCl_3$ at 298 K.

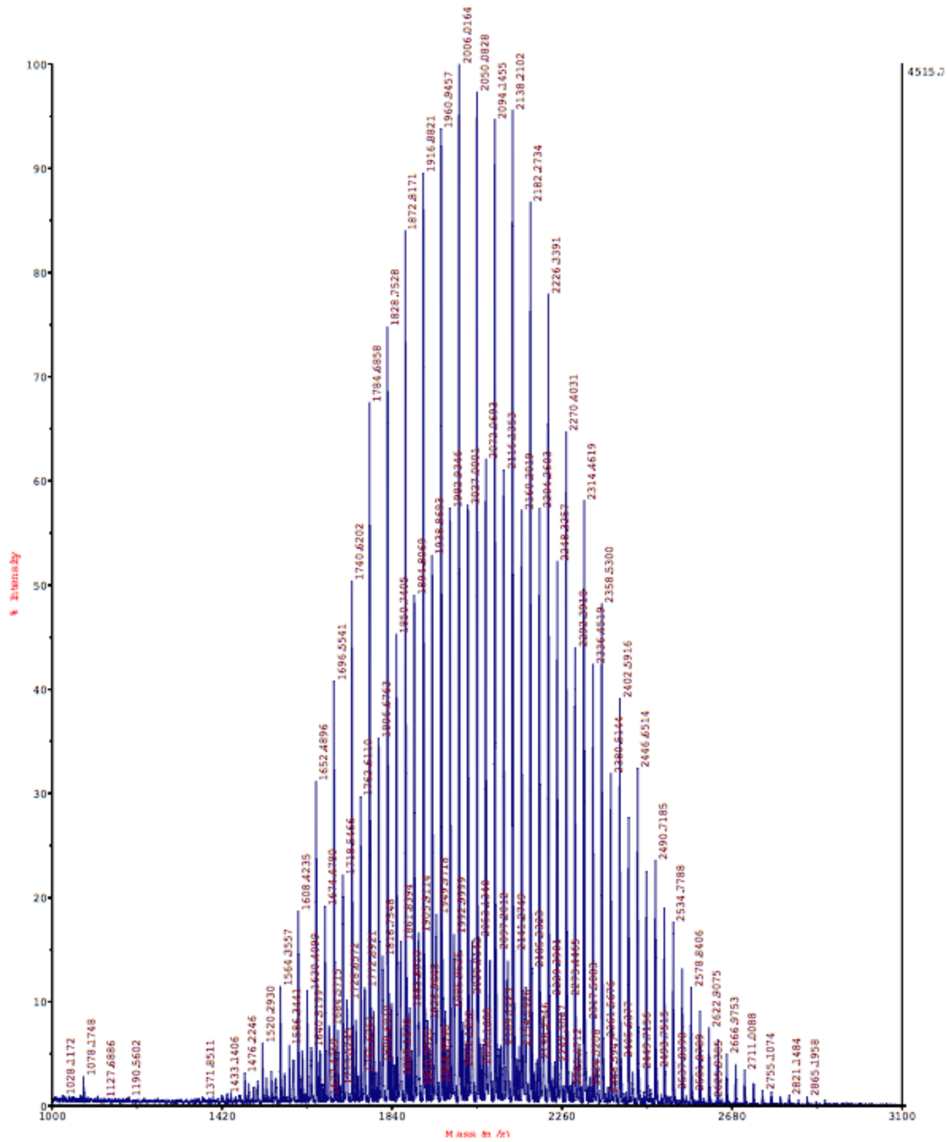


Figure S 12. MALDI-TOF mass spectra (down) of the reagent carbossyl-PEG(2kDa)-OCH_3

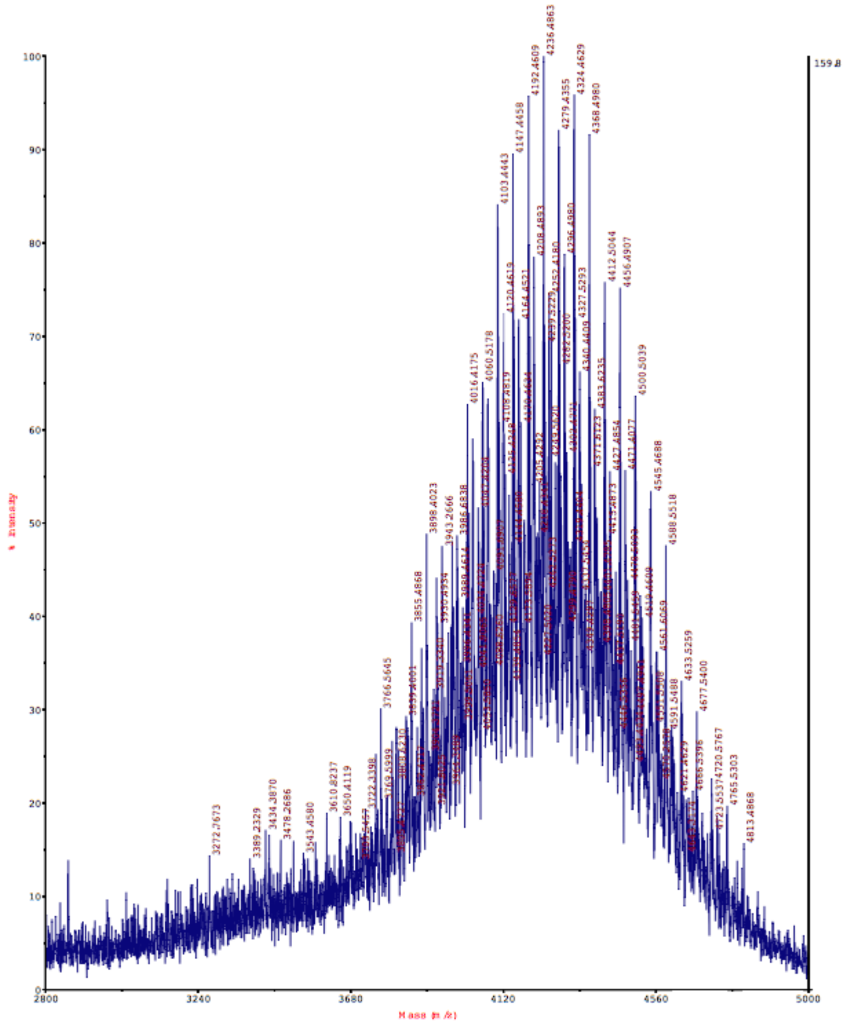


Figure S 13. MALDI-TOF mass spectra (down) of the product (**2**), DHAP-TFA used as matrix. Expected mass distribution is centered at 4390 Da and experimental mass distribution is found centered at (m/z) 4350 Da.

Characterization of DOTA-DBCO: Mass spectrum and HPLC

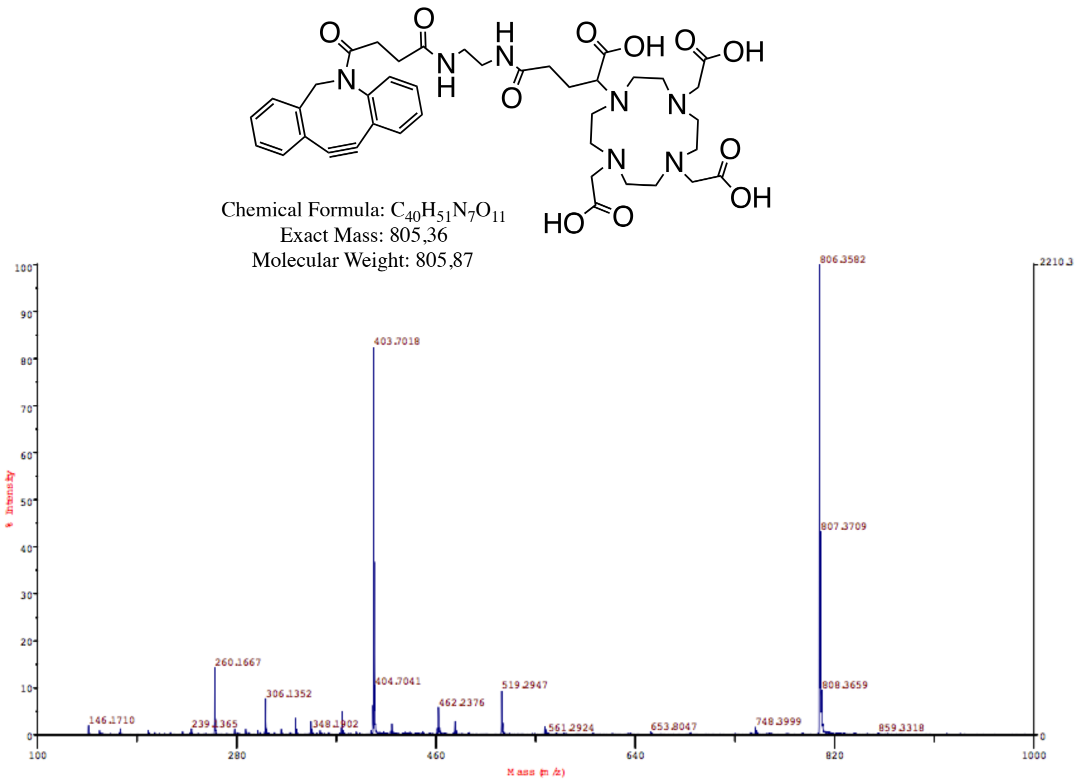


Figure S 14. Molecular structure and ESI-TOF mass spectrum (down) of the product DOTA-DBCO. Calc. (m/z) 805.36 Da, found (m/z) 806.36 Da (molecular ion + H).

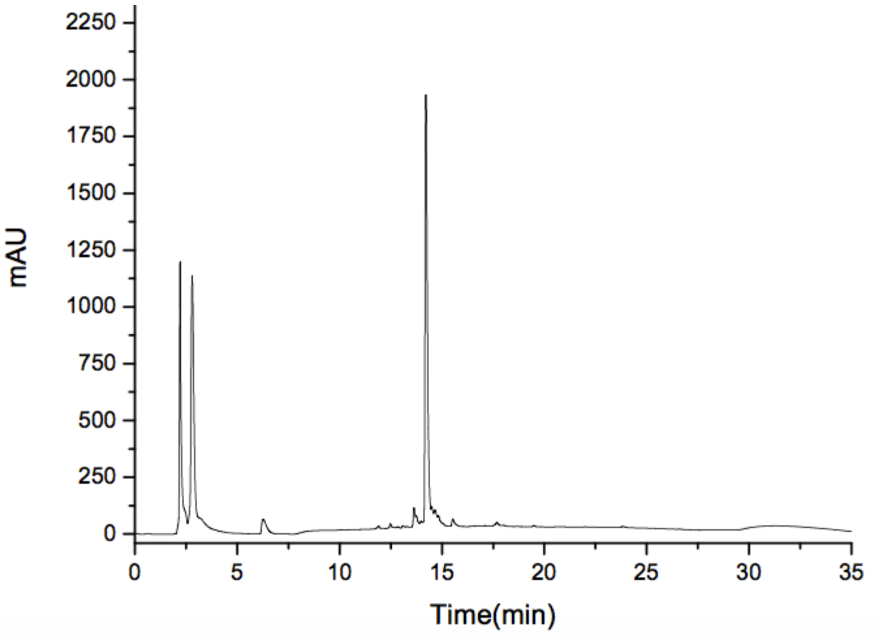


Figure S15. HPLC chromatogram of the purified DOTA-DBCO product.

Characterization of MultiCAs

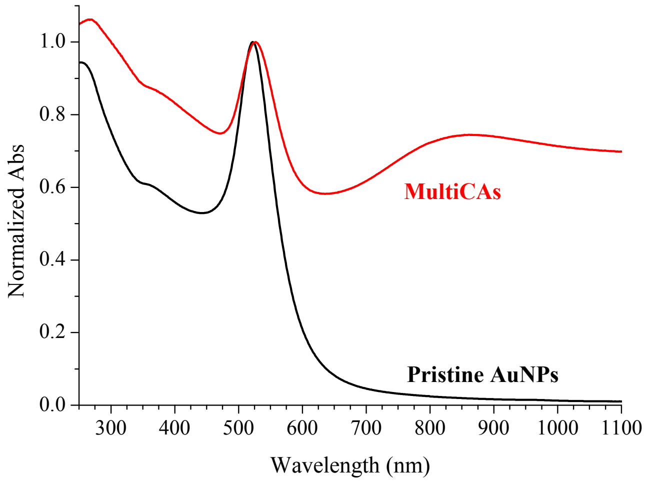


Figure S16. UV-vis-NIR extinction spectra of MultiCAs and of laser ablated gold nanoparticles used for their synthesis. Samples are water dispersed colloids. The MultiCA solution shows the characteristic broad absorption over 700nm, due to aggregated particles, that falls into the so called optical window for biological samples.

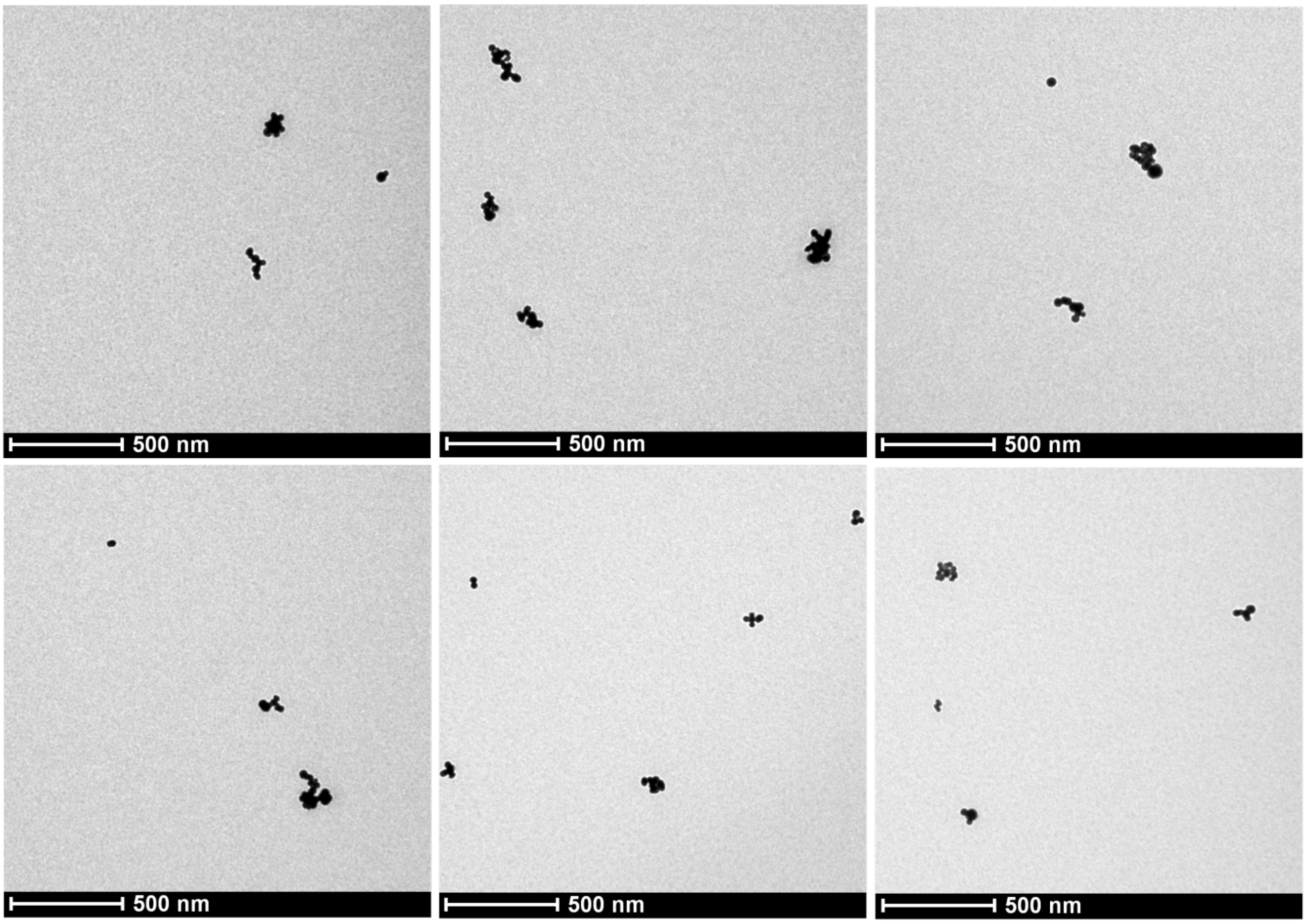


Figure S17. TEM images of MultiCA.

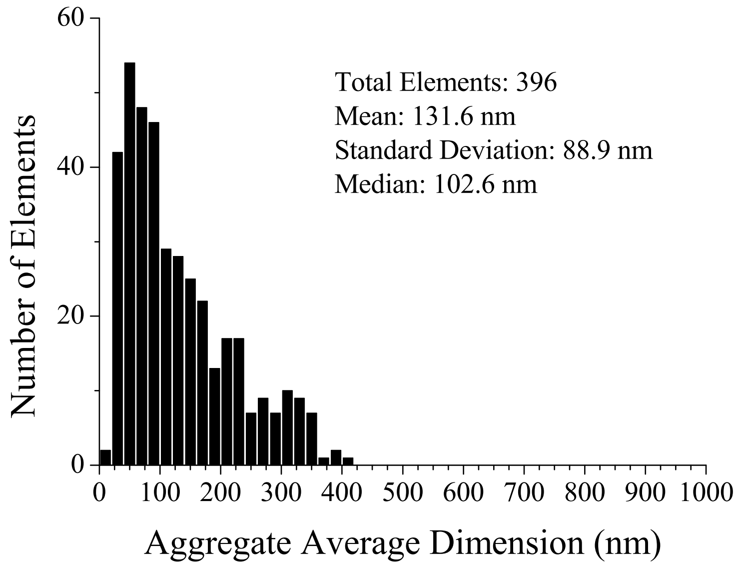


Figure S18. Distribution of the average dimension of MultiCA obtained by TEM.

Table S2. ICP evaluation of Au and Gd content into MultiCA sample.

Sample	Au, mmol/L - g/L	Gd, mmol/L - mg/L
Au	602 - 119	-
Gd	-	0.80 - 126

With the values reported in Table S2 it could be estimated a molar ratio of 1.33 • 10^{-3} Gd ions / Au ions. One could also estimate an average content of about 300 – 400 Gd ions per single AuNP (average diameter of 20 nm).
The average AuNPs dimension were estimated by fitting the extinction spectra (Figure S16) with Mie-Gans model.[1]

Table S3. r_1 and r_2 magnetic relaxivities per Gd atom for MultiCA.

Field	r_1 ($mM^{-1}\cdot s^{-1}$)	r_2 ($mM^{-1}\cdot s^{-1}$)
4 T (200 MHz)	14.8	127
14 T (600 MHz)	5.3	271

Table S4. r_1 and r_2 magnetic relaxivities at 14 T (600 MHz).

Sample	r_1 ($mM^{-1}\cdot s^{-1}$)	r_2 ($mM^{-1}\cdot s^{-1}$)
3(DOTA-Gd^{III})-PEG	2.5	2.4
MultiCA	5.3	271

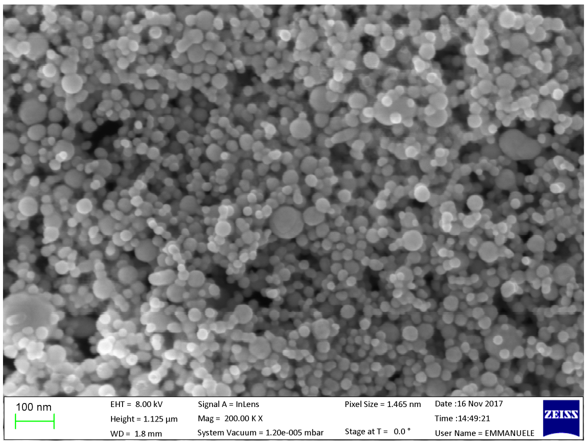


Figure S19. FEG-SEM image of MultiCA.

Table S5. EDS analysis of MultiCA

element	*Atomic Concentration (%)*
Au	99.51
Gd	0.49
	Gd/Au=4.9 10^{-3}

Magnetic Resonance Imaging, T_2 maps

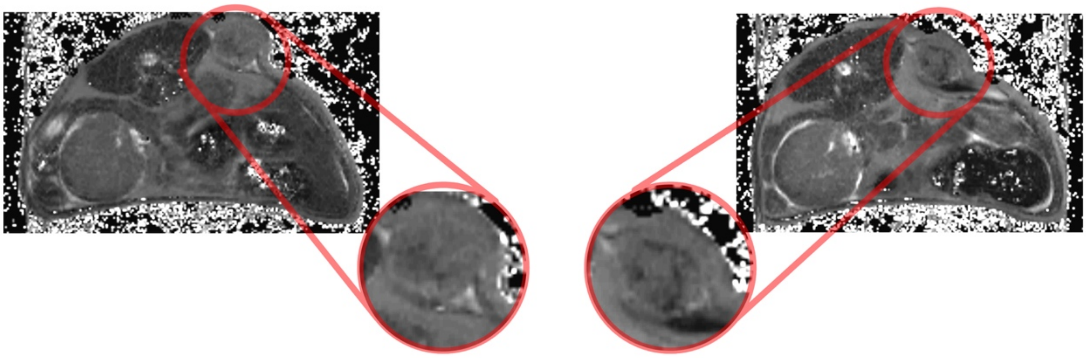


Figure S 20. Representative T_2 maps of a mouse from "Treated" group, before intratumoral injection of MultiCA (left) and 1 hour (right) after the nanoparticle injection. The tumor site images are enlarged to highlight the differences.

References:

1. V. Amendola and M. Meneghetti, *The Journal of Physical Chemistry C*, 2009, **113**, 4277-4285.