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Dual contrasting ability of $NaGd_{0.7}Eu_{0.3}F_4$ nanocrystals tuned by their hydrophilic coating mode

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Materials and methods

Materials

Anhydrous chlorides of the rare earth elements (YCl_3, $EuCl_3$, $GdCl_3$, 99.999%) were purchased from Chemcraft (Russia), NaOH, NH_4F, citric acid, Bovine serum albumin, Polyethylenimine(PEI), Polylysine (PL) and ethanol were purchased from Sigma-Aldrich Pty Ltd. (Germany), and used without additional purification.

Methods.

Electronic absorption (UV-Vis) spectra were recorded at room temperature on a Perkin-Elmer Lambda 35 spectrometer with a scan speed of 480 nm/min, using a spectral width of 1 nm..

Dynamic light scattering (DLS) measurements were performed by means of the Malvern Mastersize 2000 particle analyzer. A He–Ne laser operating at 633 nm wavelength and emitting vertically polarized light was used as a light source. The measured autocorrelation functions were analyzed by Malvern DTS software and the second-order cumulant expansion methods. The effective hydrodynamic radius (RH) was calculated by the Einstein–Stokes relation from the first cumulant: $D = k_BT/6\pi\eta RH$, where D is the diffusion coefficient, kB is the Boltzmann constant, T is the absolute temperature, and η is the dynamic viscosity. The diffusion coefficient was measured at least three times for each sample. The average error in these experiments is approximately 4%.

The proton relaxation times T_1 and T_2 were measured using pulsed NMR-relaxometer Minispec MQ20 from Bruker with operational frequency of 19.65 MHz (0.47 T) by applying the standard radio frequency pulse sequences: inversion-recovery method (spin-lattice relaxation time T_1), and Carr-Purcell sequence, modified by Meiboom-Gill (spin-spin relaxation time T_2) with the measuring accuracy error smaller than 3%. The temperature was maintained with the Thermo/Haake DC10 circulator.

The emission spectra were recorded on a fluorescence spectrophotometer Hitachi F-7100 (Japan). Excitation of samples was performed at 350 nm and emission was detected at 400–700 nm.

M-HeLa cells in the amount of 1×10^5 cells / well in a final volume of 500µl were sown in 24-well plates (Eppendorf). After 24-hour incubation, PC-**7** aggregates at a concentration of 7.5 µM were added to the wells and incubated for 24 hours in a CO_2 incubator. Cellular uptake of test compounds was analyzed by flow cytometry (Guava easy Cyte 8HT, USA). Flow cytometry was used to set up statistics on the uptake of drug by cancer cells. Untreated cells were used as negative control.

M-HeLa cells at 1 x 10^5 cells / well in a final volume of 2 ml were seeded into 6-well plates at the bottom of each well. After 24 h of incubation, solution of PC-**7** (7.5 µM) was added to the wells and cultured for 24 hours in a CO_2 incubator. Then, M-HeLa cells were fixed and stained with DAPI (blue). The studies were carried out using a Nikon Eclipse Ci-S fluorescence microscope. (Nikon, Japan) at 1000x magnification.

Synthesis of $NaGd_{0.7}Eu_{0.3}F_4$

The synthesis of $NaGd_{0.7}Eu_{0.3}F_4$ was carried out in accordance with the previously published work 0.225 mmol of $EuCl_3$, 0.525 mmol of $GdCl_3$, and 3 mmol of citric acid were dissolved in distilled water to obtain 5 mL solution in total. Then, 2.5 mL of an aqueous solution containing 9 mmol of NaOH was added to the reaction mixture. After vigorous stirring for 30 min, 8 mL of aqueous solution containing 11 mmol of NaOH and 11 mmol of NH_4F was added into the above solution. The solution was maintained after vigorous stirring for 30 min at room temperature before being transferred to a Teflon-lined autoclave with an internal volume of 20 mL and heated for 17 h at the temperature of 180 °C. After that, the precipitate was separated from the reaction mixture by centrifugation, washed with ethanol and deionized water.

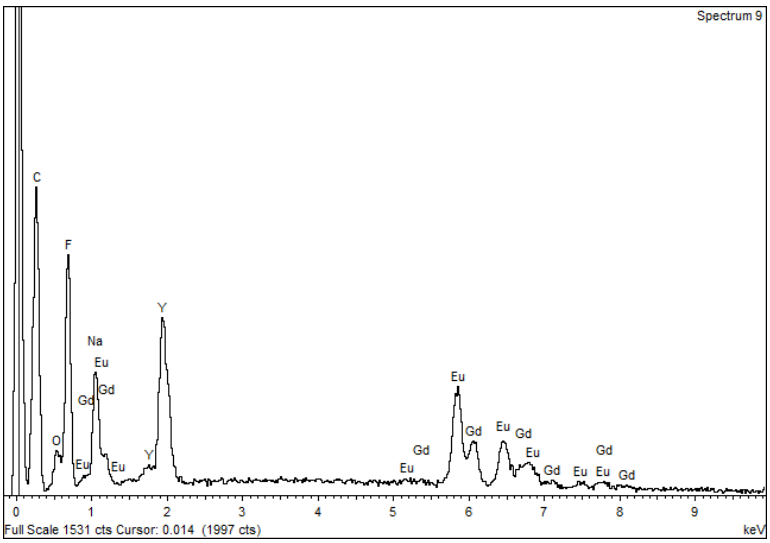

Figure S1. EDX spectra of NaGd0.7Eu0.3F4

Table 1. Elemental composition of the $NaGd_{0.7}Eu_{0.3}F_4$, obtained by X-ray fluorescence analysis.

Theoretical values		Practical implications	
%Eu	%Gd	%Eu	%Gd
30	70	28	72

Table 2. Elemental composition of the $NaGd_{0.7}Eu_{0.3}F_4$, obtained by atomic emission analysis.

Theoretical values		Practical implications					
%Eu	%Gd	C_{Eu}, mg/L	C_{Gd}, mg/L	n(Eu), mM/L	n(Gd), mM/L	%Eu	%Gd
30	70	22,6±0,9	41,1±1,3	0,149±0,006	0,262±0,008	36,3	63,4

Synthesis of NPs@PEI and NPs@PL.

The solution of $NaGd_{0.7}Eu_{0.3}F_4$ (0.1 ml, 100 g/L) and Polyethyleneimine (1.4 mL, 1 g/L) or DL-Polylysine bromide (1.4 mL, 1 g/L) were mixed in eppendorf. The resulting solutions were kept in an ultrasonic bath for 10 minutes, then were precipitated in a centrifuge at 10000 rpm for 10 minutes. The supernatant solution

with excess Polyethyleneimine or DL-Polylysine bromide were drained. 1.5 ml of water was added to the precipitate obtained and kept in an ultrasonic bath for 10 minutes.

Determination of NPs:PEI/PL ratio

Two portions of 0.1 ml (100 g/L) of $NaGd_{0.7}Eu_{0.3}F_4$ were evaporated and the mass of the dry residual was weighed, which amounted to 0.01011 and 0.01013. Then 1.4 ml of PEI or PL solution and 0.1 ml of H_2O were added to the resulting solution and the synthesis technique of NPs@PEI and NPs@PL was reproduced. The luminescence spectrum of the supernatants did not exhibit Eu-based luminescence, which indicates complete sedimentation of nanoparticles during centrifugation. For determination the nanoparticles–polymer ratio, 1.5 ml of NPs@PEI and NPs@PL solution were evaporated and the dry residue mass was again weighted. In the case of NPs@PI and NPs@PL, it was 0.01128 g and 0.01149 g, respectively. The final ratio is for NPs@PI and NPs@PL: 26.5 mM $NaGd_{0,7}Eu_{0,3}F_4$ per 0.0276 mM PEI and 26.5 mM $NaGd_{0,7}Eu_{0,3}F_4$ per 0.0224 mM PL

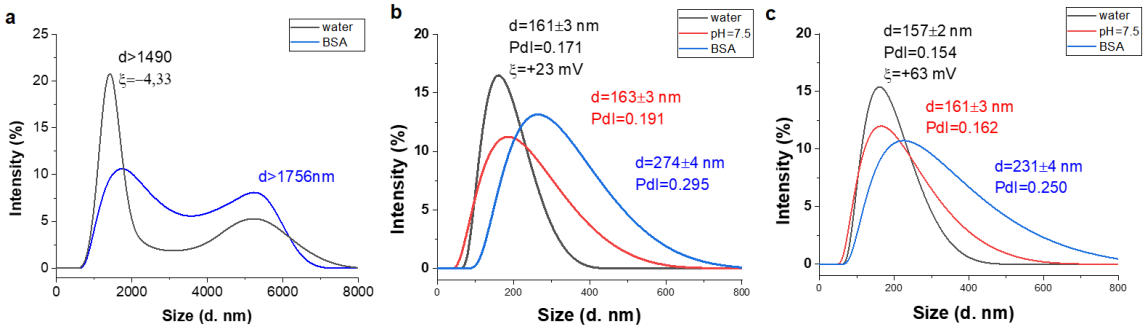

Figure S2. Size distribution of NPs, NPs@PL and NPs@PEI in different solution

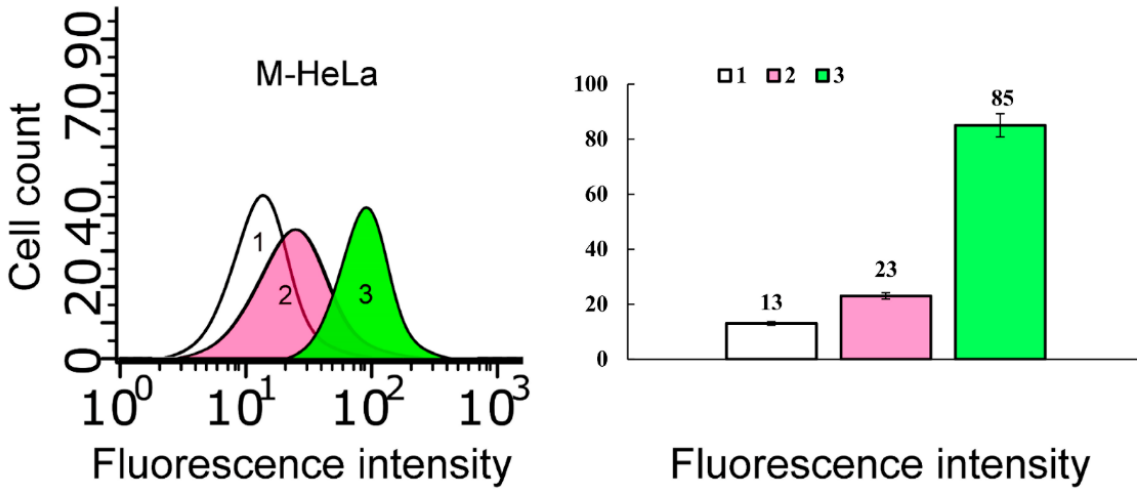

Figure S3. Cellular uptake study: 1 – Control; 2 - NPs@PEI; 3 – NPs@PL

Table S3. Cell viability of M-Hela and Chang Liver cells in presence of different concentration of NPs@PEI and NPs@PL

	Compound	Concentration g/L	Cell Viability %	IC_{50} g/L
M-HeLa	NPs @PEI	3.30	28,6	2,50
		1,65	61,5	
		0,82	62,5	
		0,41	63,6	
		0,21	69,9	
		0,10	71,6	
	NPs@PL	3.30	66,1	>3,30
		1,65	67,5	
		0,82	67,7	
		0,41	69,3	
		0,21	70,4	
		0,10	73,4	
Ch, Liver	NPs @PEI	3.30	22,5	1,70
		1,65	57,4	
		0,82	63,5	
		0,41	71,1	
		0,21	71,1	
		0,10	88,6	
	NPs@PL	3.30	72,9	>3,30
		1,65	74,5	
		0,83	77,2	
		0,41	77,7	
		0,21	78,9	
		0,10	81,5	