Electronic supporting information for the article

Polymeric nanoparticles with embedded Eu(III) complexes as molecular probes for temperature sensing

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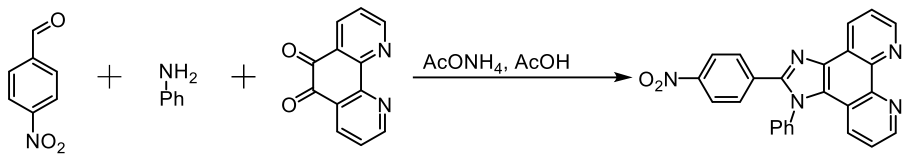
Synthesis of ligands and complexes



Scheme S1. Scheme of **L3** ligand synthesis.

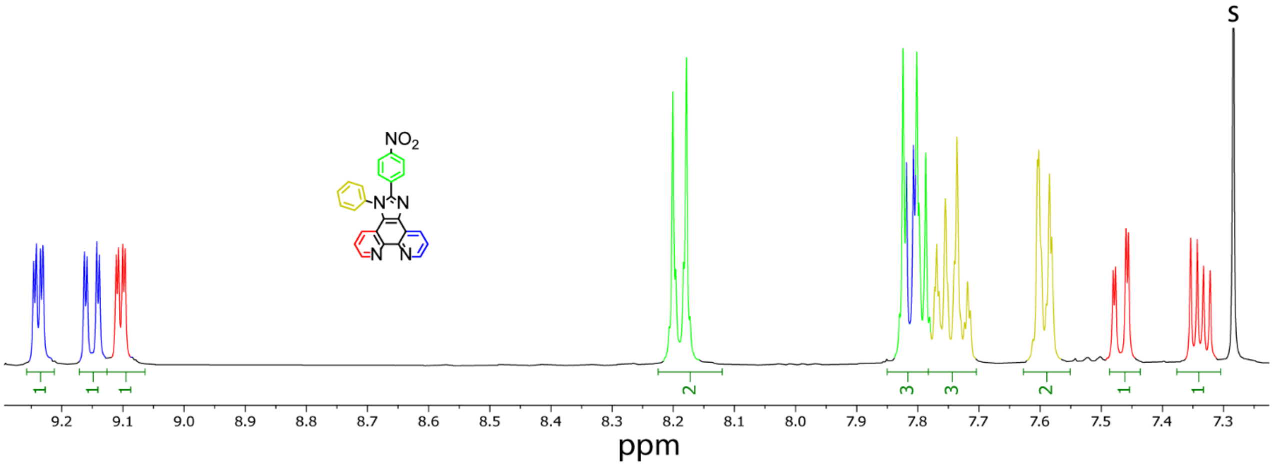


Figure S1. [1]H NMR spectrum of ligand **L₃** in CDCl₃. S – residual solvent peak.

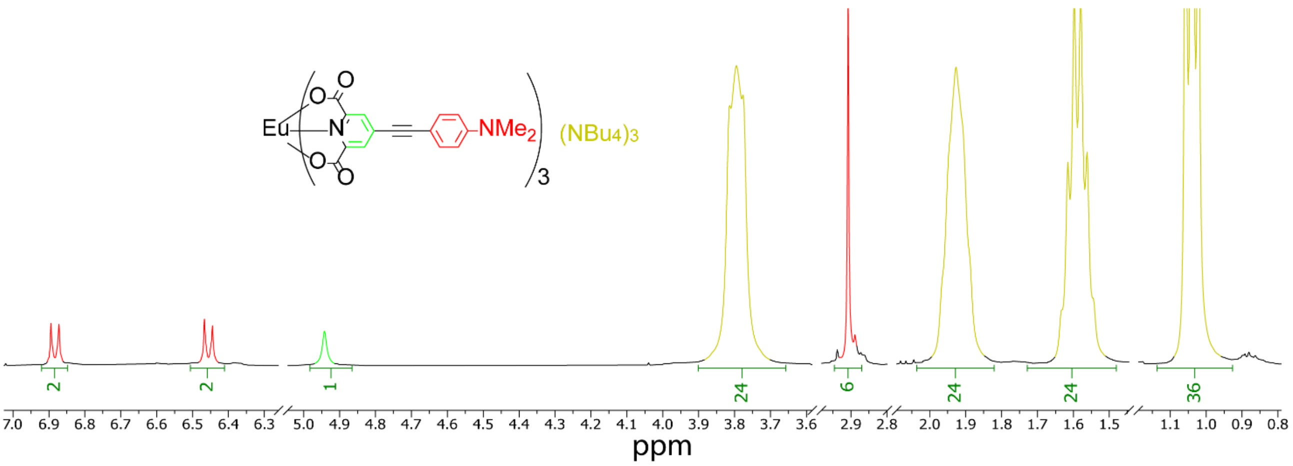


Figure S2. [1]H NMR spectrum of complex **Eu1** in CDCl₃.

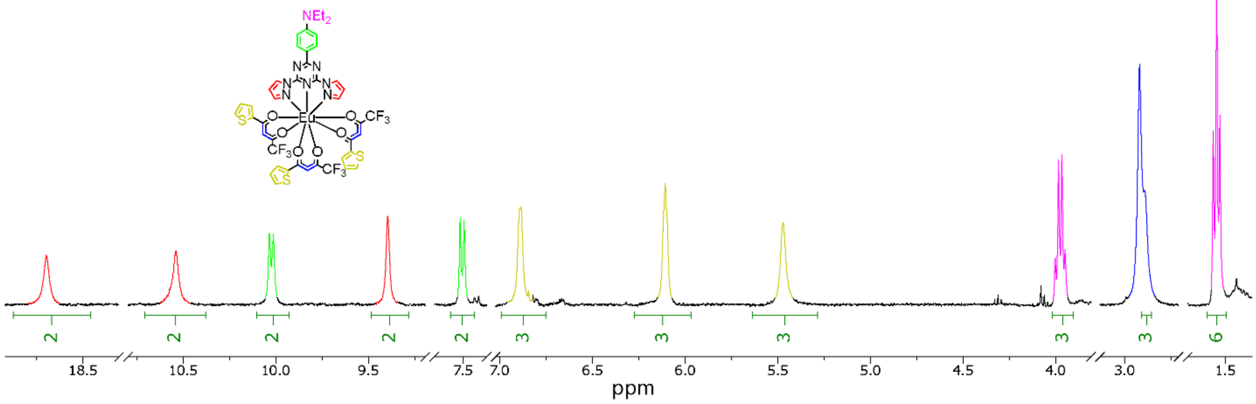


Figure S3. ^{1}H NMR spectrum of complex **Eu2** in DMSO-d_6.

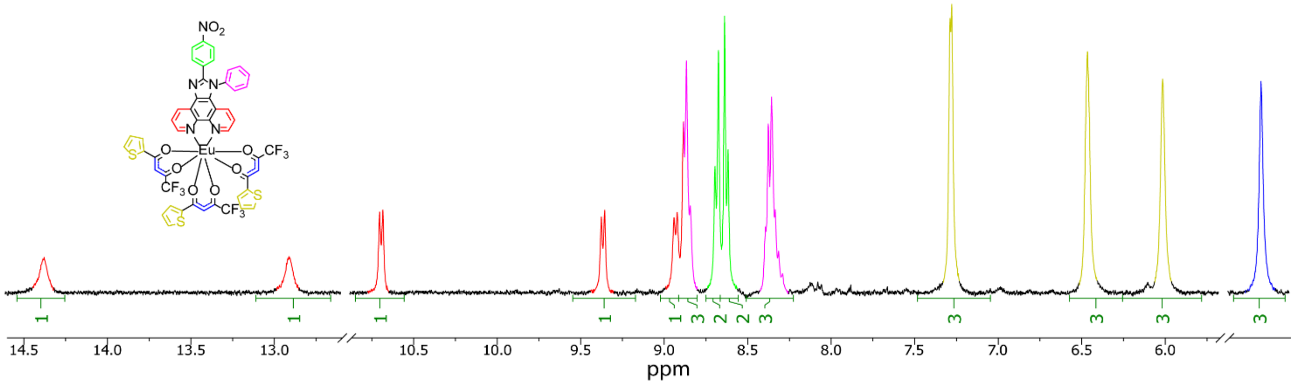


Figure S4. ^{1}H NMR spectrum of complex **Eu3** in Acetone-d_6.

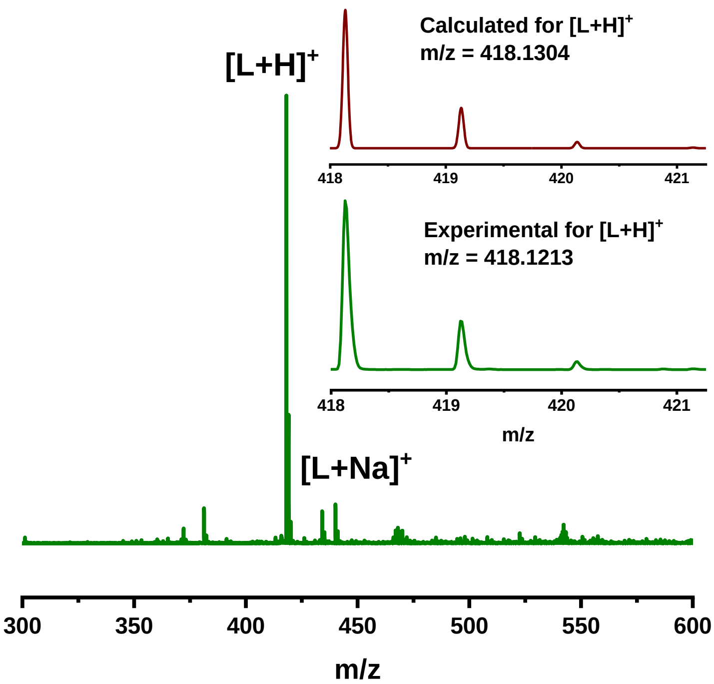


Figure S5. Fragment of ESI^{+} spectrum of ligand **L3**.

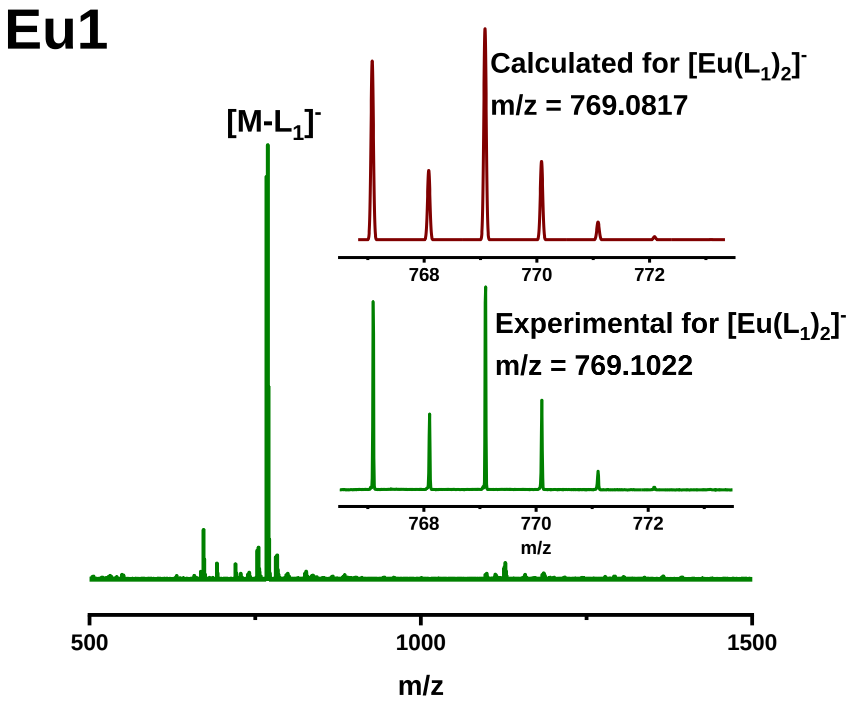


Figure S6. Fragment of ESI⁻ spectrum of complex **Eu1**.

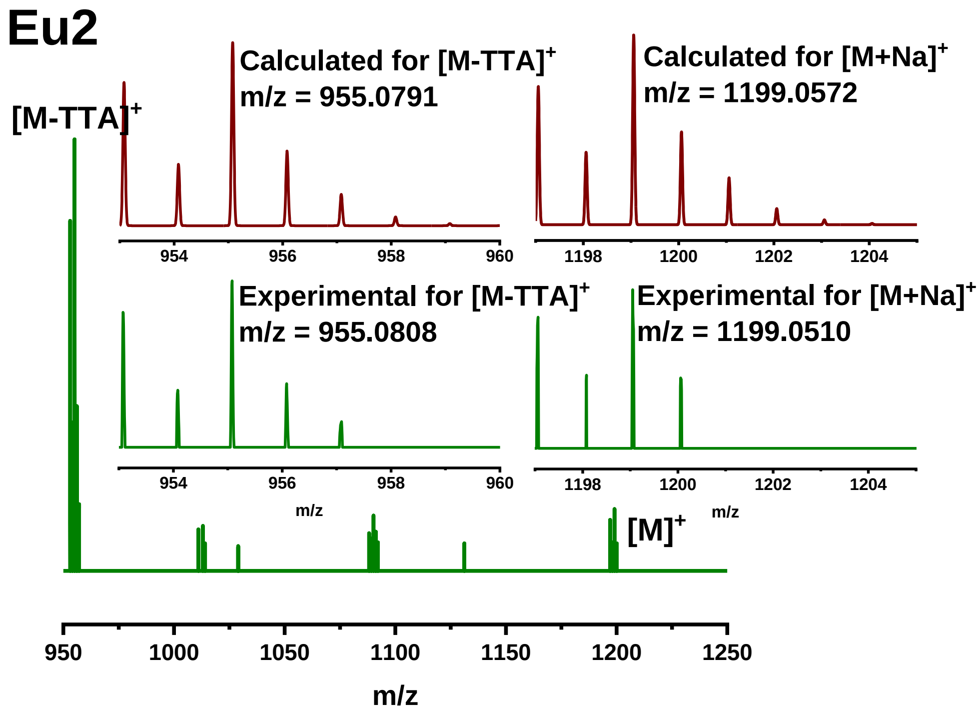


Figure S7. Fragment of ESI$^+$ spectrum of complex **Eu2**.

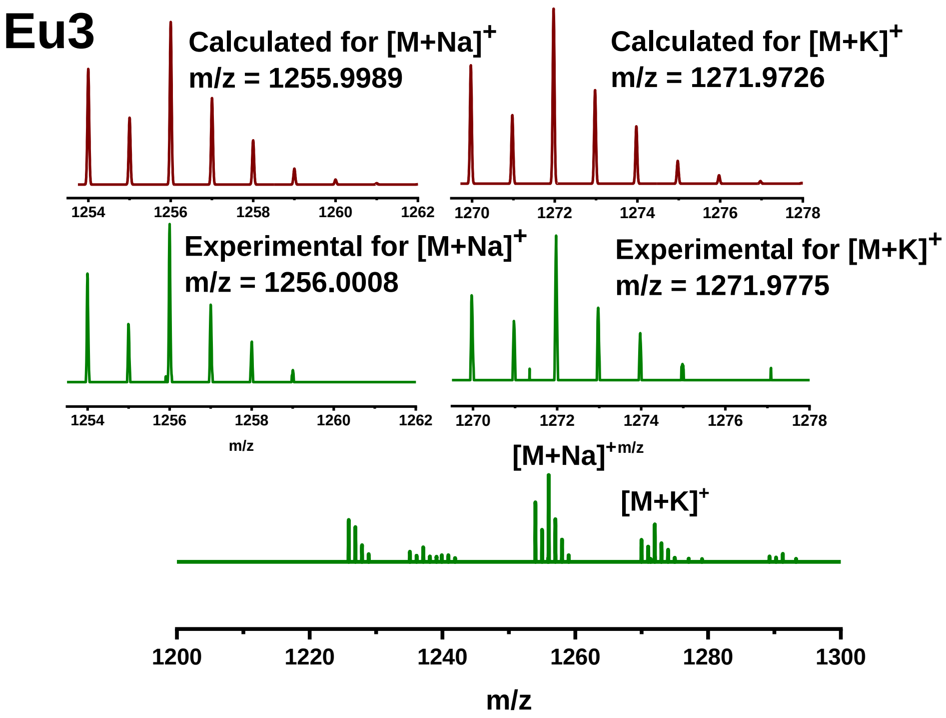


Figure S8. Fragment of ESI^+ spectrum of complex **Eu3**.

Photophysical properties of ligands and complexes

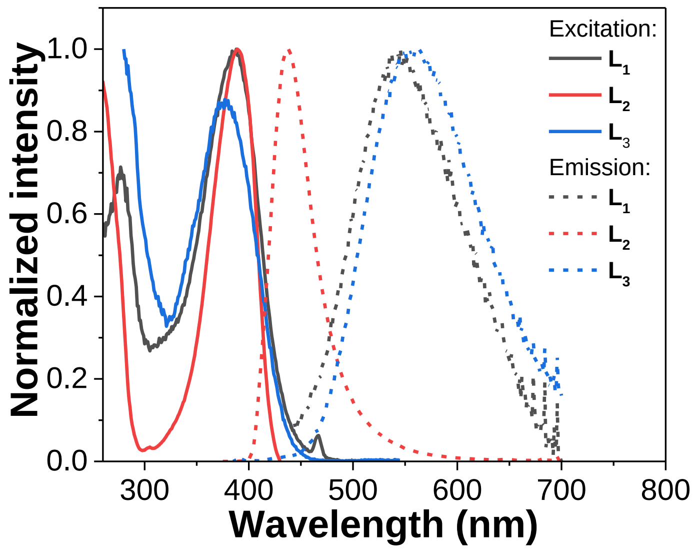


Figure S9. Excitation (solid lines) and emission (dashed lines) spectra of ligands in dichloromethane, 293K.

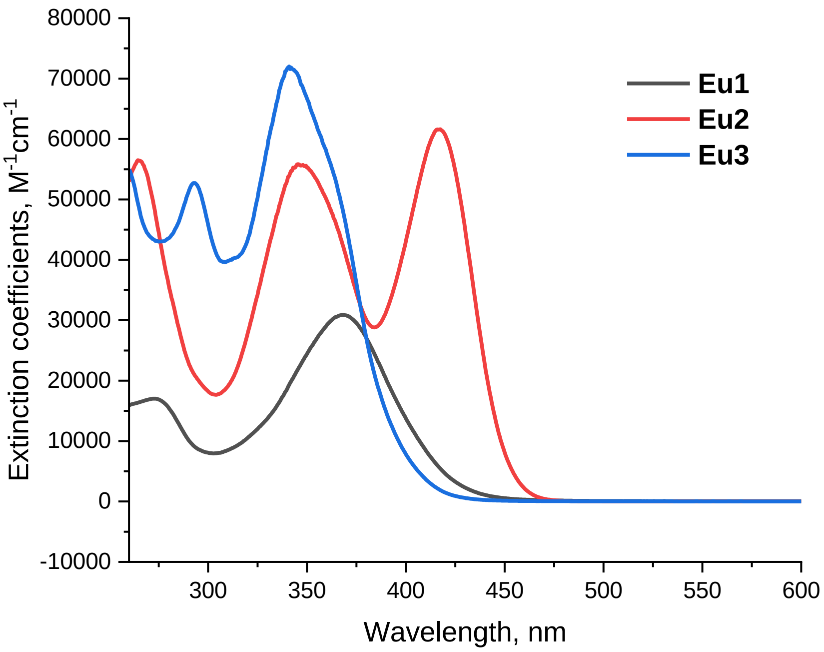


Figure S10. Absorption spectra of **Eu1-Eu3** complexes in dichloromethane, 293K.

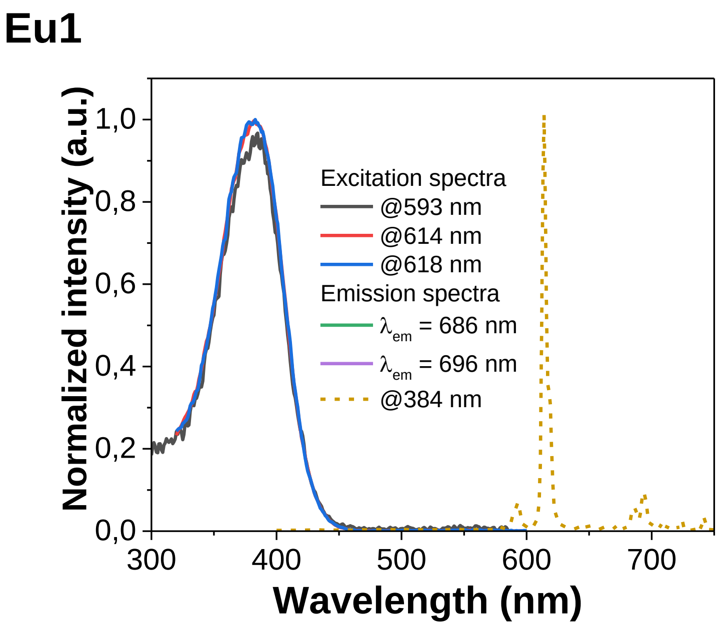


Figure S11. Excitation (solid lines) and emission (dashed lines) spectra of complex **Eu1** in dichloromethane, 293K.

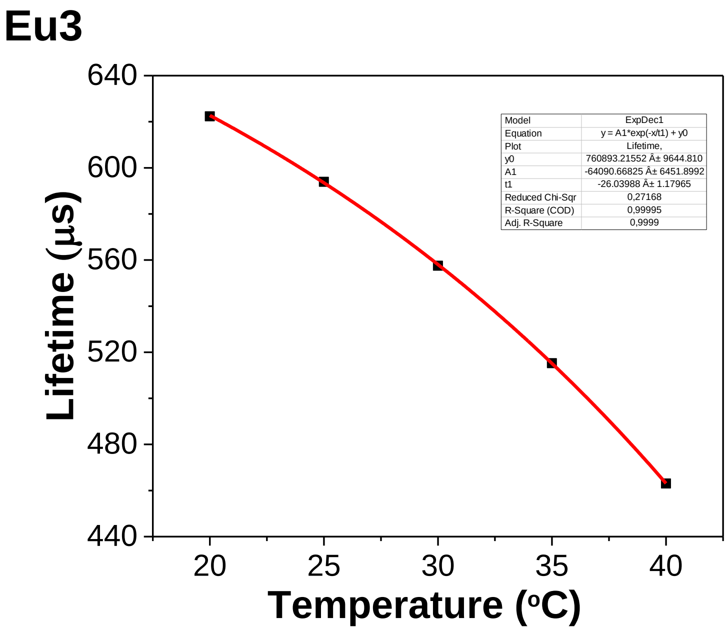


Figure 12. Correlation between lifetimes of excited state and temperature for **Eu3** in dichloromethane solution.

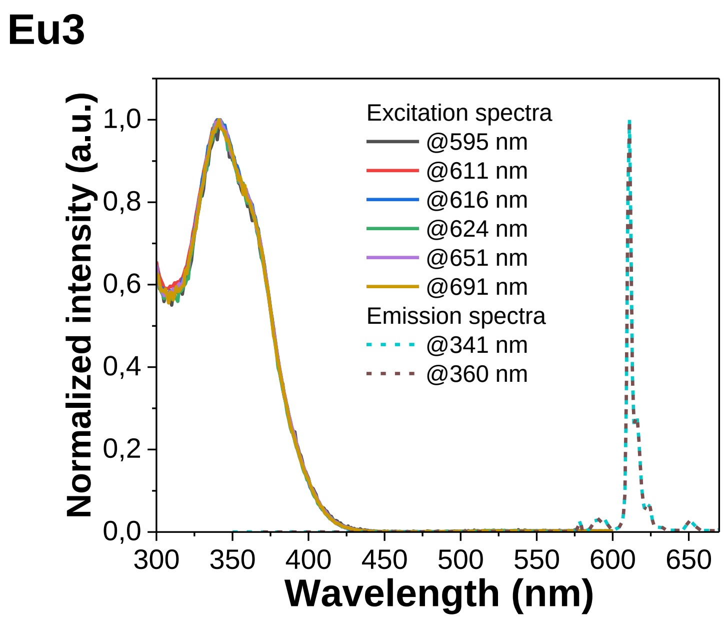


Figure S13. Excitation (solid lines) and emission (dashed lines) spectra of complex **Eu3** in dichloromethane, 293K.

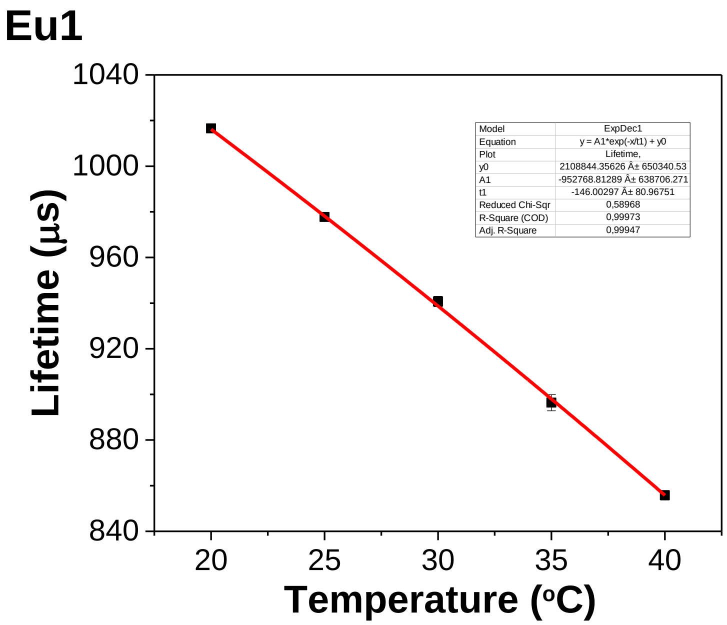


Figure S14. Correlation between lifetimes of excited state and temperature for **Eu1** in dichloromethane solution.

Eu1

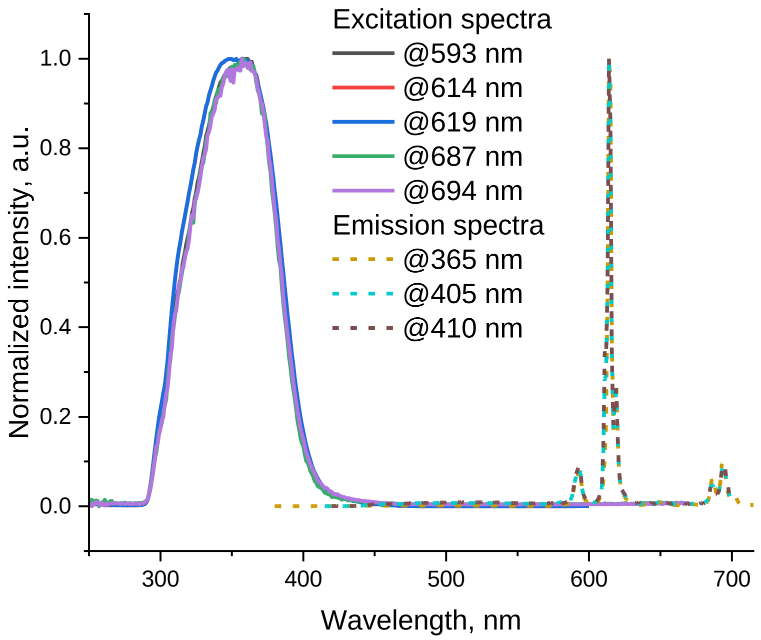


Figure S15. Excitation (solid lines) and emission (dashed lines) spectra of complex **Eu1** in monomers taken with the same amount as for polymerization, 293K.

Eu2

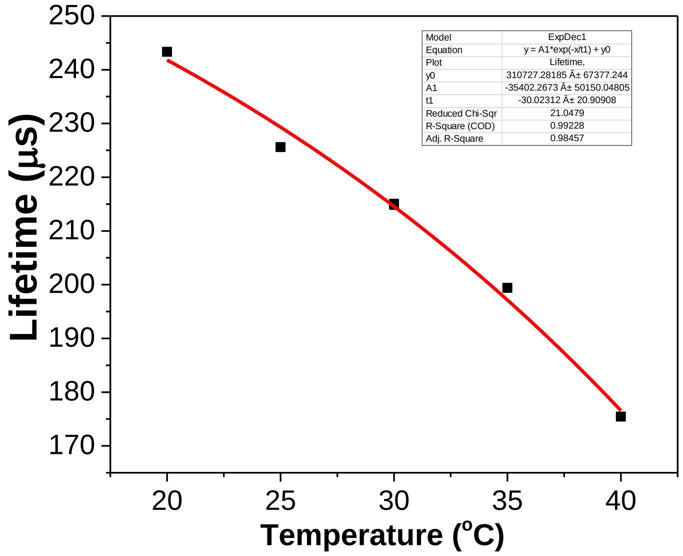


Figure S16. Correlation between lifetimes of excited state and temperature for **Eu2** in dichloromethane solution.

Eu2

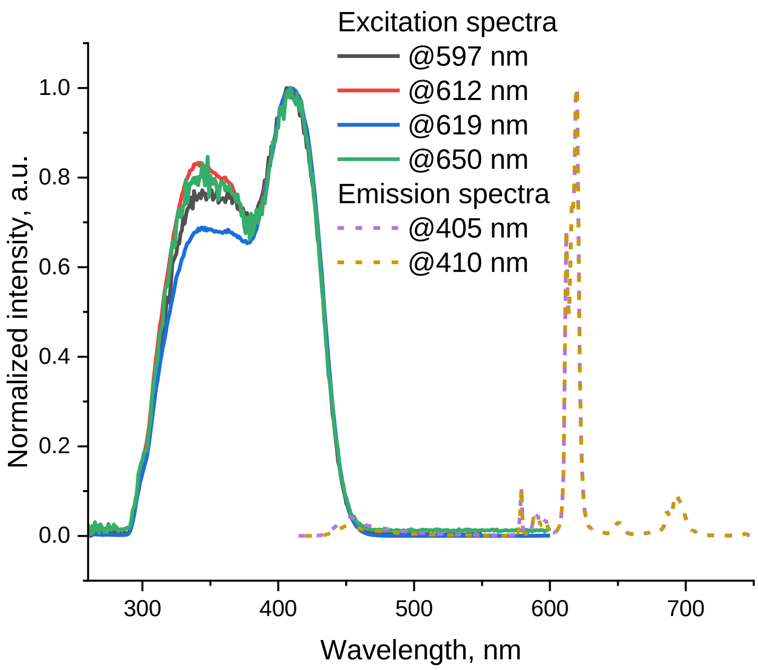


Figure S17. Excitation (solid lines) and emission (dashed lines) spectra of complex **Eu2** in monomers taken with the same amount as for polymerization, 293K.

Eu3

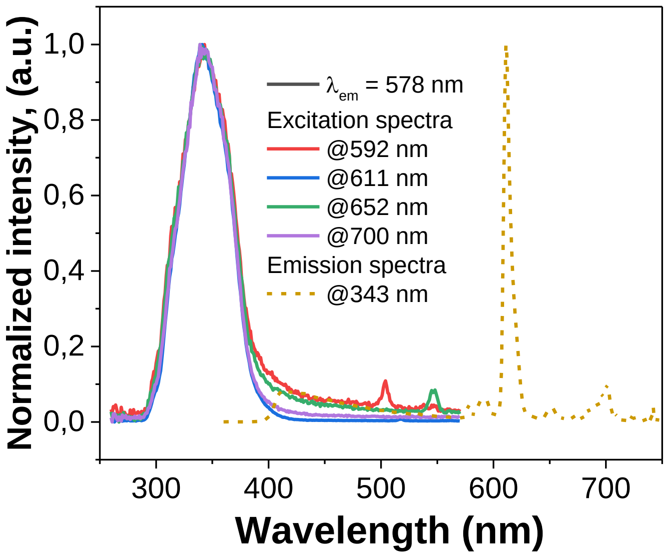


Figure S18. Excitation (solid lines) and emission (dashed lines) spectra of complex **Eu3** in monomers taken with the same amount as for polymerization, 293K.

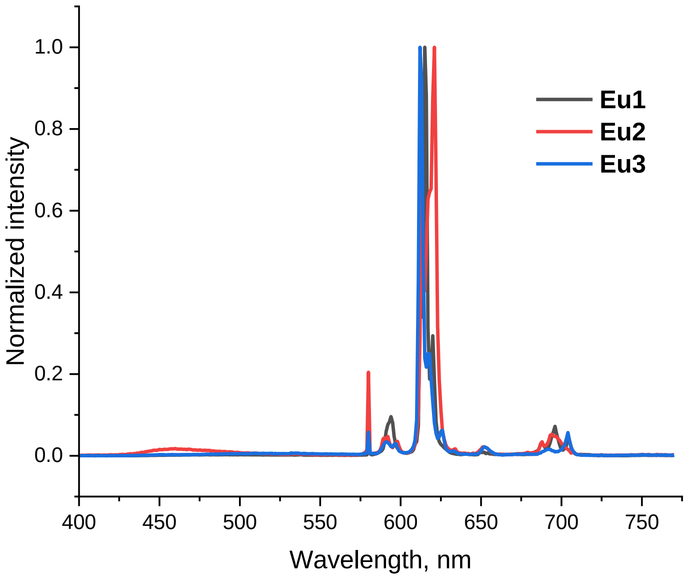


Figure S19. Two-photon emission spectra of europium complexes in dichloromethane solution using 800 nm excitation wavelength, 293K.

The temperature dependence of the excited state lifetime (τ) was approximated according to the equation:

$$\tau(T) = \frac{1}{k_r + A \cdot e^{-\frac{\Delta E}{k_B T}}} \sim A' \cdot e^{\frac{\Delta E}{k_B T}} \qquad \textbf{Equation S1}$$

where

k_r is the rate of radiative transition;

k_{nr} is the rate of nonradiative transition;

A is a constant weakly dependent on temperature;

k_B is the Boltzmann constant;

T is temperature.

Synthesis of Eu-containing nanoparticles

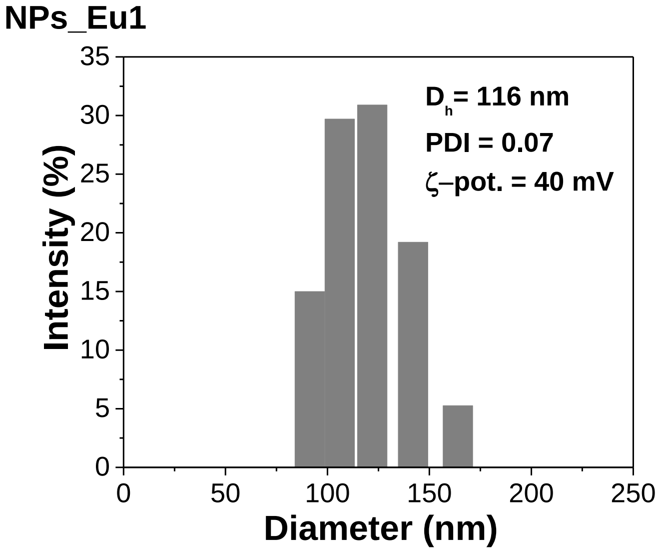


Figure S202. Main characteristics of **NPs_Eu1**. D_h – hydrodynamic diameter, PDI – polydispersity index, ζ-pot. - ζ-potential.

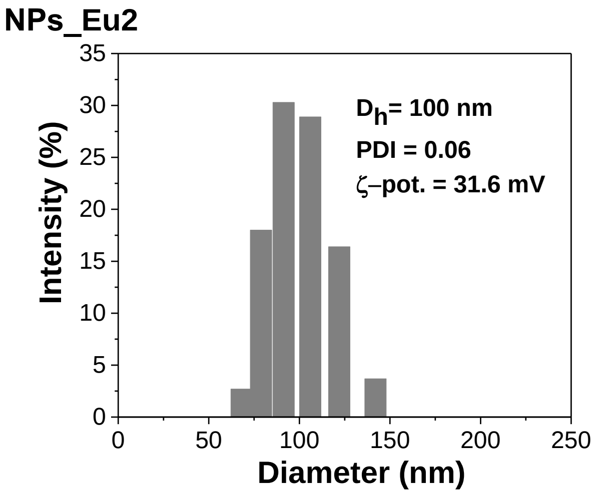


Figure S213. Main characteristics of **NPs_Eu2**. D_h – hydrodynamic diameter, PDI – polydispersity index, ζ-pot. - ζ-potential.

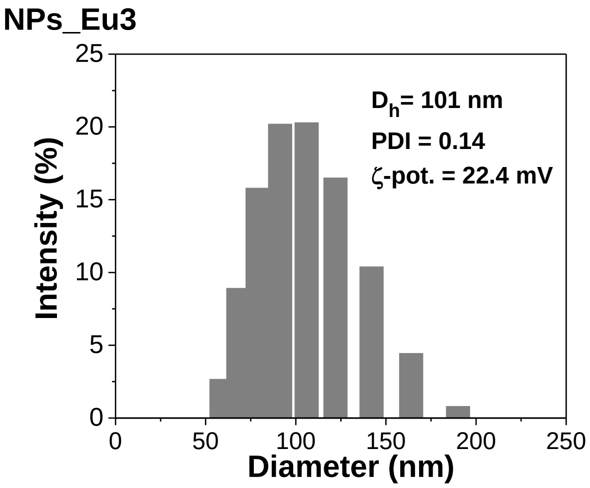


Figure S224. Main characteristics of **NPs_Eu3**. D_h – hydrodynamic diameter, PDI – polydispersity index, ζ-pot. - ζ-potential.

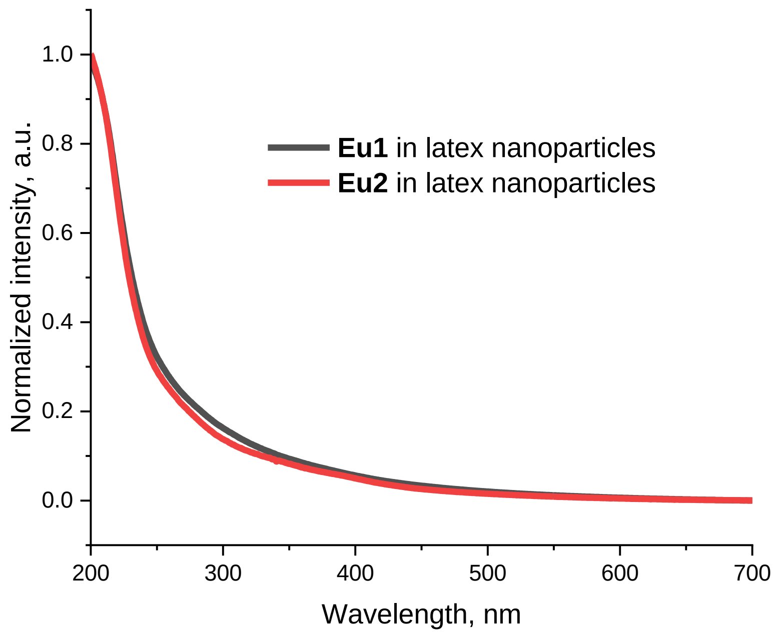


Figure S23. Absorption spectra of latex nanoparticles in water dispersion, 293K.

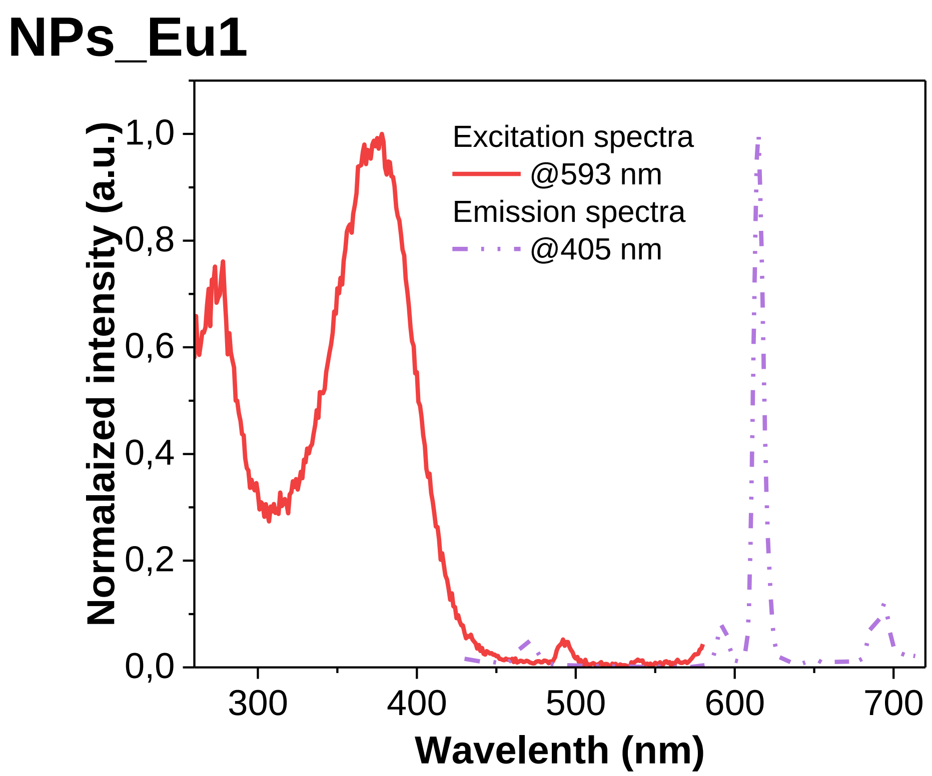


Figure S24. Excitation (solid lines) and emission (dashed lines) spectra of nanoparticles with **NPs_Eu1** in water dispersion, 293K.

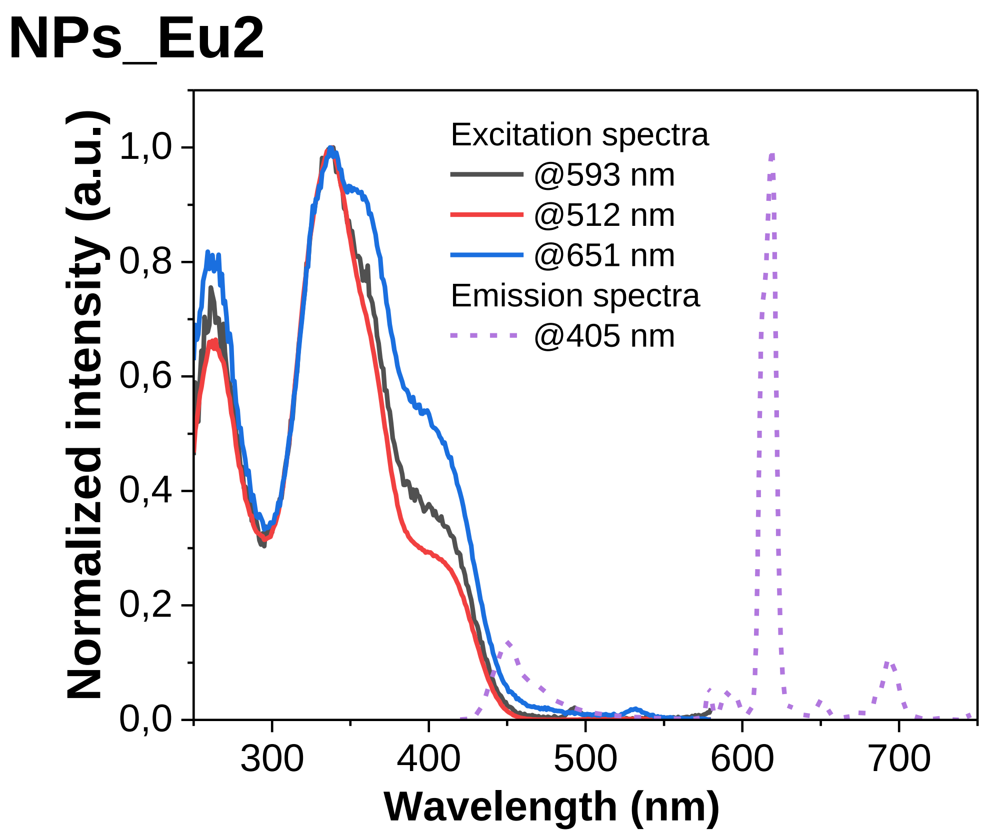


Figure S25. Excitation (solid lines) and emission (dashed lines) spectra of nanoparticles with **NPs_Eu2** in water dispersion, 293K.

NPs_Eu3

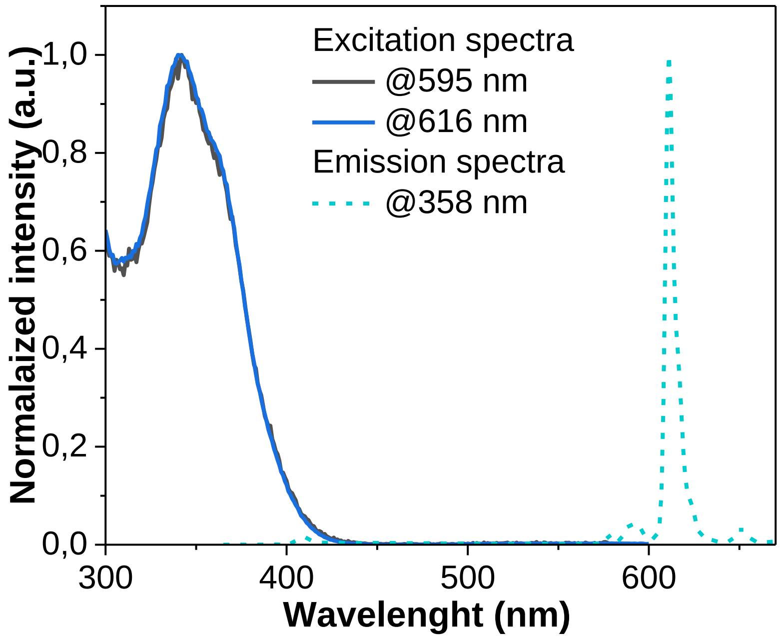


Figure S26. Excitation (solid lines) and emission (dashed lines) spectra of nanoparticles with **NPs_Eu3** in water dispersion, 293K.

Determination of the number of lanthanide complexes and triphenylphosphine per particle

The number of latex particles per milliliter is given by the following equation [bangslab.com URL https://www.bangslabs.com/sites/default/files/imce/docs/TechNote%20206%20Web.pdf (achieved on 04.10.2022)]:

$$N_p = \frac{6 \cdot 10^{10} \cdot S \cdot \rho_L}{\pi \cdot \rho_S \cdot d^3}, \qquad \textbf{Equation S2}$$

where

N_p is number of latex nanoparticles per milliliter;

S is weight % solids (for 10% solids suspension S=10);

ρ_L is density of nanoparticle suspension (g/mL);

$$\rho_L = \frac{100 \cdot \rho_S}{S \cdot (1 - \rho_S) + (100 \cdot \rho_S)};$$

ρ_L is density of solid nanoparticle (g/cm^3);

d is mean diameter (µm).

The number of europium complexes (or phosphonium salt) per particle was then calculated according to:

$$N_{Eu} = \frac{n_{Eu} \cdot N_A}{N_p} \text{ (or } N_P = \frac{n_P \cdot N_A}{N_p}\text{)},$$

where

N_p is number of latex nanoparticles per milliliter;

n_{Eu} and n_P is the quantity of incorporated europium complexes or phosphonium salt)(mol) determined by ICPOES;

N_A is the Avogadro number (mol^{-1}).

The lifetime sensor characteristics

The sensors characteristics have been calculated in accordance with the literature guidelines [Miroslav D. Dramićanin, *J. Appl. Phys.* 128, 040902 (2020); doi: 10.1063/5.0014825].

Temperature sensitivity (S_T)

$$S_T = \frac{d\tau}{dT}$$ **Equation S3**

Dividing this magnitude by the indicator value (τ_{in}), which is the minimum in the range under study, we obtain the relative sensitivity (S_r):

$$S_r = \frac{d\tau/dT}{\tau_{in}}$$ **Equation S4**

Reproducibility (R) was determined by measuring ten cycles with a change in the signal value from τ to τ_i:

$$R = 1 - \frac{\tau - \tau_i}{\tau}$$ **Equation S5**

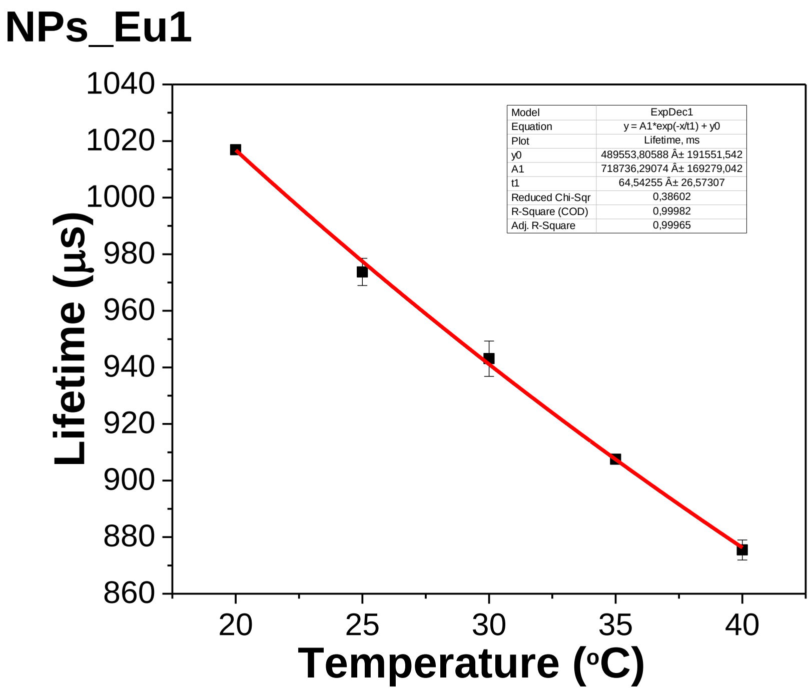


Figure S27. Correlation between lifetimes of excited state and temperature for **NPs_Eu1** in water dispersion.

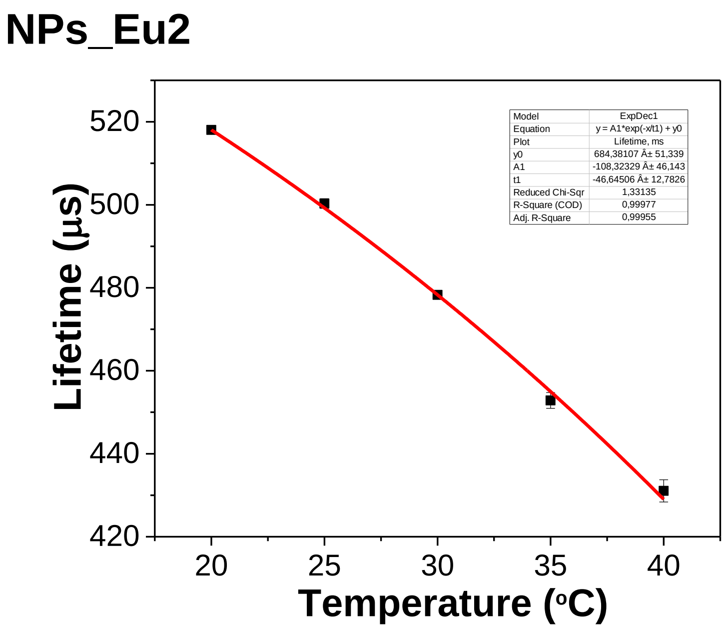


Figure S285. Correlation between lifetimes of excited state and temperature for **NPs_Eu2** in water dispersion.

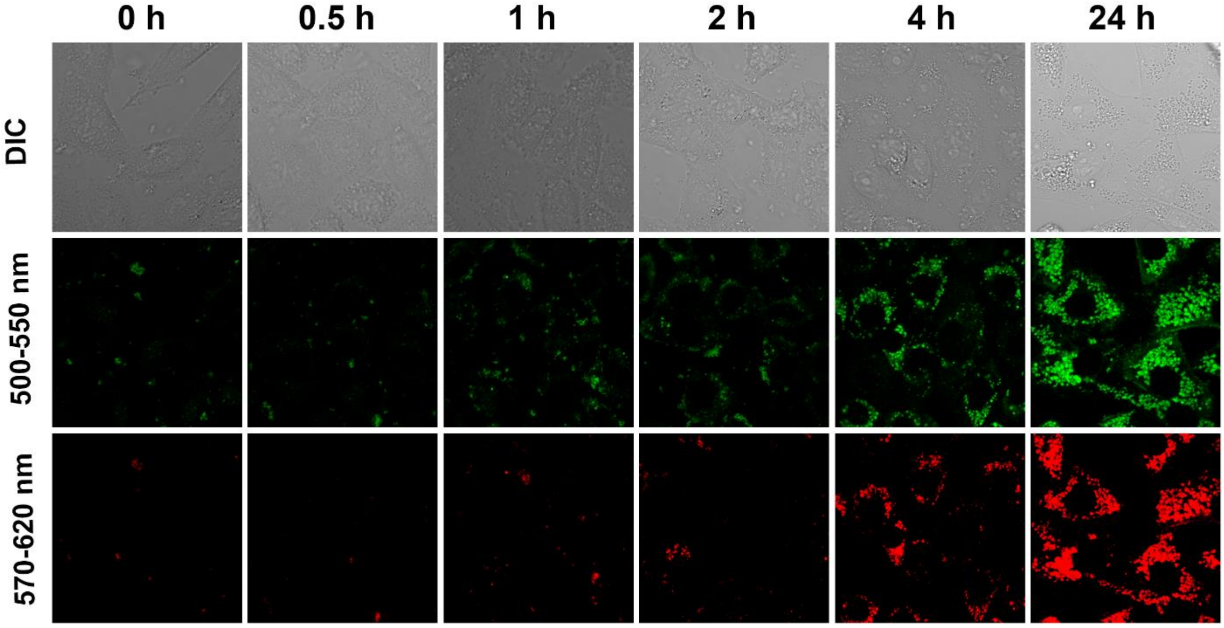
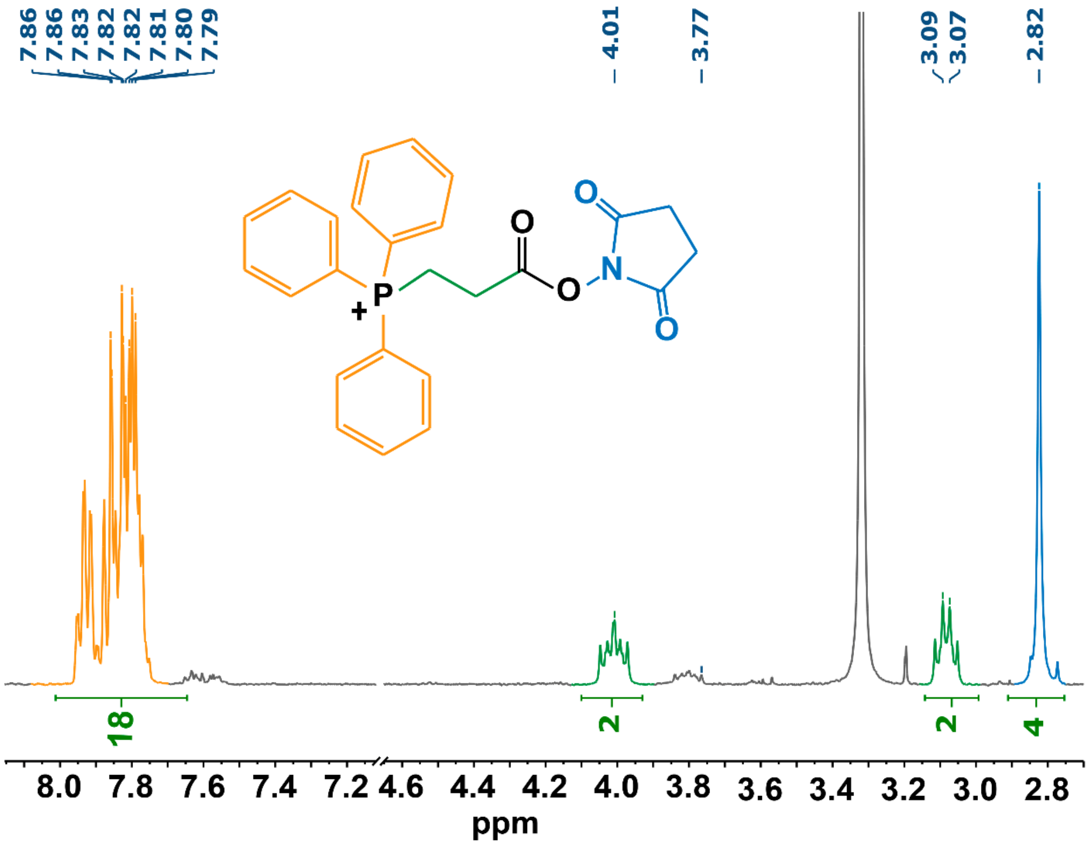
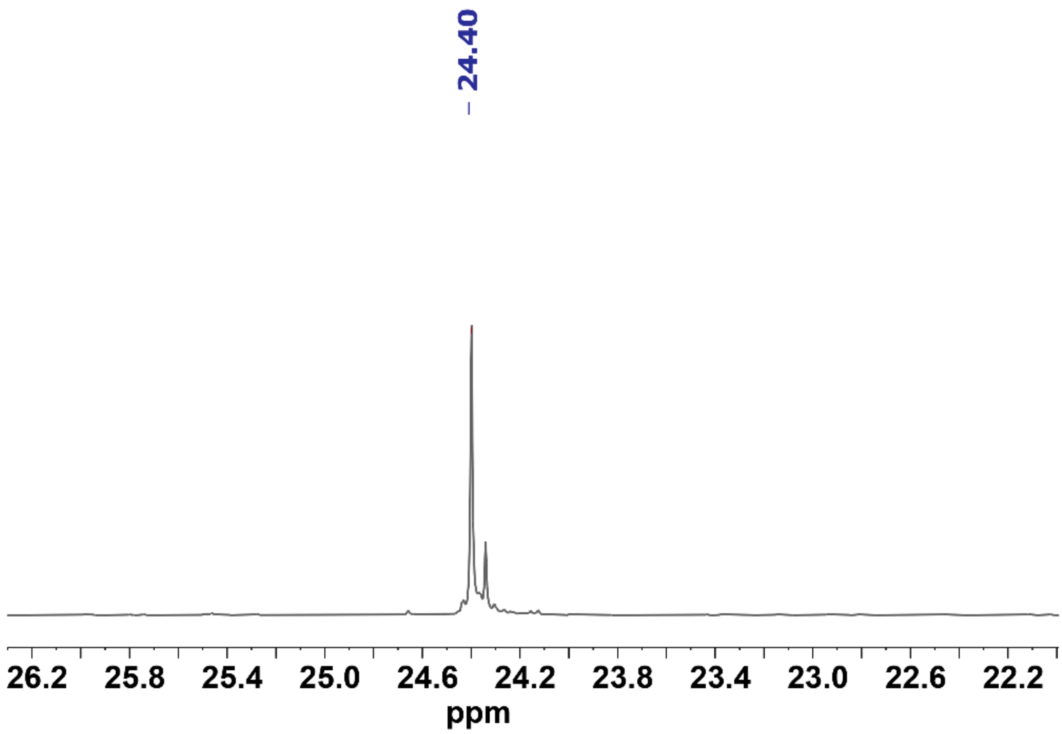


Figure S29. Dynamics of **NPs_Eu2** (0.0195 wt%,) accumulation by CHO-K1 cells during long-term incubation carried out in DMEM-F12 supplemented with 10% FBS. Confocal images: DIC image (top); green channel 500-550 nm (middle); red channel 570-620 nm (bottom). Scale bar 20 µm.



(A)



(B)

Figure S30. The NMR spectra of **TPP-NHS ester** (A) ^{1}H, (B) ^{31}P.

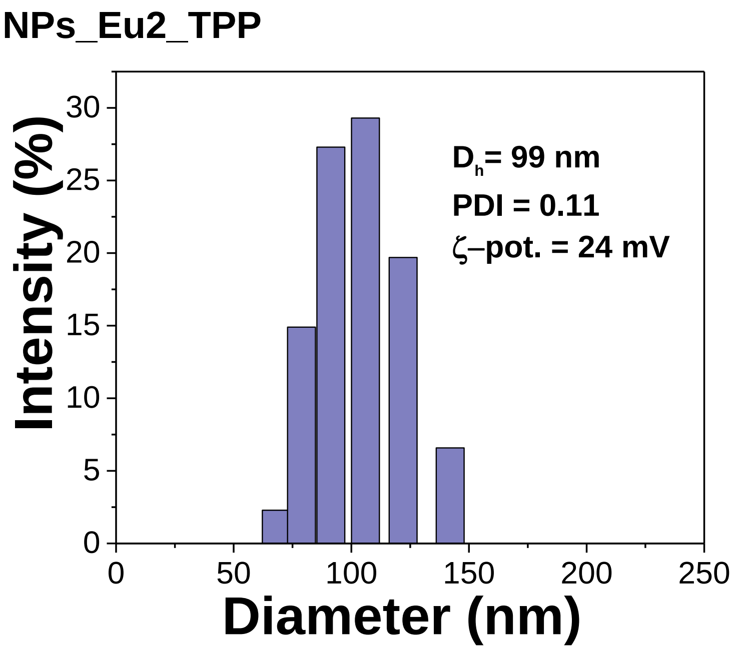


Figure S316. Main characteristics of **NPs_Eu2_TPP**. D_h – hydrodynamic diameter, PDI – polydispersity index, ζ-pot. - ζ-potential.

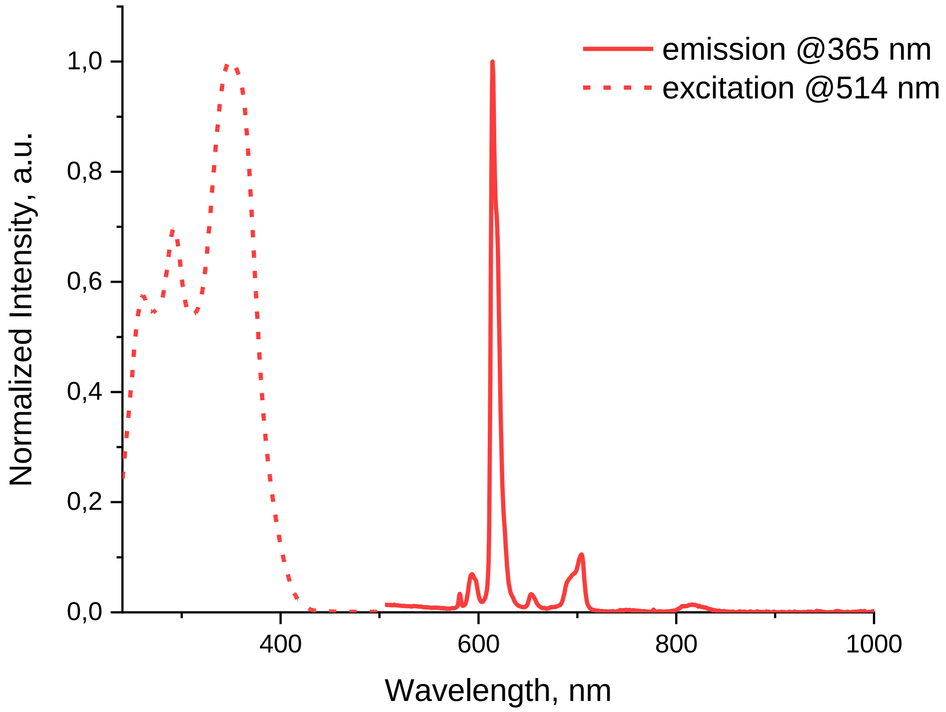
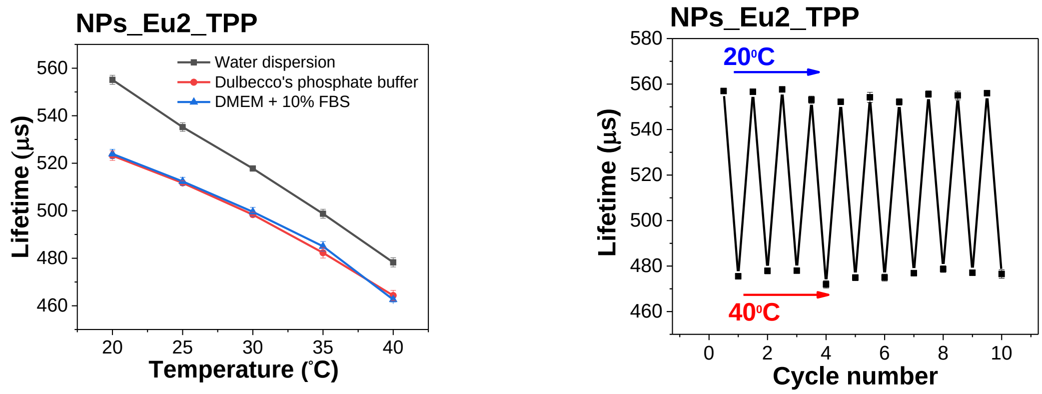
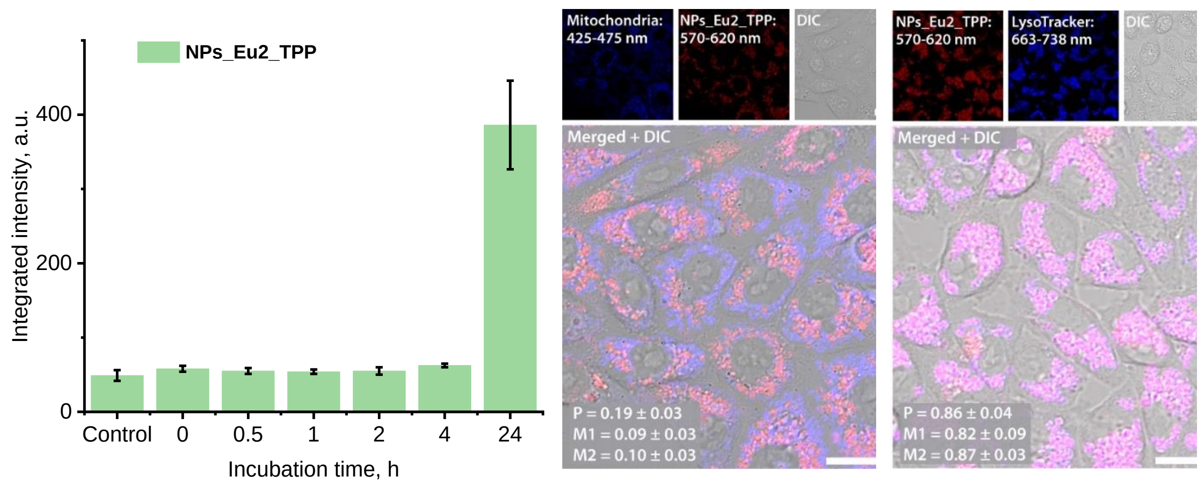


Figure S32. Excitation (dashed line) and emission (solid line) spectra of nanoparticles with **NPs_Eu2_TPP** in water dispersion, 293K.



(a) (b)

Figure S33. (a) Temperature sensitivity of NPs_Eu2_TPP lifetime in different media: water, Dulbecco's buffer and model physiological medium (DMEM+10%FBS). Interpolation of experimental data has been done using Equation 1; (b) Cyclic lifetime measurements for NPs_Eu2 between 20 and 40°C for NPs_Eu2 in water and DMEM + 10% FBS.



(a) (b) (c)

Figure S34. (a) The dependence of emission intracellular intensity vs incubation time of CHO-K1 with NPs_Eu2_TPP (b) Subcellular distribution of BioTracker 405 Blue Mitochondria Dye (blue color) and NPs_Eu2_TPP (red color) in CHO-K1 cells; (c) Subcellular distribution of LysoTracker Deep Red (blue color), and NPs_Eu2_TPP (red color) in CHO-K1 cells. Pearson's (P) and Manders' overlap coefficients (M1 – fraction of tracker signal that overlaps conjugate signal, M2 – fraction of conjugate signal that overlaps tracker signal) are presented as mean ± standard deviation calculated for 50 cells. Scale bar 20 µm.

Table S1. Temperature-dependent emission lifetimes of NPs_Eu2 and NPs_Eu2_TPP in media of various nature.

Temperature, ˚C	Lifetime, µs									
	NPs_Eu2							NPs_Eu2_TPP		
	Water	Dulbecco`s phosphate buffer (pH=7.4)	DMEM +10% FBS	Water/glycerin mixture, wt.%		Citrate-phosphate buffer		Water	Dulbecco`s phosphate buffer (pH=7.4)	DMEM +10% FBS
				90:10	50:50	pH=4.7	pH=7.2			
20	518	496	493	533	524	530	525	555	523	524
25	500	481	478	-	-	-	-	535	512	512
30	478	462	458	-	-	-	-	518	498	499
35	453	440	436	-	-	-	-	498	482	485
40	431	420	410	447	449	437	435	478	464	463

Table S2. A comparison of ***Polymer NPs-Eu2*** nanothermometer characteristics with those of published luminescent lifetime probes.

Probe	Measurment mode	Temperature range, °C	Excitation/Emission wavelengths, nm	Lifetime Range	Sensitivity, %/K	Reference
Ultra-Long-Lived Luminescent Nanocapsule loaded by $(PdPc(OBu)_8$ and PCU	Fluorescence Lifetime	20-40	375/444, 475, 508	0.3-1.52 s	7.5	[38]
HPS/Butter/ DSPE-PEG-Biotin Nanorod based on AIE material	Fluorescence Lifetime	20-60	375, 405sh/490	1-2.5 ns	6.3	[39]
Gold Nanoclusters (AuNCs)	Fluorescence Lifetime	10-50	360, 402sh/603	2.8-7.9 µs	2.8	[40]
ER thermo yellow	Fluorescence Lifetime	23-40	560/600	2.2-2.6 ns	1.04	[41]
YAG:Ce NPs	Fluorescence Lifetime	7-77	346/560	18.5-26.5 ns	0.2	[42]
C70 in PtBMA	Fluorescence Lifetime	20-90	470/700	17.5-27.0 ms	0.5	[43]
Eu-DT in BTD-PMMA	Phosphorescence Lifetime	25-45	400/616	145-350 µs	2.2	[44]
Gold Nanoclusters	Fluorescence Lifetime	15-45	580/710	460-600 ns	0.5	[45]
Fluorescent polymer	Fluorescence Lifetime	28-40	456/565	4.5-7.5 ns	0.6	[46]
First generation of luminescent polymer NPs with Eu complex	Phosphorescence Lifetime	25-45	335/614	509-580 µs	0.84	[31]
Polymer NPs-Eu2	Phosphorescence Lifetime	20-40	405/614	430-520 µs	1.3	This work

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