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ELECTRONIC SUPPLEMENTARY INFORMATION

Ytterbium 10-carboxyperylene-3,4,9-tricarboxylates for targeted NIR luminescent bioimaging

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1. Experimental details.

Materials and methods

All solvents and chemicals were purchased from commercial sources, were of reagent grade, and were used as commercially obtained without any further purification.

X-ray powder diffraction (XRD) measurements were performed on Bruker D8 Advance Vario diffractometers (λ (CuK α 1) = 1.54046 Å, Ge (111) monochromator, position-sensitive detector LynxEye, $\theta/2\theta$ geometry, with rotation). **Thermal analysis** was carried out on a thermoanalyzer STA 449 F1 Jupiter (NETZSCH, Germany) in the temperature range of 40–1000 °C in air, heating rate 10 °/min. The evolved gases were simultaneously monitored during the TA experiment using a coupled QMS 403 Aeolos quadrupole mass spectrometer (NETZSCH, Germany). The mass spectra were registered for the species with following m/z values: 18 (corresponding to H₂O), 28 (N₂), and 44 (CO₂). **Elemental analyses** (C, H, N) were performed on a Vario Micro Cube (Elementar, Germany). **The IR spectra** were recorded on an Thermo ScientificTM NicoletTM iS50 FTIR Spectrometer as powdered at ATR. **X-ray microanalysis** was carried out on a Leo Supra 50 VP scanning electron microscope equipped with an INCA Energy + Oxford 350X-Max 80 X-ray energy dispersion spectrometer. Measurements were made for each sample both at the point and in area. **Transmission Electron Microscopy** was performed on a JEOL JEM-2100 F/Cs/GIF/EDS transmission electron microscope.

Absorption spectra were recorded in the range 200-800 nm using Perkin-Elmer Lambda 650 spectrometers to determine the maximum absorption of the ligand, as well as to estimate the molar extinction coefficient of the ligand. **Emission spectra in NIR region** were measured using Hamamatsu R928P PMT as detector upon diode laser ($\lambda_{ex} = 447$ nm and $\lambda_{ex} = 365$ nm) as excitation source. **Emission spectra of K₃HPTC in** *vis* **region** were measured using FluoroMax-Plus fluorometer (HORIBA) with 1905-OFR 150-W Xenon Lamp as excitation source. **Photoluminescence quantum yield** were determined by absolute method using formula (1), when (i) L_a is the integrated intensity of Rayleigh scattering band (measurement at the excitation wavelength with empty cuvette in the sphere); (ii) L_c is the same integrated intensity at the excitation wavelength when the sample is introduced into the cuvette; (iii) E_a is the integrated intensity of the entire emission spectrum of empty cuvette; (iv) E_c is the integrated intensity of the entire emission spectrum of empty cuvette; (iv) E_c is the integrated intensity of the entire using Ocean Optics Maya 2000 upon diode laser ($\lambda_{ex} = 447$ nm) as excitation source.

$$PLQY = \frac{E_c - E_a}{L_a - L_c} \cdot 100\%$$
 (1)

2. Synthesis

2.1. Synthesis of K₃HPTC

Synthesis of $K_3HPTC \cdot xH_2O$ was carried out from the perylene-3,4,9,10-tetracarboxylic dianhydride (PTCDA) according to the methods, described in ^{1,2}. PTCDA was added to the stochiometric amount of KOH, dissolved in water (10 mmol/L) at 55 °C. After stirring the mixture for 5 min, seven times the amount of distilled water was gradually added, and the mixture was sequentially stirred for another 12 h. Then, the solution was added to 300 ml of EtOH, and the yellow precipitate of K₃HPTC was formed.

2.2. ¹H-NMR spectra

H₄PTC was obtained by dropping hydrochloric acid into an aqueous solution of K₃HPTC. The resulting yellow precipitate was washed with water and dried in air. Then, ¹H-NMR-spectra of both K₃HPTC in D₂O, as well as PTCDA and H₄PTC in DMSO-D6, were recorded.

In the spectra of all three compounds, we can see two doublets, each of which corresponds to 4 protons of the aromatic nucleus ($\delta \sim 7-9$); in addition, in the spectrum of the acid we see a signal corresponding to four protons of the carboxy-groups ($\delta \sim 13$ ppm). This corresponds to the expected composition of each of the compounds.

K₃HPTC:¹H NMR (400 MHz, D₂O) δ 8.36 (d, *J* = 7.9 Hz, 4H), 7.74 (d, *J* = 7.8 Hz, 4H). H₄PTC: ¹H NMR (400 MHz, DMSO-*d*₆) δ 13.03 (s, 4H), 8.62 (d, *J* = 8.1 Hz, 1H), 8.56 (d, *J* = 8.1 Hz, 4H), 7.99 (d, *J* = 7.9 Hz, 4H), 7.95 (d, *J* = 8.9 Hz, 1H), 7.24 (d, *J* = 8.9 Hz, 1H). PTCDA: ¹H NMR (400 MHz, DMSO-*d*₆) δ 7.96 (d, *J* = 8.5 Hz, 4H), 7.24 (d, *J* = 8.3 Hz, 4H).





Fig. S 1. ¹H-NMR spectra of a) K₃HPTC, b) H₄PTC, c) PTCDA

2.3. Absorption spectra

The absorption spectra of K₃HPTC, H₄PTC, PTDA (C = 10^{-5} M) at 200-1000 nm were recorded with Perkin–Elmer Lambda 650 spectrometer.



Fig. S2. Absorption data (a) and photo (b) for K₃HPTC solution in water (green), H₄PTC solution in DMSO (yellow), PTCDA solution in DMSO (red)

Ligand has absorbance in bluish-green range for about 370-500 nm with maxima at 440 and 465 nm with $\varepsilon \sim 23\ 000\ M^{-1}\ cm^{-1}$. Excitation in safer for cells green range was $\sim 500\ M^{-1}\ cm^{-1}$, which is significantly higher than typical values for inorganic materials.

2.4. Synthesis of (Yb_xGd_{1-x})(HPTC)(H₂O)₂

The CCs $(Yb_xGd_{1-x})(HPTC)(H_2O)_2$ (x=0.1...1 with a step of 0.1) were obtained by the exchange reaction between aqueous solutions of potassium 10-carboxyperylene-3,4,9-tricarboxylate (K₃HPTC·xH₂O, *in situ* from KOH and PTCDA) and a mixture of lanthanide chlorides (GdCl₃·6H₂O, YbCl₃·6H₂O), taken in a stoichiometric ratio.

$$x \text{ YbCl}_3 \cdot 6H_2O + (1-x) \text{ GdCl}_3 \cdot 6H_2O + K_3\text{HPTC} \rightarrow (\text{Yb}_x\text{Gd}_{1-x})(\text{HPTC})(H_2O)_2 \downarrow + 3\text{KCl} + 4H_2O$$

After mixing the solutions, a red powder immediately precipitated. The resulting suspension was stirred for 20 minutes on a heated magnetic stirrer at 95°C, then the precipitate was separated by centrifugation, washed with distilled water, and dried in air.

Yb(HPTC)(H₂O)₂: Elemental analysis (%), calcd. for Yb(C₂₄H₉O₈)(H₂O)₂ (635.3): C, 45.37; H, 2.05. Found: C, 45.02; H, 2.57.

2.5. Luminescence titration

To confirm the Yb:HPTC ratio in the complex, luminescent titration was carried out. The green luminescence of K_3 HPTC was the analytical signal, since in this case, changes during titration occur in solution, and it is possible to neutralize the influence of the insoluble product on the scattering of the exciting radiation and analytical signal by waiting for its precipitation. HPTC³⁻ luminescence was quenched upon the formation of a coordination compound due to the energy transfer to the lanthanide ion.

2 ml of YbCl₃ water solution (10⁻⁴ M) was titrated by $2 \cdot 10^{-2}$ M water solution of K₃HPTC, and green luminescence of K₃HPTC was measured upon 280 nm excitation (**Fig. 1 a**). According to titration curve, where luminescence intensity was plotted against the HPTC:Yb ratio (**Fig. 1 b**), the equivalence point corresponds to the ratio Yb:HPTC=1:1.

In a similar manner, titration was carried out using ytterbium luminescence upon excitation at 365 nm. Ytterbium chloride practically does not absorb ultraviolet radiation, however, when a ligand solution is added, a complex is formed, as a result of which we can observe the characteristic luminescence of ytterbium. Such a measurement gives a significantly larger error during titration, since we detect the luminescence of a precipitate that deposited unevenly, as a result of which at each moment of measurement we receive a signal from a different amount of emitter. However, the general appearance of the titration curve is in good agreement with the data obtained for the

luminescence of the ligand. When the ligand is added to the Yb:HPTC=1:1 ratio, the ytterbium luminescence intensity increases. After reaching an equimolar ratio of Yb^{3+} and ligand in the mixture, the luminescence intensity decreases slightly, which is due to the quenching of the luminescence of the complex by the free ligand, which is present in excess in the mixture. Thus, titration by ytterbium luminescence also shows a metal-ligand ratio of 1:1.



Fig. S3. Luminescence titration of YbCl₃ by K₃HPTC: a) spectra upon 365 nm excitation, b) titration curve.

2.6. EDX data

The experimental ratios of metals in $(Yb_xGd_{1-x})(HPTC)(H_2O)_2$ were found by EDX (**Fig. S4**) and they were identical to theoretically assumed (

Table S1). Bimetallic CCs were analyzed to confirm the theoretical ratio of ytterbium and gadolinium fractions. The actual ratio of metals was similar to the estimated.



Fig. S4. EDX data for (Yb_xGd_{1-x})(HPTC)(H₂O)₂.

Sample	Yb fraction, %	Gd fraction, %	Yb fraction, %	Gd fraction, %
	Theoretical		Experimental	
(Yb _{0.9} Gd _{0.1})(HPTC)(H ₂ O) ₂	90	10	90±2	10±2
(Yb _{0.8} Gd _{0.2})(HPTC)(H ₂ O) ₂	80	20	81±2	19±2
(Yb _{0.7} Gd _{0.3})(HPTC)(H ₂ O) ₂	70	30	74±2	26±2
(Yb _{0.6} Gd _{0.4})(HPTC)(H ₂ O) ₂	60	40	63±2	37±2
(Yb _{0.5} Gd _{0.5})(HPTC)(H ₂ O) ₂	50	50	50±2	50±2
(Yb _{0.4} Gd _{0.6})(HPTC)(H ₂ O) ₂	40	60	40±2	60±2
(Yb _{0.3} Gd _{0.7})(HPTC)(H ₂ O) ₂	30	70	31±2	69±2
(Yb _{0.2} Gd _{0.8})(HPTC)(H ₂ O) ₂	20	80	20±2	80±2
(Yb _{0.1} Gd _{0.9})(HPTC)(H ₂ O) ₂	10	90	9±2	91±2

Table S1. EDX data of (Yb_xGd_{1-x})(HPTC)(H₂O)₂ samples

2.7. Synthesis of Yb(HPTC)(Phen)

 $YbCl_3 \cdot 6H_2O + K_3HPTC + Phen \rightarrow Yb(HPTC)(Phen) \downarrow + 3KCl + 6H_2O$

To obtain Yb(HPTC)(Phen), a solution of potassium K₃HPTC was added to solution ytterbium chloride (YbCl₃·6H₂O) and phenanthroline (Phen·H₂O), mixed in the stoichiometric ratio. An orange precipitate was immediately formed. The reaction mixture was stirred for 20 minutes on a heated magnetic stirrer at 95 °C, then the precipitate was separated by centrifugation, washed with distilled water, and dried in air.

Yb(HPTC)(Phen): Elemental analysis (%), calcd. for Yb₄(C₂₄H₉O₈)(C₁₂H₈N₂) (779.4): C, 55.48; N, 3.59; H, 2.18. Found: C, 54.97; N, 3.86; H, 2.48.

2.8. Measurement of solubility

For both coordination compounds, solubility in several organic solvents was measured: H₂O, C₂H₅OH, DMSO, THF, CH₂Cl₂, CH₃CN. The procedure for measuring solubility was as follows: a suspension of 100 mg of each compound in 10 mL of solvent was refluxed for 1 h and cooled to room temperature, then 5 mL of the solution the solution was poured onto the pre-weighed watch glass. After evaporation of solvent, the glass was weighed again and the change of the mass corresponded to the amount of the dissolved product. However, for both compounds in all solvents studied, no change in glass mass was measured.

2.9. Obtaining of suspensions Alg@Yb(HPTC)(H2O)2, Alg@Yb(HPTC)(Phen)

Freshly synthesized CC powder (from 2.7 mg of YbCl₃·6H₂O and 3.5 mg of K₃HPTC for Yb(HPTC)(H₂O)₂; from 2.1 mg of YbCl₃·6H₂O, 1 mg of Phen and 2.7 mg of K₃HPTC for Yb(HPTC)(Phen)) was added with stirring and heating at 60°C to 2 ml aqueous sodium alginate solution ([NaAlg]:[CC]=2:1; 7 mmol/L for Yb(HPTC)(H₂O)₂; 5.5 mmol/L for Yb(HPTC)(Phen)). After several minutes of stirring, the suspension was placed in the ultrasonic bath for 5 minutes, and then several cycles of heating at 60°C and keeping in an ultrasonic bath at 40°C for 5 minutes were carried out to stabilize it.

 $Yb(HPTC)(H_2O)_2 \xrightarrow{NaAlg} Alg@Yb(HPTC)(H_2O)_2$ $Yb(HPTC)(Phen) \xrightarrow{NaAlg} Alg@Yb(HPTC)(Phen)$





Fig. S5. XRD data for 1) Yb(HPTC)(H₂O)₂, 2) Yb(HPTC)(Phen), 3) K₃HPTC.

4. MALDI MS data

Powders were analyzed by MALDI MS to confirm the composition of obtained CCs. The set of signals with m/z=301, corresponded to the isotopic distribution of Yb³⁺ with HPTC⁻, was found in both Yb(HPTC)(H₂O)₂ (**Fig. S6**) and Yb(HPTC)(Phen) (**Fig. S7**) spectra. Another sets of signals with m/z=354 and 389 (**Fig. S7 c,d**) in Yb(HPTC)(Phen) spectrum corresponded to Yb(Phen)²⁺ and Yb(HPTC)(Phen)²⁺ fragments, which confirms ligand coordination and supplement FTIR data. Other recorded signals corresponded to the organic residues of the ligand and adducts of

compounds and matrix, which was clear from their isotopic distribution. Thus, the MALDI MS analysis made it possible to state that the selected ligands were coordinated by ytterbium, and confirmed the formation of the CCs with composition Yb(HPTC)(H₂O)₂ and Yb(HPTC)(Phen).



Fig. S6 MALDI MS data: a) full spectrum of Yb(HPTC)(H₂O)₂, b) Yb³⁺ with ligand signals.



Fig. S7 MALDI MS data: a) full spectrum of Yb(HPTC)(Phen), b-c) Yb³⁺ with HPTC and Phen signals.

5. TGA data

The first step on $(Yb_xGd_{1-x})(HPTC)(H_2O)_2$ TG curve in the range of 150-200 °C coincided to 18 m/z ionic currents and was counted as splitting off 2 H₂O per Ln atom (**Fig. S8**). This step was

absent in TG curve of Yb(HPTC)(Phen), which confirmed lack of water in CC (**Fig. S9**). The second huge stepwise at 400-500 °C compared to ionic currents with m/z = 18 and 44 was obviously the stage of decomposition of an organic ligand and presented in curve of each CCs. With the obtained TGA data and the assumption that the dry residue is Yb₂O₃ gross formula was calculated for CCs as (Yb_xGd_{1-x})(HPTC)(H₂O)₂ and Yb(HPTC)(Phen), respectively.



Fig. S8. TGA data for a) Yb(HPTC)(H₂O)₂, b) (Yb_{0.7} Gd_{0.3})(HPTC)(H₂O)₂



Fig. S9. TGA data for Yb(HPTC)(Phen)

6. FTIR spectra

The FTIR spectra of the CCs demonstrate that all the ligands were coordinated to the metal (Fig. **S10**). A clear formation of coordination compounds could be observed by the broadening of the bands in the spectra of CCs compared to the spectra of ligands, this is especially noticeable by the shift of bands at 1415 cm⁻¹, 1550 cm⁻¹, corresponding to vibrations of the COO⁻ group [1]. There is a slight change in the position of the bands of the carboxyl group between ligand and CCs spectra, which indicates monodentate coordination. At 1420 cm⁻¹ C-N band of phenanthroline overlaps with the bands in K₃HPTC spectrum, so it is difficult to find the presence of these bonds existing in Yb(HPTC)(Phen). However, the formation of Yb(HPTC)(Phen) can be proved both by the disappearance of the water band at 3600-2500 cm⁻¹ in its spectrum, and by the large shift of the v(COO⁻) band 1415 cm⁻¹ comparing to Yb(HPTC)(H₂O)₂. In addition, in the fingerprint region, there was a strong absorption at 736 cm⁻¹ for phenanthroline and Yb(HPTC)(Phen), which was absent in the other two spectra. So, this fact evident coordination of both Phen and HPTC³⁻ in the obtained substances. Remarkable the disappearance of the characteristic wide band of water v(H₂O) at 3600-2500 cm⁻¹ in Yb(HPTC)(Phen) spectrum, presenting in spectra of $Yb(HPTC)(H_2O)_2$, indicated the complete replacement of water by phenanthroline in the ytterbium coordination sphere. Thus, the goal of disposing of water in CCs with the introduction of phenanthroline was achieved.



Fig. S10. FTIR spectra of CCs and ligands: 1) K₃HPTC, 2) Phen·H₂O, 3) Yb(HPTC)(H₂O)₂, 4) Yb(HPTC)(Phen)

7. Luminescence properties

7.1. Luminescence of Yb(HPTC)(H₂O)₂

Luminescence spectra in the NIR range were recorded using 447 nm diode laser as an excitation source. Obtained spectrum of Yb(HPTC)(H₂O)₂ confirmed the characteristic luminescence of Yb³⁺ appearing at 980 nm and corresponding to its ${}^{2}F_{5/2}$ - ${}^{2}F_{7/2}$ transition.



Fig. S11. Luminescence spectrum of Yb(HPTC)(H₂O)₂ powder ($\lambda_{ex} = 447$ nm).

7.2. Study of concentration quenching in CCs

Due to the low position of the resonant level of ytterbium, its compounds suffer from different types of quenching, including concentration quenching [2–4]. Therefore, it was assumed that for complexes with HPTC³⁻ ion it also leads to a decrease in the luminescence intensity, and the change in luminescent properties with a decrease in the concentration of Yb³⁺ in the powder was studied. Since ytterbium and gadolinium ions have close radii due to lanthanide compression, compounds of composition (Yb_xGd_{1-x})(HPTC)(H₂O)₂ form a continuous series of solid solutions. It turned out that a large volume of the organic ligand provides a sufficient Yb-Yb distance for this effect to be negligible. Therefore, only monometallic CCs were used to obtain functional materials.

Indeed, a comparison of the integral intensities showed that maximum value was reached for the compound $(Yb_{0.7}Gd_{0.3})(HPTC)(H_2O)_2$. Although the luminescence intensity was not powerful enough for biometric applications, so we continued to explore ways to increase the intensity.



Fig. S12. Luminescence intensity dependence of $(Yb_xGd_{1-x})(HPTC)(H_2O)_2$ on Yb^{3+} fraction





Fig. S13. Luminescence spectra of $(Yb_{0.7}Gd_{0.3})(HPTC)(H_2O)_2$ (blue) and $Yb(HPTC)(H_2O)_2$ (black) powders ($\lambda_{ex} = 447$ nm).

8. Photostability of complexes

To study the photostability of the obtained compounds, the complexes were kept under constant exposure to ultraviolet radiation (365 nm) with a specific power of 200 mW/cm² (298 K). After 6 hours of exposure, the intensity of both Yb(HPTC)(H₂O)₂ and Yb(HPTC)(Phen) decreased by 16%. At the same time, after 17 hours of rest, the intensity slightly increases.



Fig. S14. Photostability studies for $Yb(HPTC)(H_2O)_2$ (a-c) and Yb(HPTC)(Phen) (d-f): luminescence spectra and changes in intensity when kept powders under 365 nm excitation

9. Suspensions in Na(Alg)

9.1. Stability



Fig. S15. ζ-potential of particles in suspensions of Alg@Yb(HPTC)(H₂O)₂ (blue) and Alg@Yb(HPTC)(Phen) (red).

9.2. Size of particles



Fig. S 16. Size distribution of Alg@Yb1 (blue) and Alg@Yb2 (red) a) intensity, b) volume

9.3. TEM microphotographs

Aqueous suspensions of complexes with a concentration of $200 \ \mu g/ml$ were placed in the ultrasonic bath for 10 minutes, and then deposited upon 300 mesh copper nets. After 24 hours air drying, samples were examined using a transmission electron microscope.



Fig. S17. TEM micrograph of $Yb(HPTC)(H_2O)_2$ (a), Yb(HPTC)(Phen) (b), $Alg@Yb(HPTC)(H_2O)_2$ (c), Alg@Yb(HPTC)(Phen) (d)

10. Cell study

10.1. In vitro test

Breast adenocarcinoma (MCF-7) and human embryonic kidney (293T) cells (ATCC® Cat. No. HTB-22TM) were cultivated in complete growth DMEM or MEM medium respectively (Gibco, UK) supplemented with 10% of FBS (Gibco, UK), 4.5 g/L glucose, 100 U/ml penicillin, 100 μ g/ml streptomycin and 0.25 μ g/ml Gibco amphotericin at 37 °C, 5 % CO₂.

10.2. Cytotoxicity test

MCF-7 or 293T cells were seeded $3x10^4$ cells/well in a 96-well plate in 200 µl/well complete growth medium and incubated at 37 °C with 5 % CO₂ for 48 h. Then the medium was replaced with 100 µl/well complete DMEM or MEM medium in the absence (control) or presence of various amounts of the tested compounds and incubated at 37 °C with 5 % CO₂. After 24 h 10 µl/well WST-1 solution (CELLPRO-RO Roche, Switzerland) was added to each well and incubated in culture conditions for 2 h. The absorbance of the samples was measured at 450 nm. Data are presented as Mean ± StD for 3 independent experiments.

10.3. Cellular accumulation

MCF-7 or 293T cells were harvested in complete growth medium in T-25 flask at 37 °C with 5 % CO_2 up to 80 % of cell confluency before the treatment with tested compounds. After cell incubation with 20 µ/ml samples for 12 h at 37 °C with 5 % CO_2 cells were washed with DPBS (Gibco, UK), detached from the flask with Tryple (Gibco, UK), centrifugated (5 min, 125×g) and resuspended in PBS (10 mM, pH 7.4). Cells concentration was measured with Countess automated cell counter (Invitrogen, Korea). An aliquot of cell suspension was treated with 63 % nitric acid at 60 °C overnight, centrifugated (20 min, 125×g) and Yb concentration was measured in supernatant by ICP-MS. Cells treated by sodium alginate were set as a control. Data are presented as Mean ± StD for 3 independent experiments.



Fig. S18. NIR luminescence spectra of MCF-7 (black) and 293T cells (red) after 24 hours incubation with Alg@Yb1 ($\lambda_{ex} = 447 \text{ nm}$)

11. Literature

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