Electronic Supplementary Material (ESI) for Nanoscale.
This journal is © The Royal Society of Chemistry 2016

Electronic supplementary information to

Synthesis and functionalization of monodisperse near-ultraviolet and visible excitable multifunctional Eu^{3+}:Bi^{3+}:$REVO_4$ nanophosphors for bioimaging and biosensing applications

Alberto Escudero,[1,2]* Carolina Carrillo-Carrión,[1,3] Mikhail V. Zyuzin,[1] Sumaira Ashraf,[1] Raimo Hartmann,[1] Nuria O. Núñez,[2] Manuel Ocaña,[2] and Wolfgang J. Parak.[1]

1. AG Biophotonik, Fachbereich Physik, Philipps-Universität Marburg. Renthof 7. D-35037, Marburg, Germany.
2. Instituto de Ciencia de Materiales de Sevilla. CSIC – Universidad de Sevilla. C. Américo Vespucio 49. E-41092, Seville, Spain.
3. CIC biomaGUNE. Paseo Miramón 182. E-20009, San Sebastian, Spain.
* Corresponding author: alberto.escudero@csic.es

Index

1. Synthesis of the nanoparticles

Europium and bismuth doped YVO_4 and $GdVO_4$ nanoparticles were synthesised from appropriate RE precursors and sodium orthovanadate in ethylene glycol – water mixtures, as described in the experimental section of the paper.

Table S1: Experimental conditions for the synthesis of all prepared samples.

Sample	$[Eu^{3+}]$ (M)	$[Bi^{3+}]$ (M)	$[Y^{3+}]$ (M)	$[VO_4^{3-}]$ (M)	PAA (mg/mL)
Bi0Y	0.0016	0	0.0184	0.1	0
Bi5Y	0.0016	0.001	0.0174	0.1	0
Bi10Y	0.0016	0.002	0.0164	0.1	0
Bi15Y	0.0016	0.003	0.0154	0.1	0
Bi20Y	0.0016	0.004	0.0144	0.1	0
Bi0YPAA2	0.0016	0	0.0184	0.1	2
Bi5YPAA2	0.0016	0.001	0.0174	0.1	2
Bi10YPAA2	0.0016	0.002	0.0164	0.1	2
Bi15YPAA2	0.0016	0.003	0.0154	0.1	2
Bi20YPAA2	0.0016	0.004	0.0144	0.1	2
Bi20YPAA5	0.0016	0.004	0.0144	0.1	5
Bi20YPAA8	0.0016	0.004	0.0144	0.1	8
	$[Eu^{3+}]$ (M)	$[Bi^{3+}]$ (M)	$[Gd^{3+}]$ (M)	$[VO_4^{3-}]$ (M)	PAA (mg/mL)
Bi0Gd	0.0016	0	0.0184	0.1	0
Bi5Gd	0.0016	0.001	0.0174	0.1	0
Bi10Gd	0.0016	0.002	0.0164	0.1	0
Bi15Gd	0.0016	0.003	0.0154	0.1	0
Bi20Gd	0.0016	0.004	0.0144	0.1	0
Bi0GdPAA	0.0016	0	0.0184	0.1	2
Bi5GdPAA	0.0016	0.001	0.0174	0.1	2
Bi10GdPAA	0.0016	0.002	0.0164	0.1	2
Bi15GdPAA	0.0016	0.003	0.0154	0.1	2
Bi20GdPAA	0.0016	0.004	0.0144	0.1	2

2. Physicochemical characterization of the nanoparticles

Table S2: Morphological and colloidal characterization data of all the synthesized samples. DLS (number distribution) and ζ-potential measurements were recorded in water at pH 7. Errors refer to standard deviations or in case of the unit cell volume to absolute errors. N. r. = not recorded.

Sample	Particle size (TEM) (nm)	Hydrodynamic diameter (nm)	Crystallite size (nm)	ζ-potential (mV)	Unit cell volume ($Å^3$)
Bi0Y	N.r.	142 (9)	N.r.	N.r.	N.r.
Bi5Y	N.r.	139 (7)	N.r.	N.r.	N.r.
Bi10Y	N.r.	148 (4)	N.r.	N.r.	N.r.
Bi15Y	N.r.	137 (5)	N.r.	N.r.	N.r.
Bi20Y	107 (14)	138 (7)	N. r.	-19 (1)	N.r.
Bi0YPAA2	88 (14)	82 (3)	62	-37 (2)	319.24 (3)
Bi5YPAA2	N.r.	94 (4)	77	N.r.	321.01 (4)
Bi10YPAA2	N.r.	98 (3)	79	N.r.	322.55 (4)
Bi15YPAA2	N.r.	110 (4)	77	N.r.	324.03 (2)
Bi20YPAA2	93 (7)	108 (10)	79	-42 (3)	325.58 (2)
Bi20YPAA5	N.r.	79 (4)	N.r.	-37 (2)	N.r.
Bi20YPAA8	51 (5)	51 (2)	57	-39 (2)	325.61 (3)
Bi0Gd	N.r.	47 (2)	N.r.	N.r.	N.r.
Bi5Gd	N.r.	52 (1)	N.r.	N.r.	N.r.
Bi10Gd	N.r.	57 (4)	N.r.	N.r.	N.r.
Bi15Gd	N.r.	58 (3)	N.r.	N.r.	N.r.
Bi20Gd	51 (15)	58 (4)	N. r.	-25 (1)	N.r.
Bi0GdPAA	39 (7)	40 (2)	53	N.r.	330.32 (4)
Bi5GdPAA	N.r.	41 (3)	54	N.r.	331.65 (3)
Bi10GdPAA	N.r.	45 (2)	56	N.r.	332.79 (5)
Bi15GdPAA	N.r.	46 (1)	54	N.r.	333.74 (2)
Bi20GdPAA	40 (8)	41 (2)	65	-41 (2)	335.37 (2)

Table S3: ICP-determined europium, bismuth, yttrium, gadolinium, and vanadium content of selected samples. RE = Y or Gd.

Sample	Eu/(Eu + Bi + RE) (%)	Bi/(Eu+ Bi+ RE) (%)	V/(Eu+Bi+RE)
Bi20YPAA2	7.96	20.6	1.17
Bi20YPAA8	7.91	20.4	1.05
Bi20GdPAA	8.07	19.7	1.15
Bi20Y	8.04	19.9	1.03
Bi20Gd	7.88	20.0	1.06

Table S4: Mean hydrodynamic diameter (nm) of freshly prepared suspensions of PAA-functionalized and LbL-functionalized Eu, Bi-doped YVO_4 and $GdVO_4$ NPs obtained by DLS in water and in different buffer media of biological interest. C.a = aproximate results.

	Bi20YPAA2	Bi20GdPAA	Bi20Y@PAH @PAA@PAH	Bi20Gd@PAH @PAA@PAH
Water	108	41	157	84
MES pH 6.5	109	48	164	125
PBS pH 7.4	125	81	c.a. 700	c.a. 1250
Cell medium	> 1100	> 1900	c.a. 1400	c.a. 1450
Serum-supplemented cell medium	182	113	c.a. 250	c.a. 530

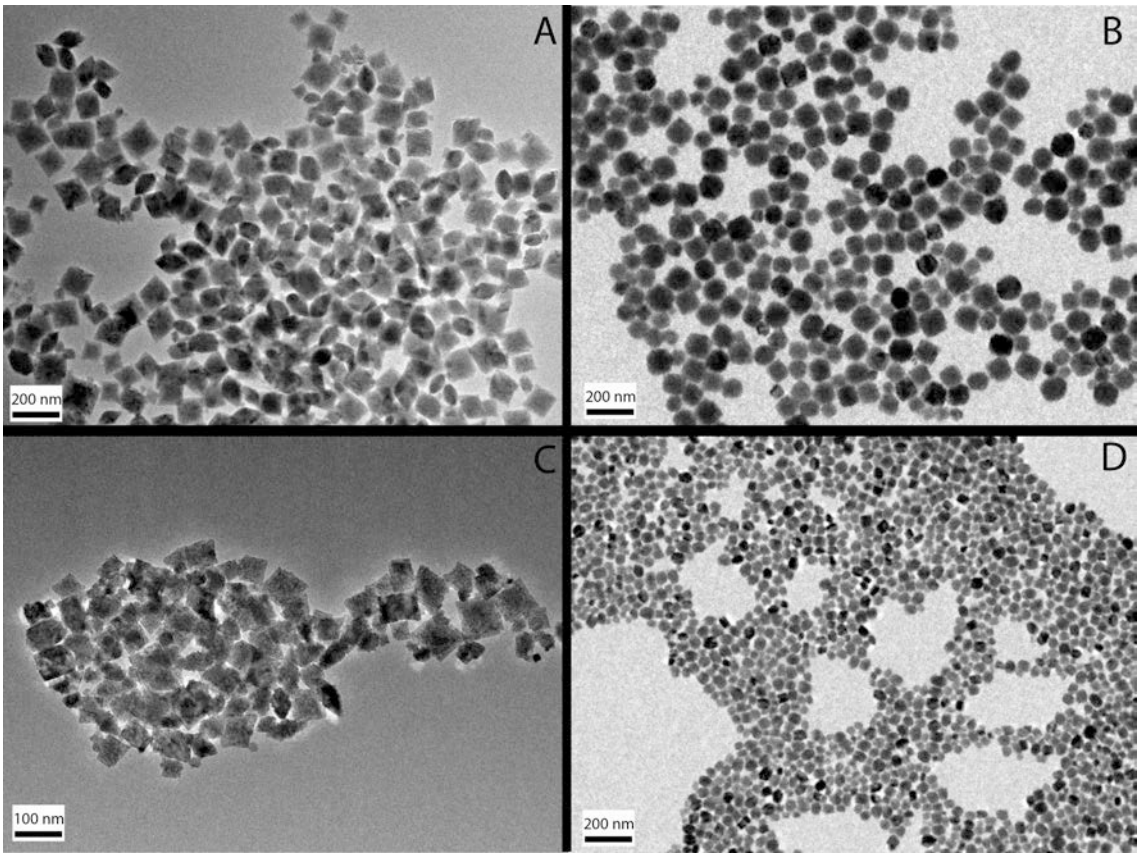

Figure S1: TEM micrographs of the Bi20Y (A), Bi0YPAA2 (B), Bi20Gd (C), and Bi0GdPAA (D) samples.

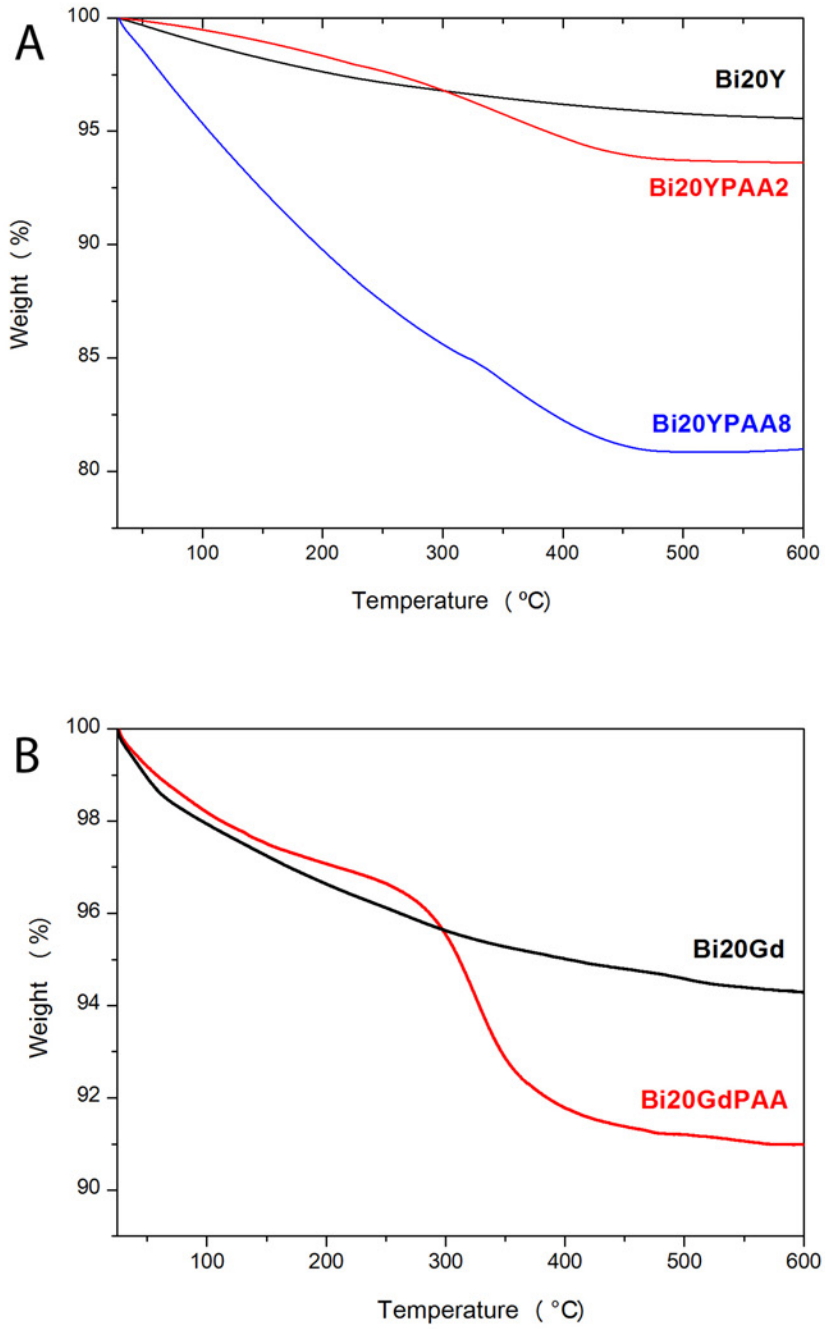

Figure S2: Thermogravimetry (TG) analysis of selected NPs functionalised with PAA. The curves corresponding to the samples synthesized in the absence of PAA (Bi20Y and Bi20Gd) are also included.

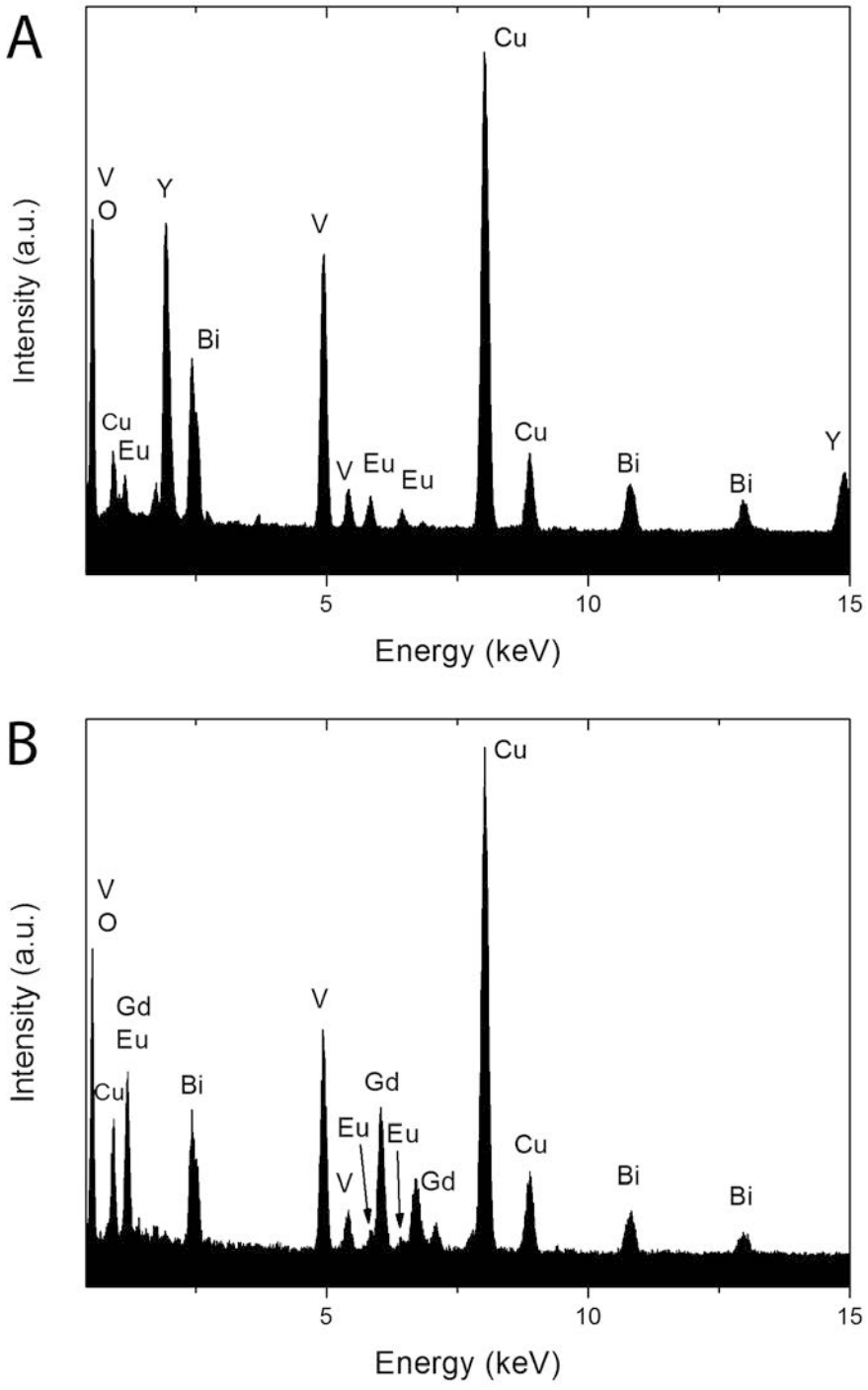

Figure S3: Energy-dispersive X-ray (EDX) spectra of single particles of Bi20YPAA2 (A) and Bi20GdPAA (B) samples.

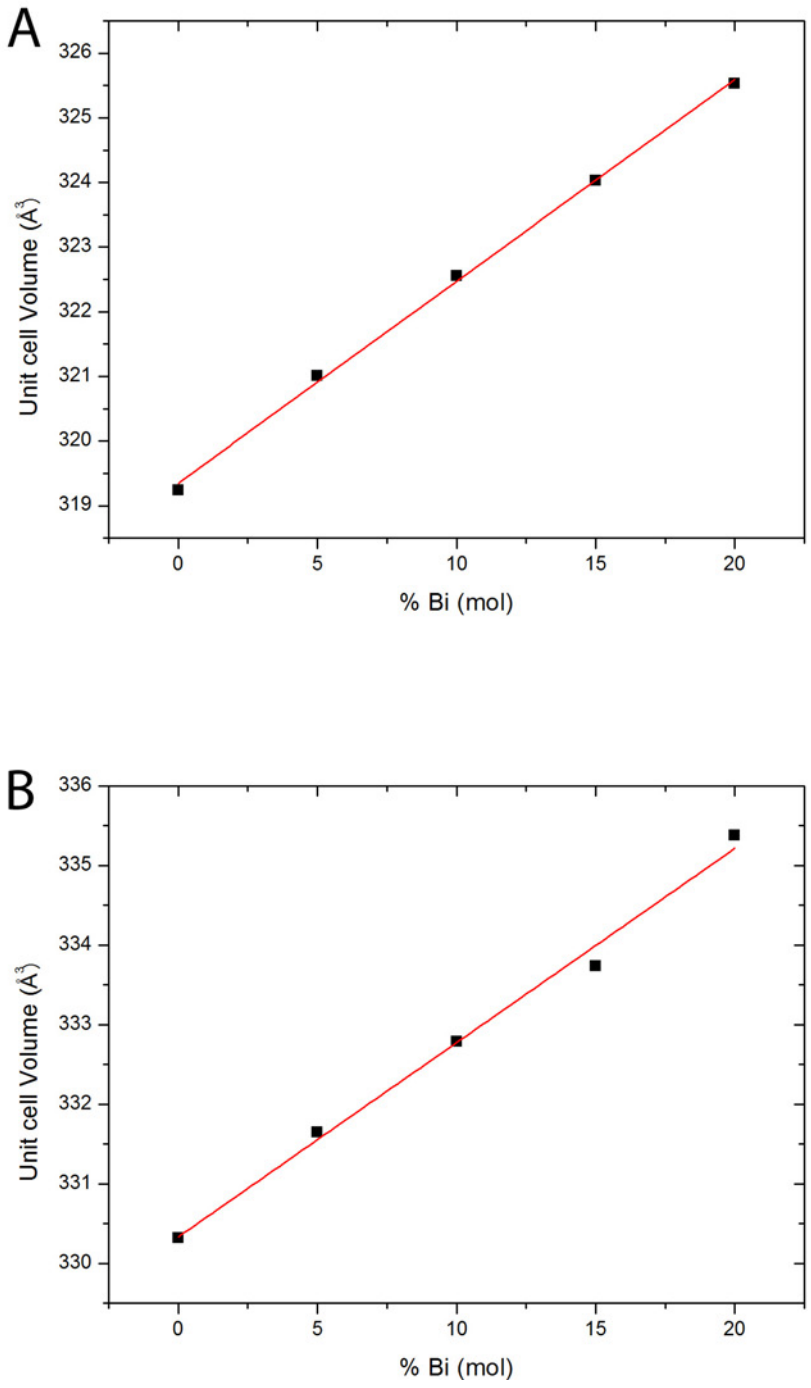

Figure S4: Unit cell volume of the Eu- and Bi- doped YVO_4 (A) and $GdVO_4$ (B) samples sythesized in presence of PAA (2 mg/mL) with increasing the bismuth content. The error bars are smaller than the symbol size.

3. Colloidal stability studies

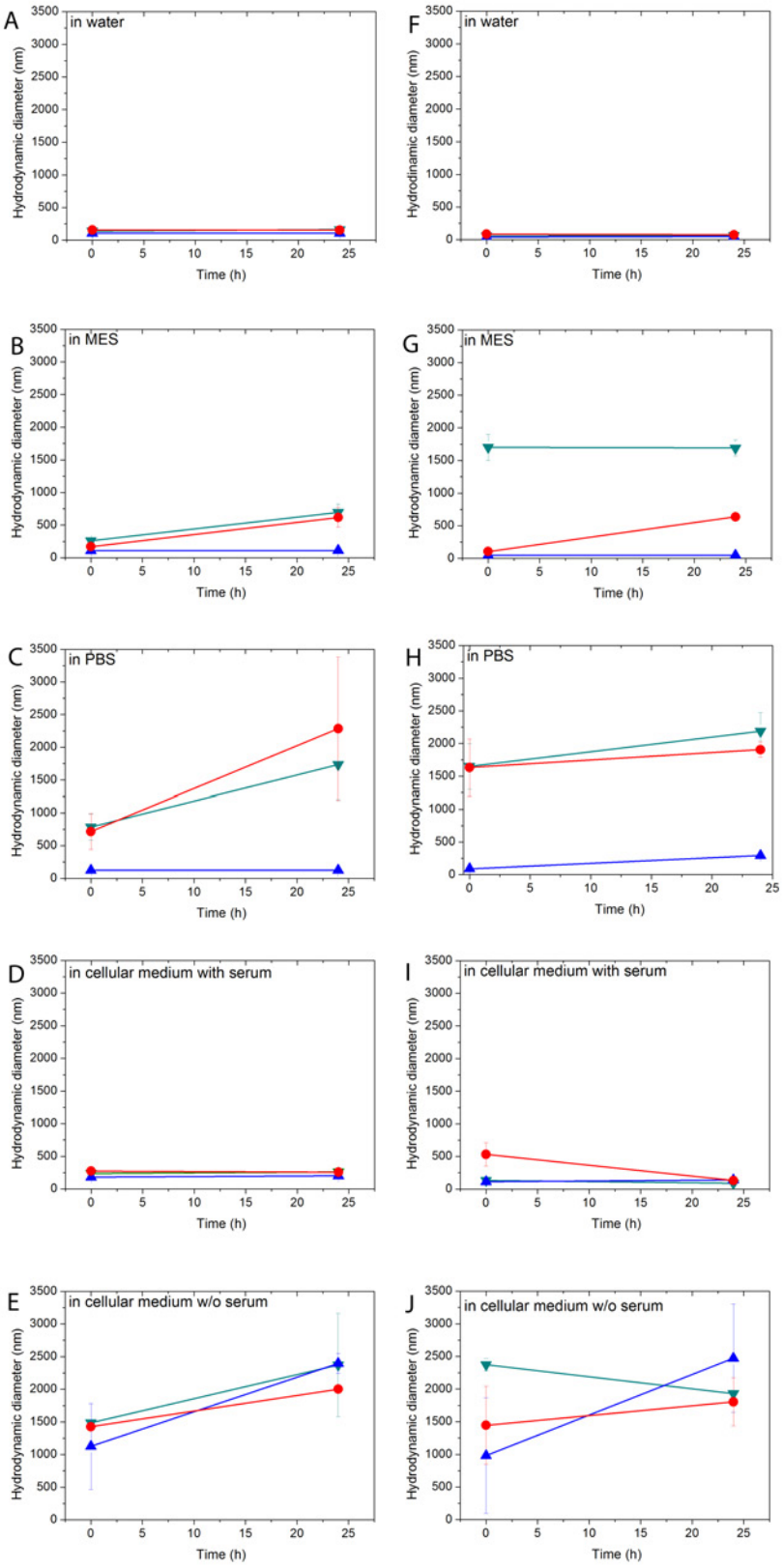

Figure S5: Evolution of the hydrodynamic diameter obtained from DLS (dinamic light scattering) analyses of the YVO_4-based nanoparticles (A to E; blue triangles = Bi20YPAA2, green triangles = Bi20Y, red circles = Bi20Y@PAH@PAA@PAH samples), and $GdVO_4$-based nanoparticles (F to J; blue triangles = Bi20GdPAA, green triangles = Bi20Gd, red circles = Bi20Gd@PAH@PAA@PAH samples) samples in water (pH 7), MES (N-Morpholino)ethanesulfonic acid hydrate) 50 mM at pH 6.5, PBS (phosphate buffered saline) at pH 7.4, serum-supplemented cell medium, and serum-free cell medium.

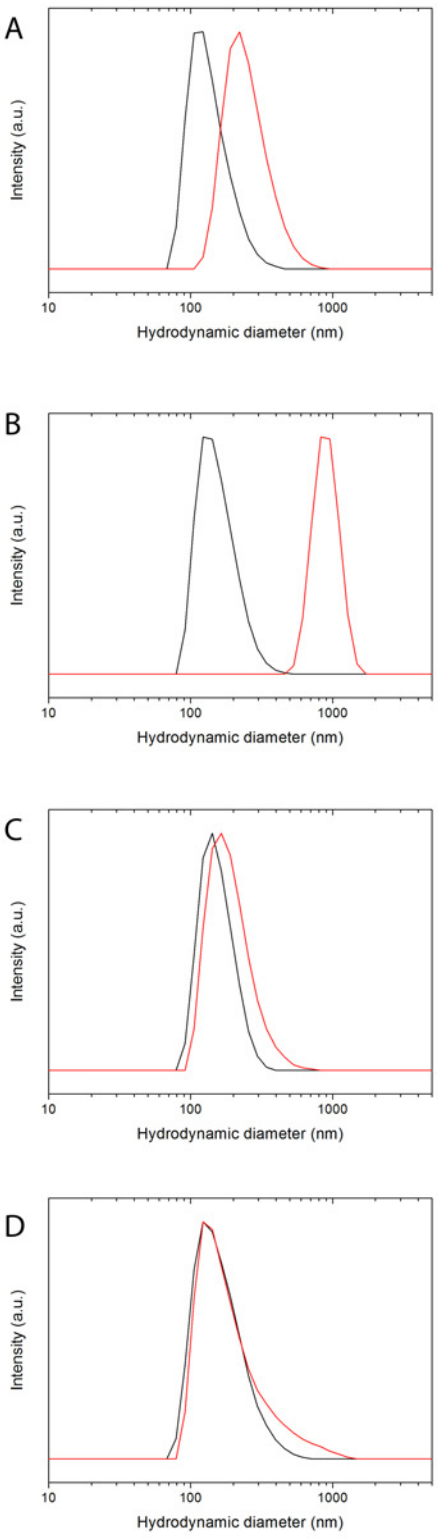

Figure S6: Hydrodynamic diameter obtained by DLS of the Bi20Y sample (A), and after the deposition of one PAH layer (B), one extra PAA layer (C) and one extra PAH layer (D). Red lines correspond to analyses carried out in MES buffer 50 mM at pH 6.5 and black lines correspond to milli-Q water at pH 7.

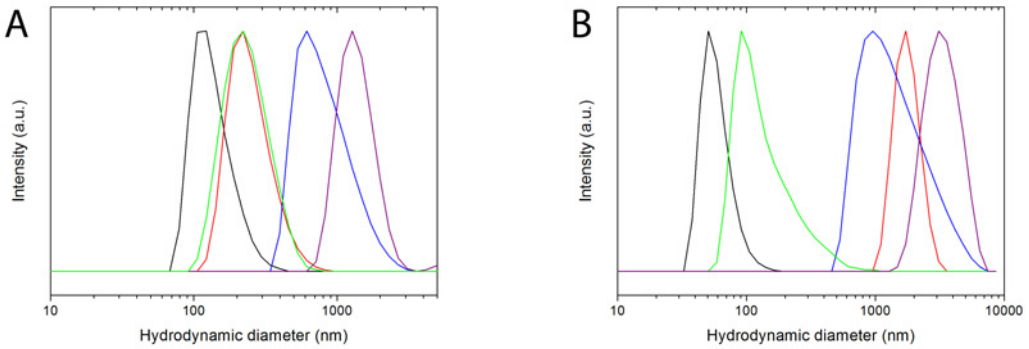

Figure S7: Hydrodynamic diameter obtained by DLS of the Bi20Y sample (A) and Bi20Gd sample (B) in water at pH 7 (black lines), MES 50 mM at pH 6.5 (red lines), PBS at pH 7.4 (blue lines), serum-containing cell medium (green lines), and cell medium (purple lines).

4. Optical properties

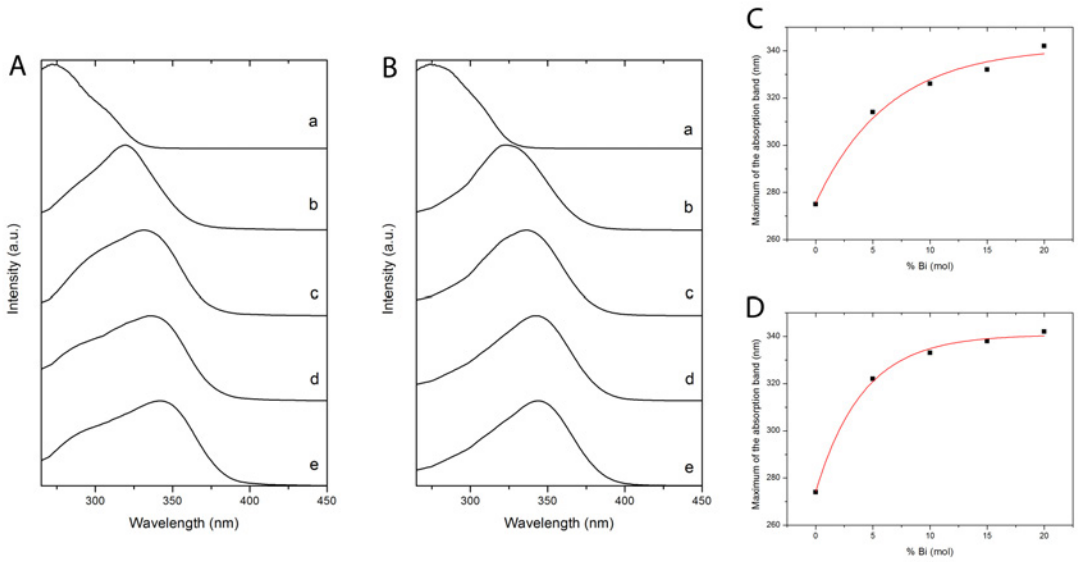

Figure S8: (A) Excitation spectra (emission recorded at λ_{em} = 622 nm) of the Eu – Bi-doped YVO_4 samples functionalized with PAA (2 mg/mL) with increasing the Bi content. a = Bi0YPAA2, b = Bi5YPAA2, c = Bi10YPAA2, d = Bi15YPAA2, e = Bi20YPAA2. (B) Excitation spectra (λ_{em} = 622 nm) of the Eu – Bi- doped $GdVO_4$ samples functionalized with PAA (2 mg/mL) with increasing the Bi content. a = Bi0GdPAA, b = Bi5GdPAA, c = Bi10GdPAA, d = Bi15GdPAA, e = Bi20GdPAA. (C) Evolution of the absorption maximum of the Eu-Bi-YVO_4 samples functionalized with PAA with increasing the Bi content. (D) Evolution of the absorption maximum of the Eu-Bi-$GdVO_4$ samples functionalized with PAA with increasing the Bi content. The lines representing the fits are only intented as guides for the eye.

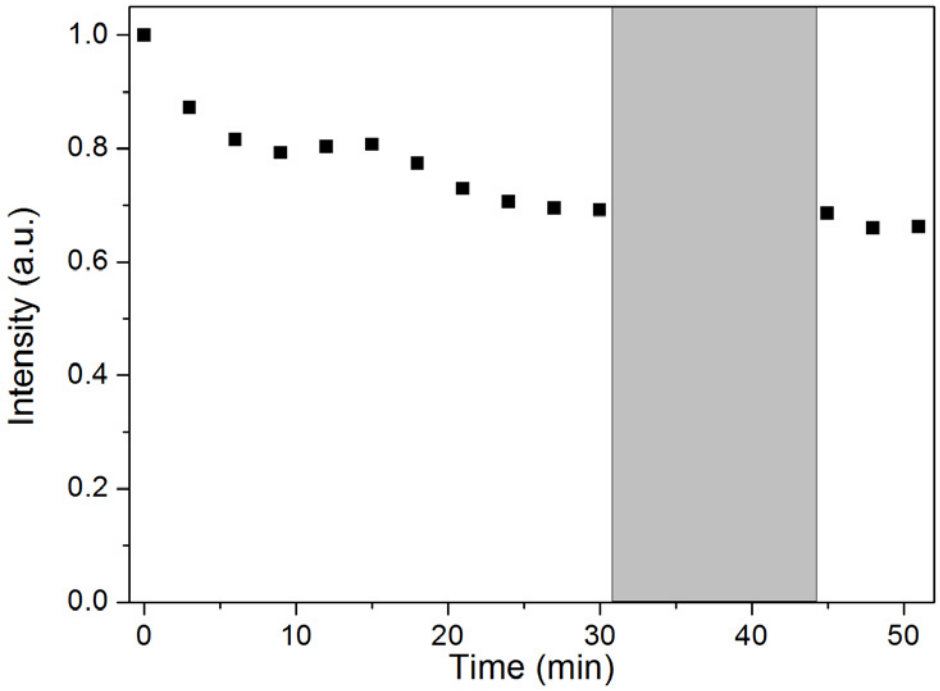

Figure S9: Evolution of the emission intensity (λ_{em} = 622 nm) of Bi20YPAA2 NPs under continuous UV irradiation in a in a wide-field fluorescence microscope (Axiovert 200M, Zeiss, Germany). The particles were inmobilised with Fluoromount – G (SouthernBiotech). The UV irradiation was stopped from minutes 31 to 44.

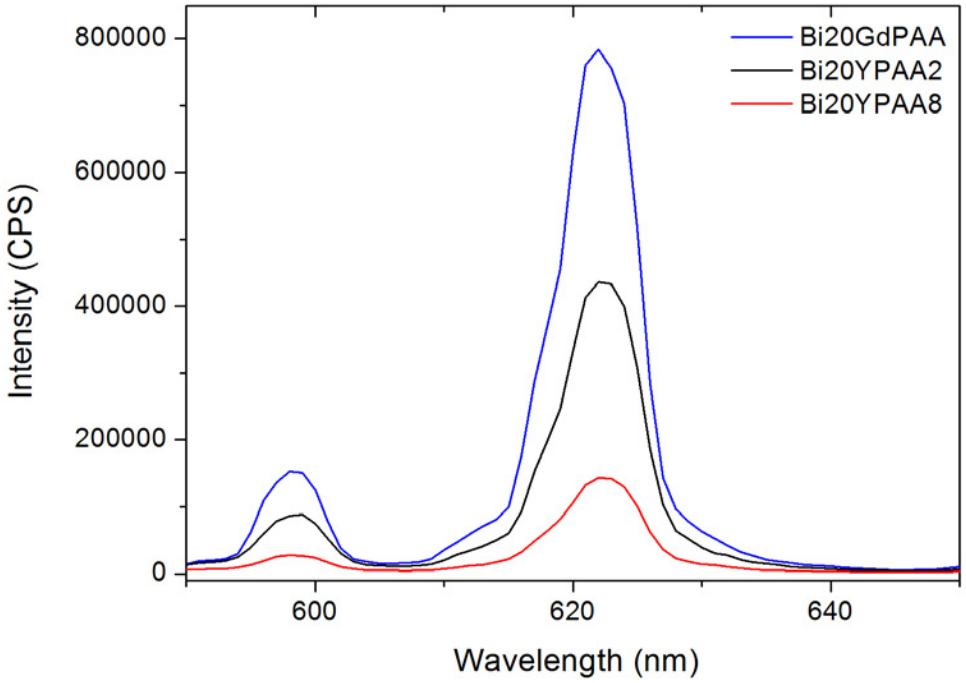

Figure S10: Emission spectra (λ_{ex} = 355 nm) of aqueous suspensions (0.05 mg/mL) of the Bi20GdPAA (blue), Bi20YPAA2 (black) and Bi20YPAA8 (red) samples.

5. Imaging set-up

5.1 Fluorescence microscopy

Imaging was performed with a wide-field fluorescence microscope (Axiovert 200M, Zeiss, Germany). A Plan Apochromat 63x/1.4 OIL DIC ∞ /0.17 oil objective was used while imaging the stained fixed samples (cells with luminescent particles inside). The immersion oil was ImmersolTM 518F. The tripod was equipped with a mercury arc lamp of the type N HBO 103 and a CCD camera of the type Axiocam HRc. A 340±24 nm excitation filter and a long pass (> 600 nm) emission filter were used to detect the nanoparticles. Automatic imaging was performed and 9 to 16 images per sample were captured. The red channel images were collected with 3000 ms of exposure time. The micromanager software (based on imageJ) was used. The images were deconvoluted and optimized after acquisition.

Filter sets were obtained from AHF Analysentechnik.

Filter name	Excitation (nm)	Emission (nm)	Beam Splitters
Exciter/Emitter			
1- Chroma 31001 FITC D480 30x / D535 40m	480	535	505 DCLP
2- Zeiss Filter Set 49 G365 / 445 50	G365	BP 445-450	FT 395
3- AHF Cy5 620/60x, ET 700/75m	BP 620/30	BP 700/40	BS T 660 LPXR ET
4- PBFI / SBFI (Alexa red) LP D340-26 / 600 LP	340	600 LP	400 DCLP

where DCLP = Dichroic Long Pass
BP = band pass
LP= long pass

5.2 Confocal laser-scanning microscopy

The nanoparticle uptake by HeLa cells was live imaged using a confocal laser-scanning fluorescence microscope (CLSM 510 Meta, Zeiss) equipped with a portable incubator (Pecon, Germany) to maintain the µ-ibidi plates (Ibidi #80826, 1 cm^2/well) at 37 °C with 5% CO_2. Image series were acquired using a Plan-Apochromat 63x/1.40 Oil DIC M27 objective. The nanoparticles were excited at 405 nm (with a diode laser, 20%

power) and their fluorescence was recorded between 604 and 754 nm. Untreated cells (*i.e.* cells not incubated with nanoparticles) were used as control.

6. Nanoparticle uptake by cells and imaging

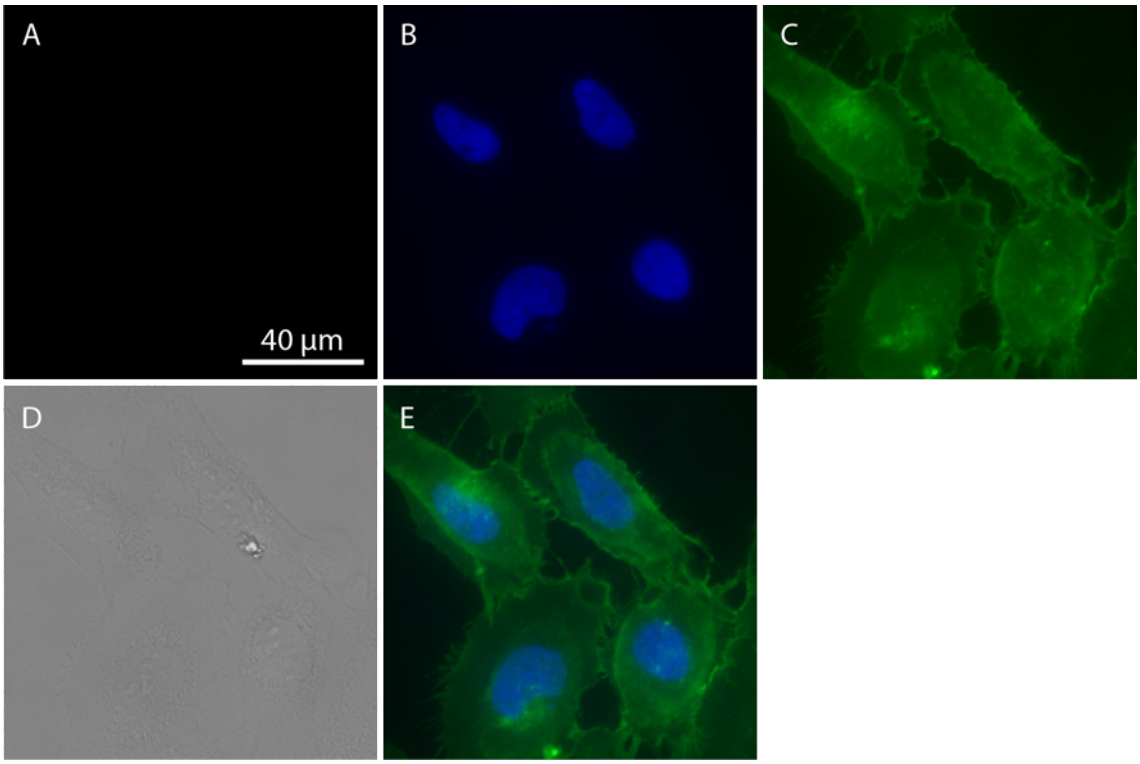

Figure S11: Fluorescence images of HeLa cells not incubated with nanoparticles (*i.e.* control experiment). (A) Red channel, nanoparticles; (B) blue channel, cell nuclei; (E) green channel, cell membranes; (D) transmission image, and (E) merged image.

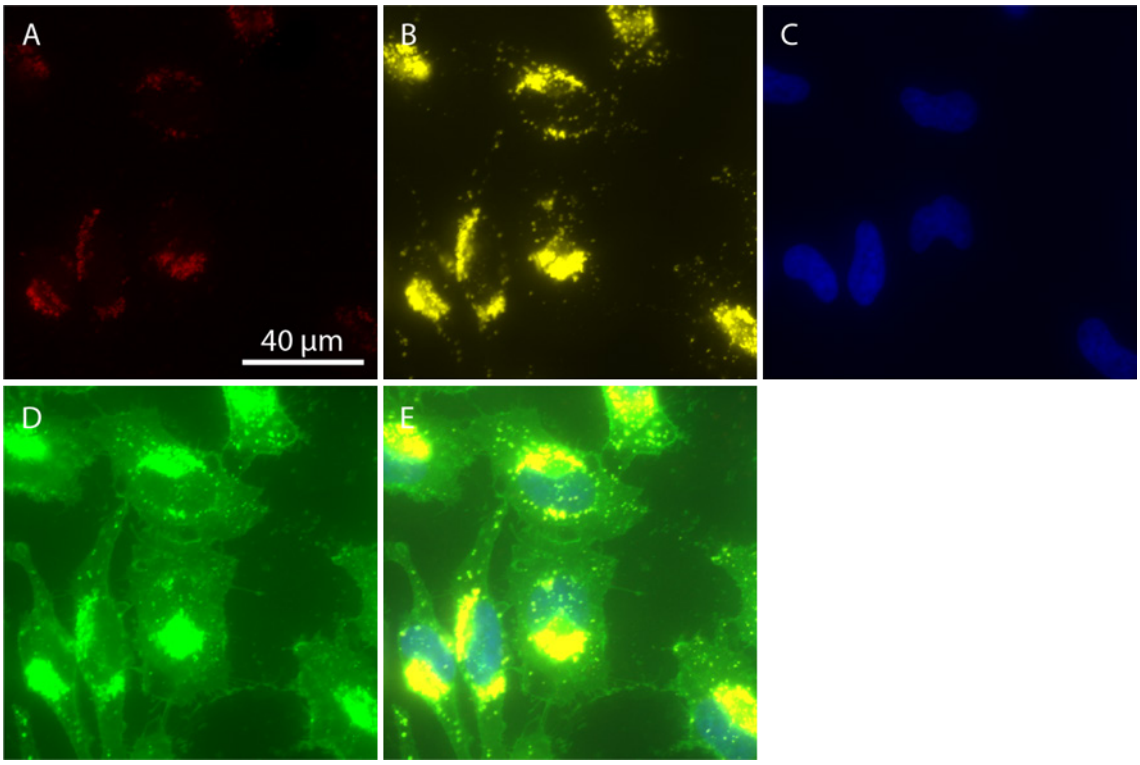

Figure S12: Fluorescence images of HeLa cells incubated with Bi20GdPAA nanoparticles for 24 h. (A) Red channel, nanoparticles (B) yellow channel, lysosomes; (C) blue channel, cell nuclei; (D) green channel, cell membranes; (F) merged image.

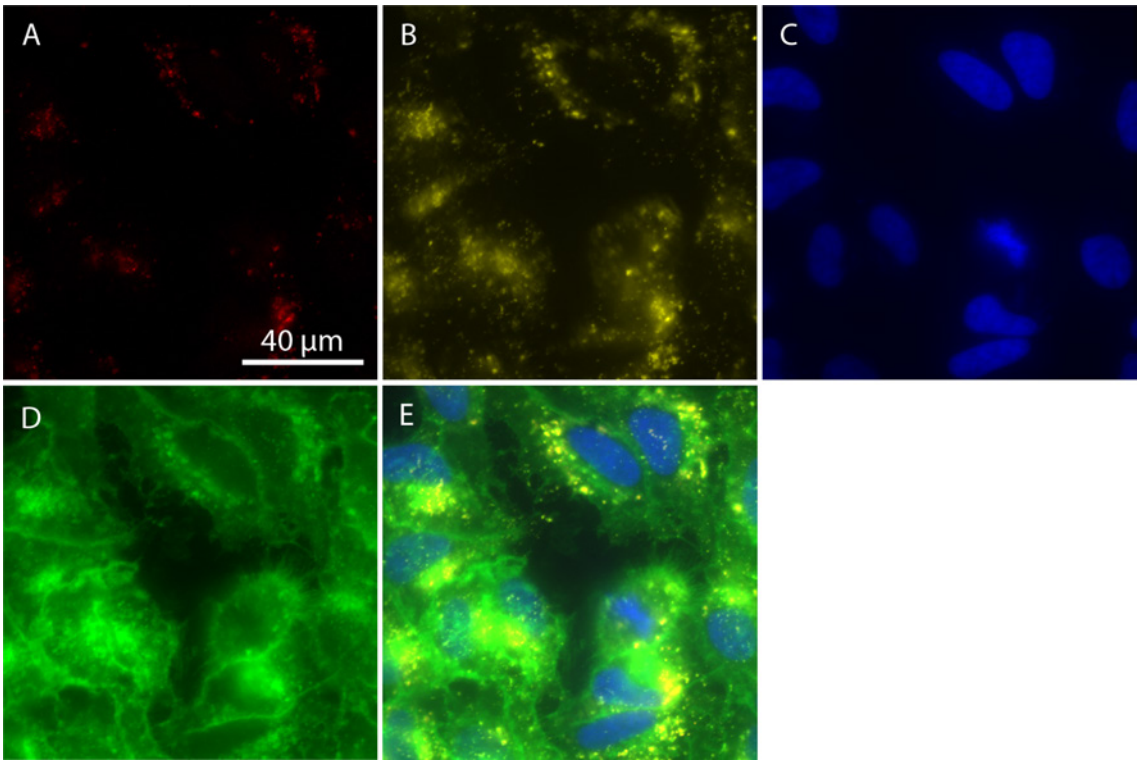

Figure S13: Fluorescence images of HeLa cells incubated with Bi20Y@PAH@PAA@PAH LbL-functionalized nanoparticles for 24 h. (A) Red channel, nanoparticles (B) yellow channel, lysosomes; (C) blue channel, cell nuclei; (D) green channel, cell membranes; and (E) merged image.

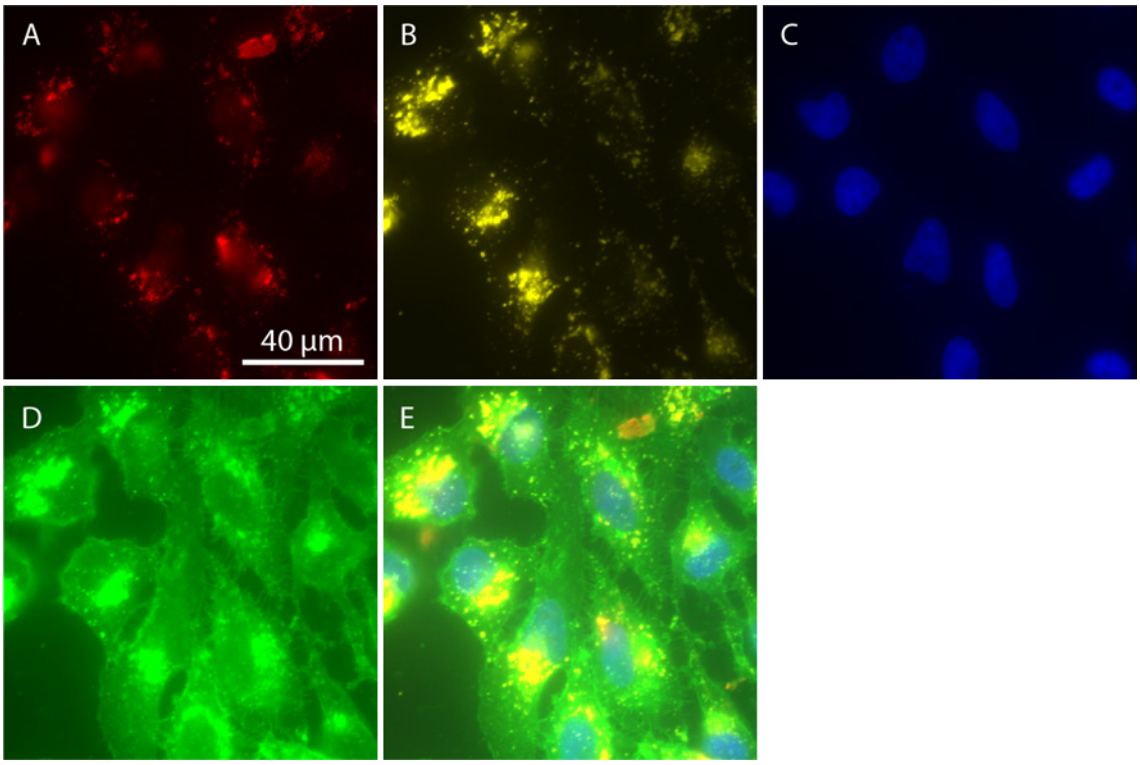

Figure S14: Fluorescence images of HeLa cells incubated with Bi20Gd@PAH@PAA@PAH LbL-functionalized nanoparticles for 24 h. (A) Red channel, nanoparticles (B) yellow channel, lysosomes; (C) blue channel, cell nuclei; (D) green channel, cell membranes; (F) merged image.

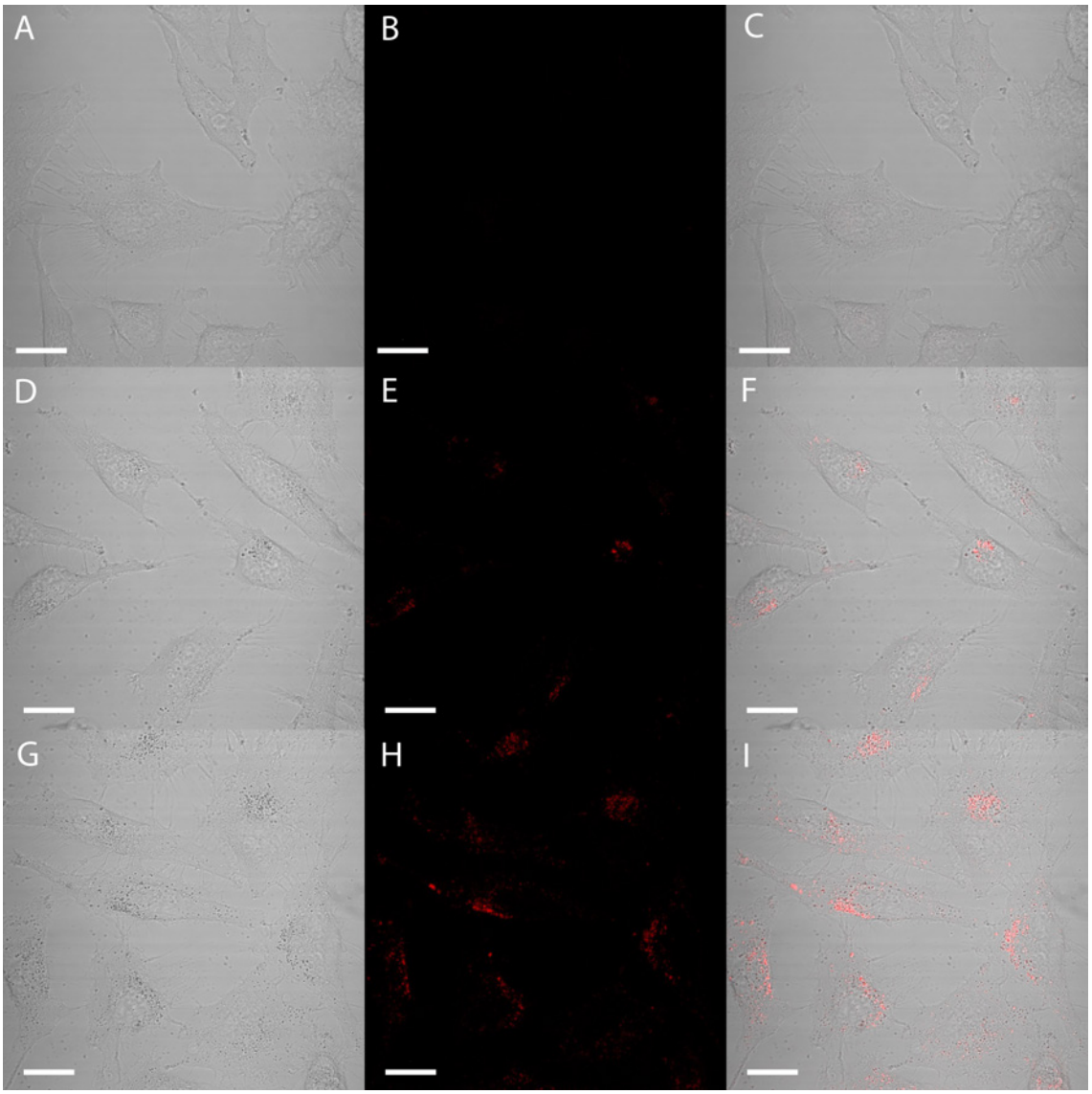

Figure S15: Confocal microscopy images of living HeLa cells without nanoparticles (A to C); and HeLa cells incubated for 24 hours with Bi20YPAA8 (D to F), and Bi20GdPAA (G to I) nanoparticles. Left column: transmission images, central column: fluorescence images; right columns: merged images. The scale bars represent 20 μm.

7. Dissolution at acidic pH

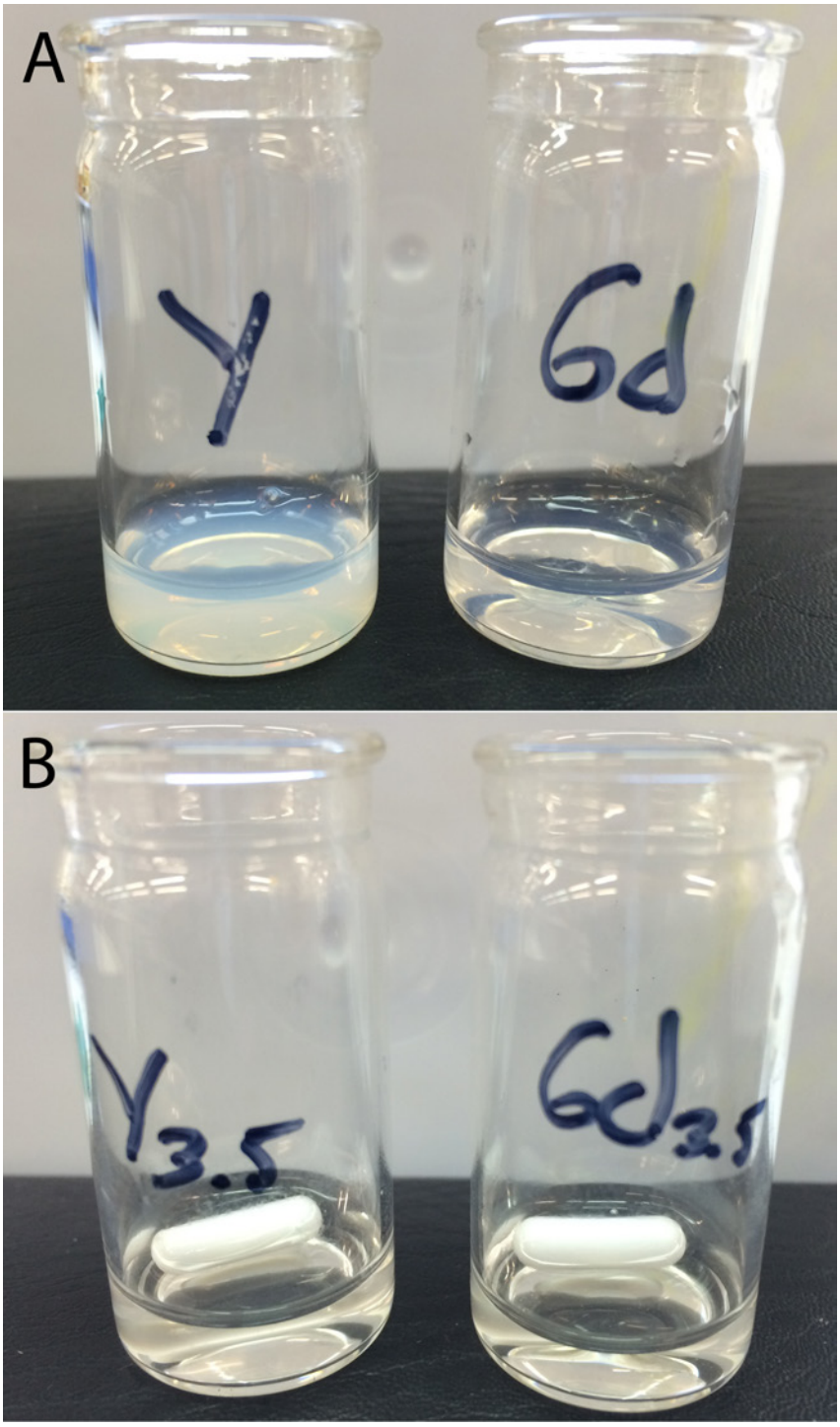

Figure S16: (A) Image of suspensions of the Bi20YPAA2 (left) and Bi20GdPAA (right) samples (initial concentration = 0.5 mg/mL) in MES 50 mM buffer at pH 3.5. (B): Same suspensions in MES 50 mM buffer at pH 3.5 after 21 days of aging.

8. Flow cytometry studies

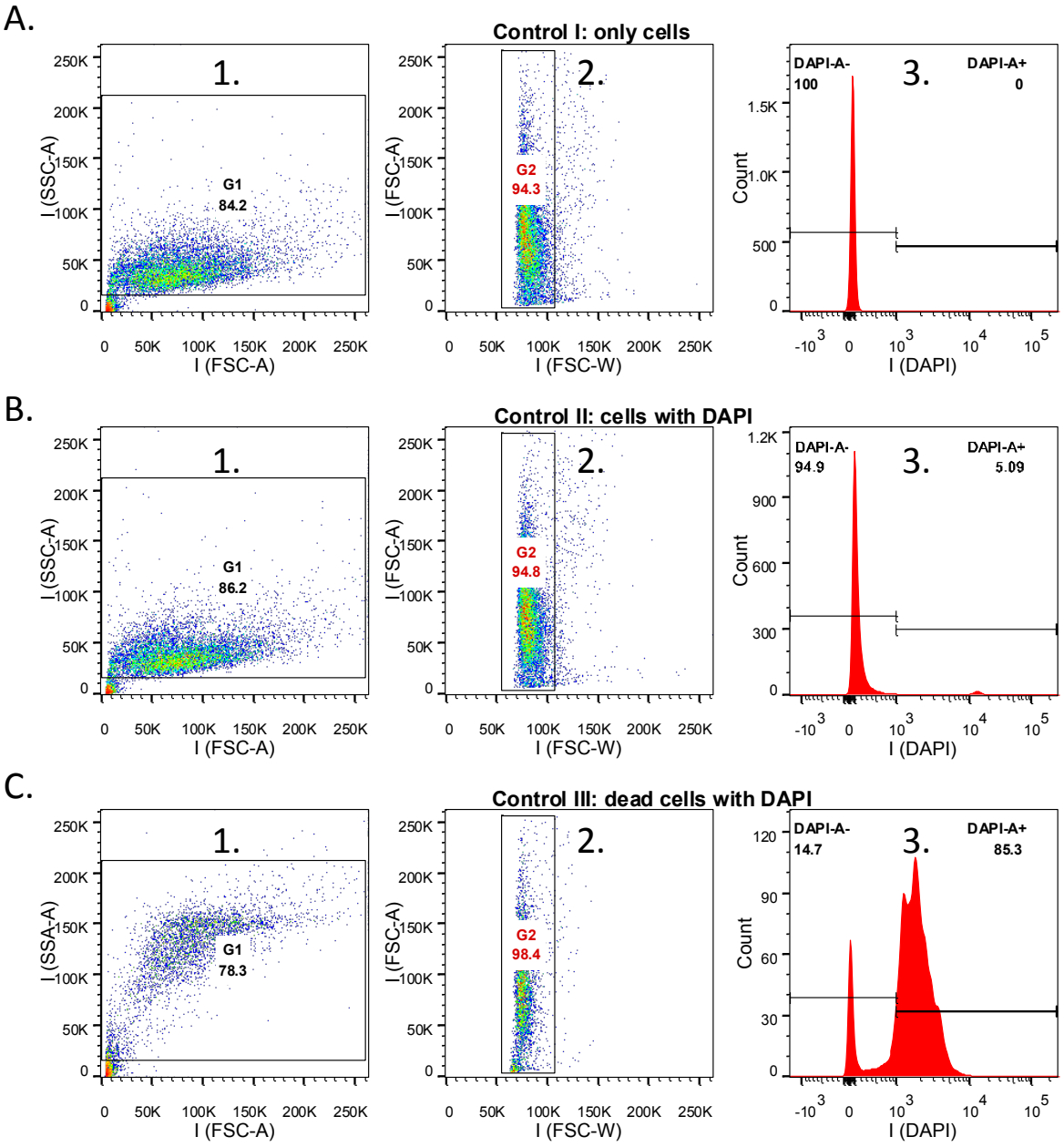

Figure S17: Flow cytometry control measurements of untreated cells (A), cells with DAPI (B), and dead cells with DAPI (C). 1, and 2 2D density plot of A. untreated cells, B. cells with DAPI, C. dead cells with DAPI. Column 1 shows the forward –area scattering versus side scattering; Column 2 shows the forward-width scattering versus forward-area scattering; Column 3 shows the DAPI intensity histogram.