

Monitoring the Nanoparticle Dissolution via Fluorescence-Colour Shift

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– SUPPORTING INFORMATION – (SI)

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1. Analytical Techniques

Dynamic light scattering (DLS) measurements of the IOH-NPs were performed in polystyrene cuvettes applying a Nanosizer ZS (Malvern Instruments, United Kingdom).

Zeta potential measurements of the IOH-NPs were conducted using an automatic titrator MPT-2 attached to a Nanosizer ZS. Titrations were performed by addition of 0.1 M HCl or 0.1 M NaOH.

Scanning electron microscopy (SEM) was conducted with a Zeiss Supra 40 VP (Zeiss, Germany), equipped with a field-emission gun and a resolution of 1.3 nm (at 15 kV). Due to the organics content, the IOH-NPs are highly sensitive to the electron beam. To minimize the sample decomposition, examinations were performed at 1 to 5 kV. Samples were prepared by placing small droplets of diluted aqueous suspensions on a silica wafer.

Energy-dispersive X-ray spectroscopy (EDXS) was performed with an Ametek EDAX device (Ametek, USA), mounted on the above described Zeiss SEM Supra 40 VP. For this purpose, powder samples of the dried IOH-NPs were fixed by conductive carbon pads on aluminium sample holders.

X-ray powder diffraction (XRD) was conducted on a Stadi-P diffractometer (Stoe, Germany) with Ge-monochromatized Cu-K_α radiation. Dried IOH-NP samples were fixed between Scotch tape and acetate paper.

Fourier-transformed infrared (FT-IR) spectra were recorded on a Bruker Vertex 70 FT-IR spectrometer (Bruker, Germany) in the range of 4000-450 cm⁻¹. To this concern, 1 mg of the dried sample was mortared with 300 mg of dried KBr and pressed to a pellet that was analyzed in transmission.

Thermogravimetry (TG) was performed with a STA409C device (Netzsch, Germany). All measurements were performed in air. The IOH-NP samples were pre-dried (70 °C, 8 h) prior to TG analysis and thereafter heated to 1200 °C with a heating rate of 5 K min⁻¹ (20 mg, corundum crucibles).

Elemental analysis (EA) (C/H/N/S analysis) was performed via thermal combustion with an Elementar Vario Microcube device (Elementar, Germany) at a temperature of about 1100 °C.

Photoluminescence (PL) was recorded with a Horiba Jobin Yvon Spex Fluorolog 3 (Horiba Jobin Yvon, France) equipped with a 450 W Xe-lamp and double grating excitation and emission monochromators.

2. IOH-NPs uniting PTC and ICG

In comparison to suspensions containing mixtures of $[\text{La}(\text{OH})]^{2+}[\text{ICG}]^{-2}$ and $[\text{La}(\text{OH})]^{2+}_2[\text{PTC}]^{4-}$ IOH-NPs, $[\text{La}(\text{OH})]^{2+}_9[(\text{PTC})^{4-}_2(\text{ICG})^{-}]_2$ IOH-NPs uniting both fluorescent dyes with an PTC : ICG ratio of 2 : 1 show two disadvantageous effects. First of all, these IOH-NPs show emission of ICG upon excitation of PTC (Figure S1a). Moreover and even more important, combining ICG and PTC in a single IOH-NP disturbs the π -stacking of PTC even at low ICG concentrations, so that the PTC emission is not quenched in the solid IOH-NPs. As a result, suspension and solution show intense green emission (Figure S1b). All in all, this points to the feasibility of the concept of mixing two different types of IOH-NPs to monitor particle dissolution via a shift of the emission.

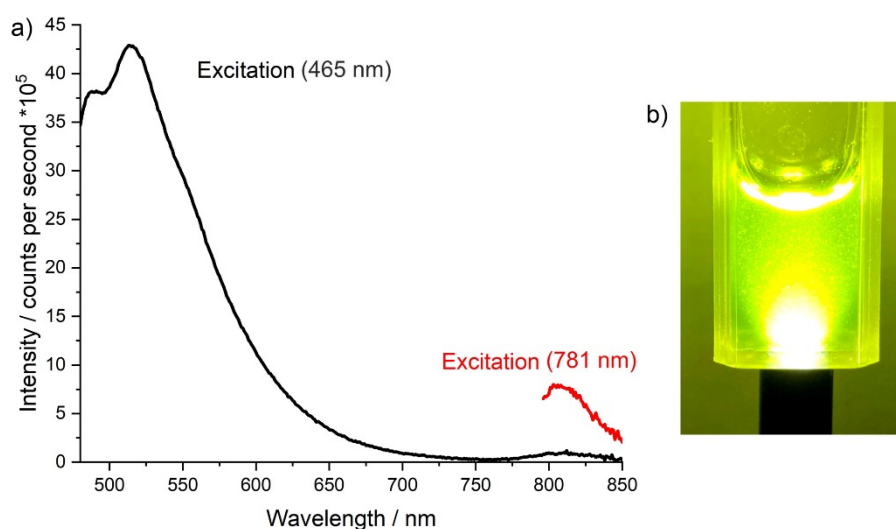


Figure S1. Emission of $[\text{La}(\text{OH})]^{2+}_9[(\text{PTC})^{4-}_2(\text{ICG})^{-}]_2$ IOH-NPs uniting both fluorescent dyes (PTC : ICG ratio of 2 : 1): a) emission spectra of aqueous suspension (spectra recorded with $\lambda_{exc}(\text{PTC})$: 465 nm, $\lambda_{exc}(\text{ICG})$: 781 nm); b) photo of aqueous suspension (with blue-light-emitting LED for excitation).

3. Material Characterization $[\text{La}(\text{OH})]^{2+}[\text{ICG}]^{-2}$

The composition of the $[\text{La}(\text{OH})]^{2+}[\text{ICG}]^{-2}$ IOH-NPs was examined by X-ray powder diffraction analysis (XRD), Fourier-transformed infrared (FT-IR) spectroscopy, thermogravimetry (TG) and elemental analysis (EA). XRD of the as-prepared $[\text{La}(\text{OH})]^{2+}[\text{ICG}]^{-2}$ IOH-NPs do not show any Bragg peak indicating their non-crystalline (Figure S2).

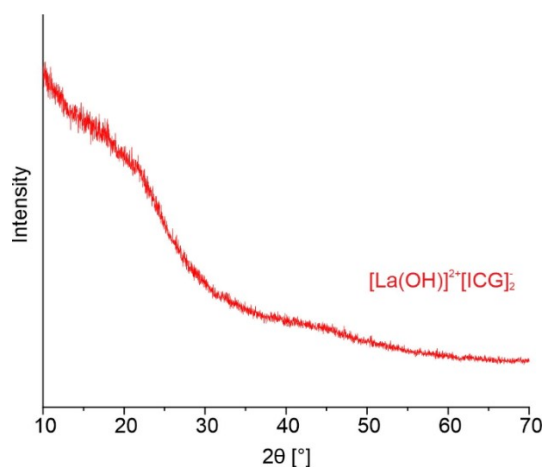


Figure S2. XRD of the as-prepared $[\text{La}(\text{OH})]^{2+}[\text{ICG}]^{-2}$ IOH-NPs.

The presence of ICG was proven by FT-IR spectra (Figure S3). Thus, spectra of the $[\text{La}(\text{OH})]^{2+}[\text{ICG}]^{-2}$ IOH-NPs fit well with the spectrum of Na(ICG) as the starting material. All characteristic vibrations of ICG are visible (especially including $\nu(\text{S}=\text{O})$: 1422 cm^{-1} , $\nu(\text{S}-\text{O})$: 1090 cm^{-1} , fingerprint area: $1050\text{-}500\text{ cm}^{-1}$). A broad vibration at $3600\text{-}3000\text{ cm}^{-1}$ indicates the presence of water.

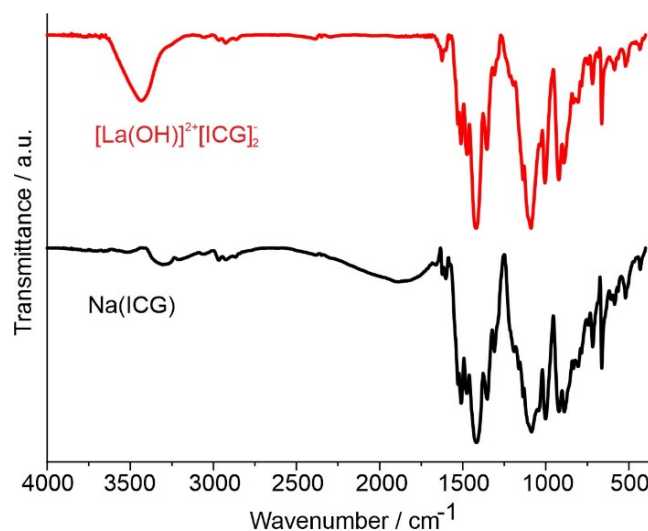


Figure S3. FT-IR spectra of the $[\text{La}(\text{OH})]^{2+}[\text{ICG}]^{-2}$ IOH-NPs (with Na(ICG) as a reference).

EA revealed the composition with C/H/N/S amounts of 61.1 wt-% C, 5.3 wt-% H, 3.4 wt-% N, and 7.3 wt-% S (calculated: 62.2 wt-% C, 5.4 wt-% H, 3.2 wt-% N, 7.7 wt-% S).

Furthermore, TG shows two-step decomposition with a first weight loss (50-200 °C, 9.0 wt-%), which can be related to the release of adsorbed water (Figure S4a). A second weight loss (200-800 °C, 82.1 wt-% or 90.4 wt-% if the total weight was corrected for the amount of adsorbed water) can be related to the decomposition of ICG and fits well with the calculated value (89.6 wt-%). Finally, La₂O₃ and (LaO)₂SO₄ were identified as thermal residues via XRD (Figure S4b). Hence, the thermal decomposition can be ascribed to the following reaction:

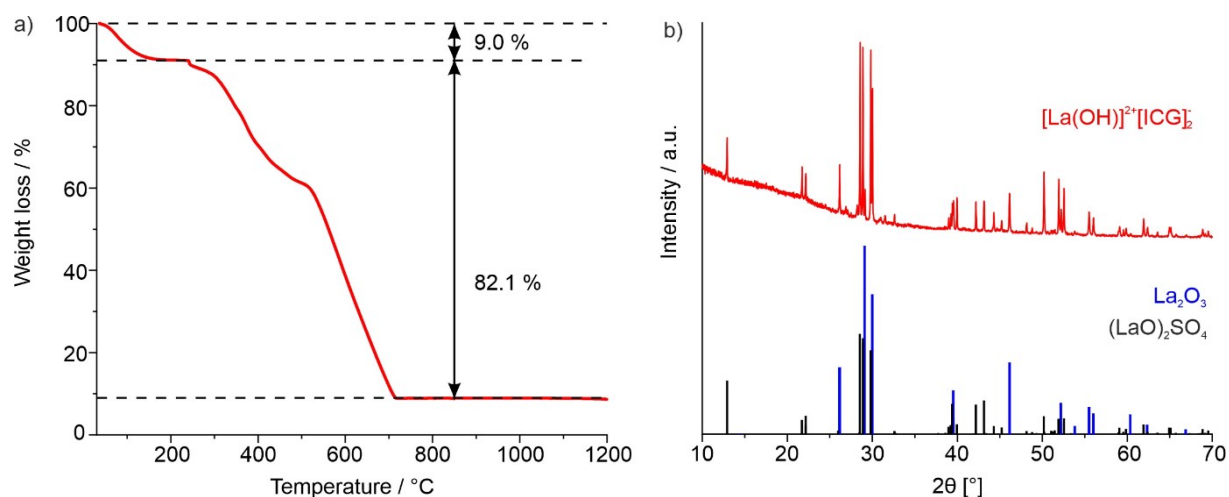
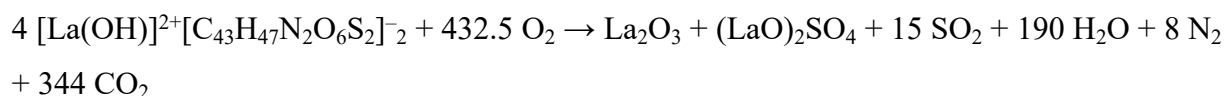


Figure S4. Total organic combustion of the $[\text{La}(\text{OH})]^{2+}[\text{ICG}]^{-}_2$ IOH-NPs: (a) TG analysis, (b) XRD of the thermal remnant after the TG analysis (La_2O_3 /ICDD-No. 01-071-5408, $(\text{LaO})_2\text{SO}_4$ /ICDD-No. 01-085-1535 as references).

The photoluminescence properties of the $[\text{La}(\text{OH})]^{2+}[\text{ICG}]^{-}$ IOH-NPs show the expected features of ICG with a strong visible absorption at 600-800 nm and deep-red emission at 800-850 nm (Figure S5; see main paper: Figure 5a).

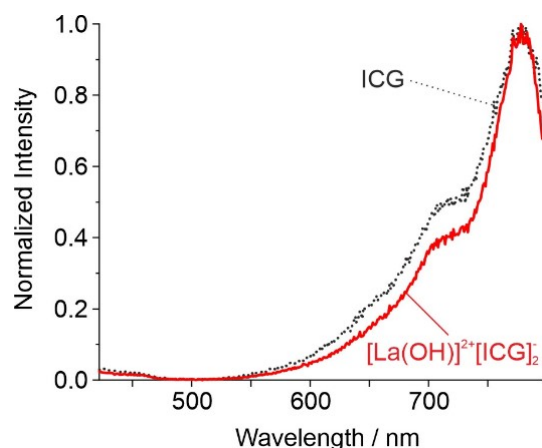


Figure S5. Excitation spectrum of the $[\text{La}(\text{OH})]^{2+}[\text{ICG}]_2$ IOH-NPs (aqueous suspension) with aqueous solution of Na(ICG) as a reference.

4. Material Characterization $[\text{La}(\text{OH})]^{2+}_2[\text{PTC}]^{4-}$

If $[\text{La}(\text{OH})]^{2+}_2[\text{PTC}]^{4-}$ was prepared without any additional measure to control the particle nucleation, micron-sized rods with a length of 5-10 μm were obtained due to π -stacking of the PTC molecules (Figure S6). Therefore, $[\text{La}(\text{OH})]^{2+}_2[\text{PTC}]^{4-}$ was prepared either at elevated temperature (i.e. injection at 95 $^\circ\text{C}$) (see main paper: Figure 4) or coated with a LaPO_4 shell to form $[\text{La}(\text{OH})]^{2+}_2[\text{PTC}]^{4-}@\text{LaPO}_4$ core-shell IOH-NPs (see Figure S11). With these measures, the particles are still rod-shaped but with a length below 100 nm.

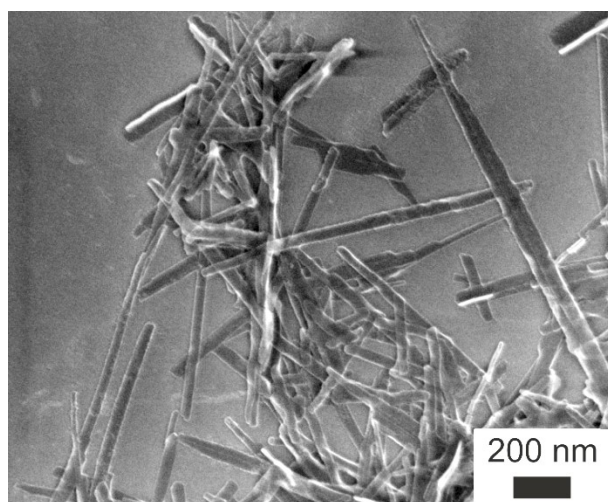


Figure S6. XRD of the as-prepared $[\text{La}(\text{OH})]^{2+}_2[\text{PTC}]^{4-}$ IOH-NPs (with PTCDA as a reference).

Similar to the $[\text{La}(\text{OH})]^{2+}[\text{ICG}]_2$ IOH-NPs, the composition of the $[\text{La}(\text{OH})]^{2+}_2[\text{PTC}]^{4-}$ IOH-NPs was examined by XRD, FT-IR spectroscopy, TG and EA. Here, XRD of the as-

prepared $[\text{La}(\text{OH})]^{2+}_2[\text{PTC}]^{4-}$ IOH-NPs shows at least weak Bragg peaks, which can be ascribed to the π -stacking of the perylene molecules (Figure S7).

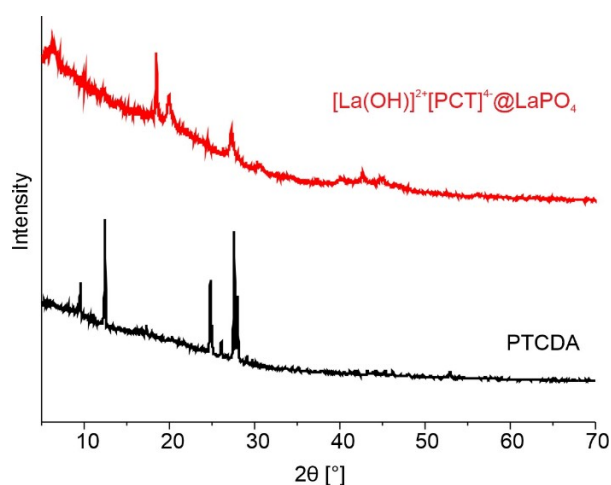


Figure S7. XRD of the as-prepared $[\text{La}(\text{OH})]^{2+}_2[\text{PTC}]^{4-}$ IOH-NPs (with PTCDA as a reference).

The presence of PTC in the $[\text{La}(\text{OH})]^{2+}_2[\text{PTC}]^{4-}$ IOH-NPs was proven by FT-IR spectroscopy and compared to spectra of $\text{Na}_4(\text{PTC})$ as the starting material (Figure S8). All characteristic vibrations of PTC are visible (especially including $\nu(\text{C}=\text{O})$: 1800-1600, $\nu(\text{C}-\text{O})$: 1600-1200, fingerprint area: 1050-500 cm^{-1}). A broad vibration at 3600-3000 cm^{-1} again indicates the presence of water.

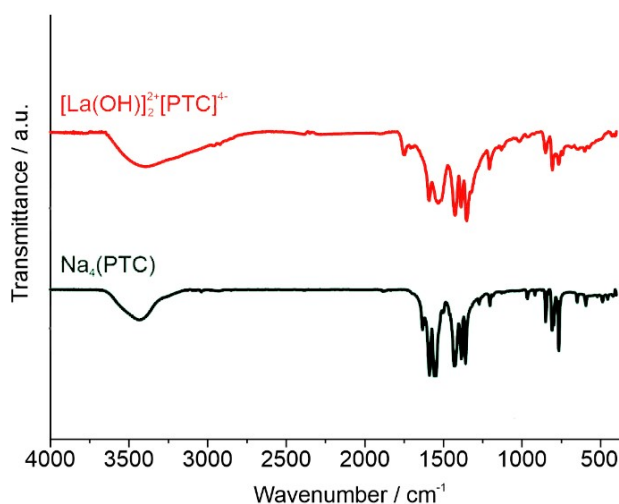


Figure S8. FT-IR spectra of the $[\text{La}(\text{OH})]^{2+}_2[\text{PTC}]^{4-}$ IOH-NPs (with PTCDA as a reference).

Elemental analysis revealed the composition with C/H/N amounts of 38.9 wt-% C and 1.8 wt-% H (calculated: 39.1 wt-% C, 1.4 wt-% H).

Furthermore, TG shows three-step decomposition with a first weight loss (50-200 °C, 9.6 wt-%), which can be related to the release of adsorbed water (Figure S9a). A second weight loss (200-600 °C, 52.2 wt-%) as well as a third weight loss (600-800 °C, 3.8 wt-%) can be related to the decomposition of PTC and together (56.0 wt-% or 62.0 wt-% if the total weight was corrected for the amount of adsorbed water) fit well with the calculated value (60.0 wt-%). Finally, La₂O₃ was identified as thermal residues via XRD (Figure S9b). Hence, the thermal decomposition can be ascribed to the following reaction:

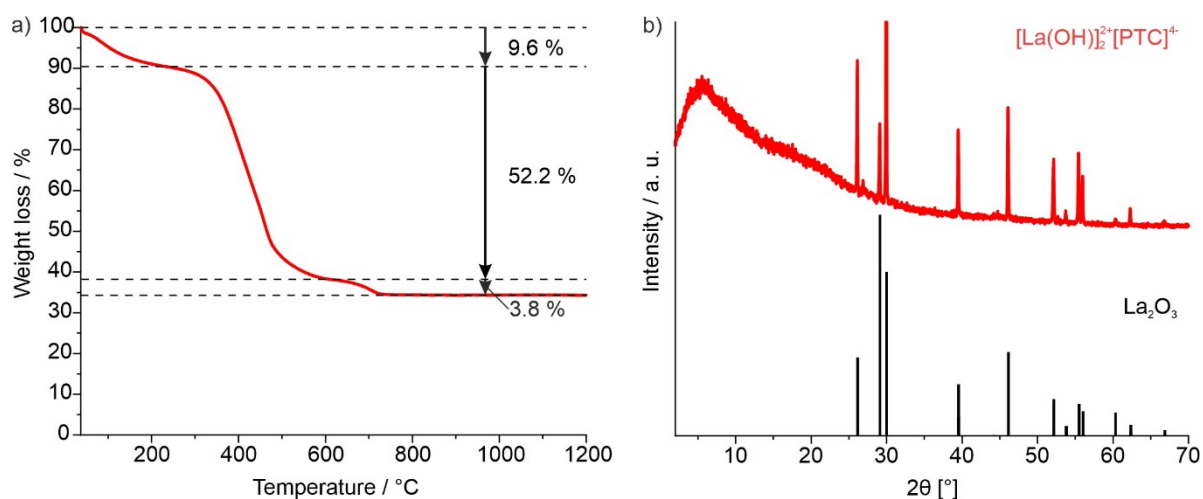
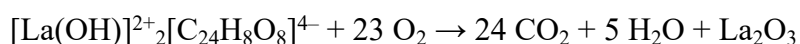


Figure S9. Total organic combustion of the $[\text{La}(\text{OH})]^{2+}_2[\text{PTC}]^{4-}$ IOH-NPs: (a) TG analysis, (b) XRD of the thermal remnant after the TG analysis (La_2O_3 /ICDD-No. 01-071-5408 as reference).

The photoluminescence properties of the $[\text{La}(\text{OH})]^{2+}_2[\text{PTC}]^{4-}$ IOH-NPs show the expected features of PTC with a strong visible absorption at 350-500 nm and very intense emission at 500-700 nm with a maximum of 517 nm, which is comparable with a solution of $\text{Na}_4(\text{PTC})$ (Figure S10; see main paper: Figure 5b).

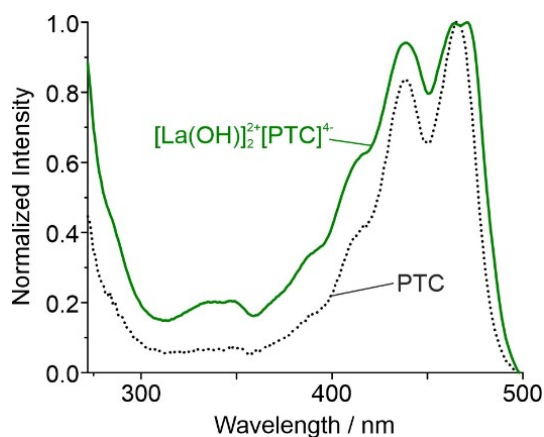


Figure S10. Excitation spectra of the $[\text{La}(\text{OH})]^{2+}_2[\text{PTC}]^{4-}$ IOH-NPs (aqueous suspension) with aqueous solution of $\text{Na}_4(\text{PTC})$ as a reference.

5. Material Characterization $[\text{La}(\text{OH})]^{2+}_2[\text{PTC}]^{4-}@\text{LaPO}_4$

Synthesis

In alternative to the synthesis of $[\text{La}(\text{OH})]^{2+}_2[\text{PTC}]^{4-}$ IOH-NPs at elevated temperature (95 °C), the nucleation of the PTC-containing IOH-NPs, and specifically the suppression of the growth of needles due to π -stacking, can be also performed by the synthesis of $[\text{La}(\text{OH})]^{2+}_2[\text{PTC}]^{4-}@\text{LaPO}_4$ core-shell IOH-NPs. To this concern, 6.3 mg (0.016 mmol) of perylene-3,4,9,10-tetracarboxylic dianhydride (PTCDA, abcr, 98%) were dissolved in 25 mL of 10 mmol/L NaOH by heating to 60 °C. After neutralisation with 10 mmol/L HCl, 15 mL of ethanol were added. Thereafter, 9.3 mg (0.025 mmol) of $\text{LaCl}_3 \cdot 7\text{H}_2\text{O}$ (Sigma-Aldrich, 99.9%) dissolved in 0.5 mL of water were injected into the aforementioned PTCDA solution. The $[\text{La}(\text{OH})]^{2+}_2[\text{PTC}]^{4-}$ IOH-NPs were separated by centrifugation (25.000 rpm, 15 min) and purified thrice by redispersion/centrifugation in/from an ethanol/water mixture (40 : 60). The IOH-NPs were resuspended in an ethanol/water mixture (40 : 60). Then, 2 mL of a $\text{Na}_2(\text{HPO}_4)/\text{Na}(\text{H}_2\text{PO}_4)$ buffer solution (2.5 mmol/L) were slowly added via a syringe pump (4 mL/h). The suspension was stirred for additional 15 h. The IOH-NPs were separated by centrifugation (25.000 rpm, 15 min) and again redispersed in an ethanol/water mixture (40:60). After redispersion in the ethanol/water mixture (40 : 60), a solution of 0.5 mg (0.001 mmol) of $\text{LaCl}_3 \cdot 7\text{H}_2\text{O}$ in 1 mL of water was slowly added via a syringe pump (1 mL/h). After an additional hour of stirring, the suspension was heated up to 95 °C for 90 min. After cooling, the as-prepared $[\text{La}(\text{OH})]^{2+}_2[\text{PTC}]^{4-}@\text{LaPO}_4$ IOH-NPs were separated by centrifugation (25.000 rpm, 15 min) and purified thrice by redispersing/centrifugating in/from water. Finally, aqueous suspensions or dried powder samples were obtained.

Characterization

The material properties of the $[\text{La}(\text{OH})]_2^{2+}[\text{PTC}]^4-\text{@LaPO}_4$ core-shell IOH-NPs is very similar to $[\text{La}(\text{OH})]_2^{2+}[\text{PTC}]^4-$, except for the presence of absence of the LaPO_4 shell. Thus, DLS and SEM result in mean sizes of 68 ± 10 nm and 63 ± 9 nm, respectively (Figure S11a). Similar to $[\text{La}(\text{OH})]_2^{2+}[\text{PTC}]^4-$ IOH-NPs (*see main paper: Figure 4*), rod-shaped particles are also observed for $[\text{La}(\text{OH})]_2^{2+}[\text{PTC}]^4-\text{@LaPO}_4$ due to the π -stacking of the PTC anion (Figure S11d,e). The formation and presence of the LaPO_4 shell are indicated by the different zeta potentials of the raw $[\text{La}(\text{OH})]_2^{2+}[\text{PTC}]^4-$ IOH-NPs and the $[\text{La}(\text{OH})]_2^{2+}[\text{PTC}]^4-\text{@LaPO}_4$ IOH-NPs (Figure S11b). Similar to $[\text{La}(\text{OH})]_2^{2+}[\text{PTC}]^4-$ IOH-NPs (*see main paper: Figure 4*), aqueous suspensions of the $[\text{La}(\text{OH})]_2^{2+}[\text{PTC}]^4-\text{@LaPO}_4$ IOH-NPs are colloiddally highly stable (Figure S11c).

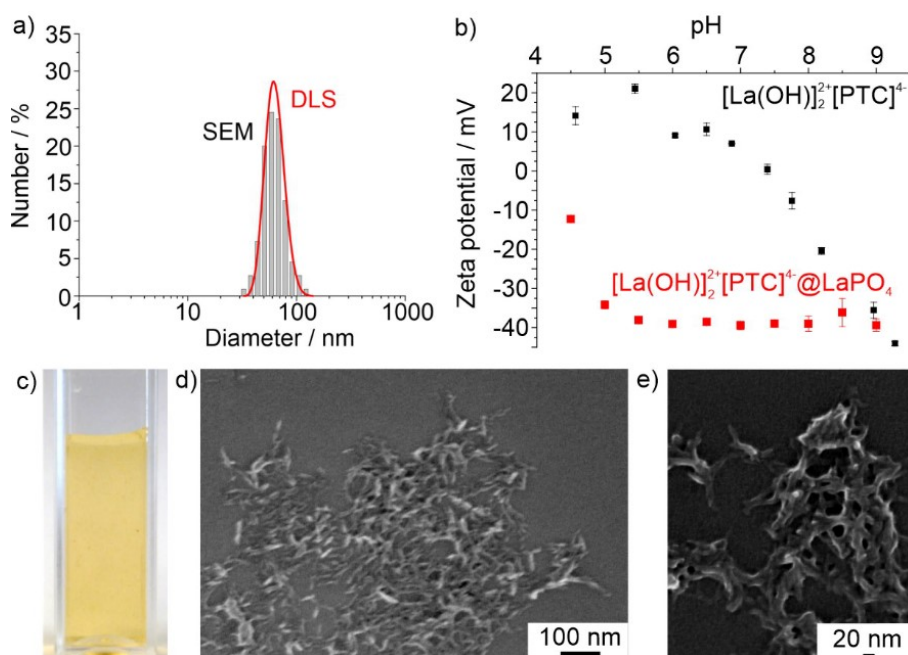


Figure S11. Particle characterization of the $[\text{La}(\text{OH})]_2^{2+}[\text{PTC}]^4-\text{@LaPO}_4$ IOH-NPs: (a) Particle size and particle size distribution according to DLS (in DEG) and SEM (statistical evaluation of 110 nanoparticles); (b) zeta potential analysis (including $[\text{La}(\text{OH})]_2^{2+}[\text{PTC}]^4-$ IOH-NPs); (c) photo of aqueous suspension; d+e) SEM images at different levels of magnification.

The emission of the $[\text{La}(\text{OH})]_2^{2+}[\text{PTC}]^4-\text{@LaPO}_4$ IOH-NPs upon dissolution is similar to $[\text{La}(\text{OH})]_2^{2+}[\text{PTC}]^4-$ IOH-NPs (Figure S12; *see main paper: Figure 5*). Similarly, the excitation spectra of the $[\text{La}(\text{OH})]_2^{2+}[\text{PTC}]^4-\text{@LaPO}_4$ IOH-NPs are similar to $[\text{La}(\text{OH})]_2^{2+}[\text{PTC}]^4-$ IOH-NPs and the freely dissolved PTC (Figure S13).

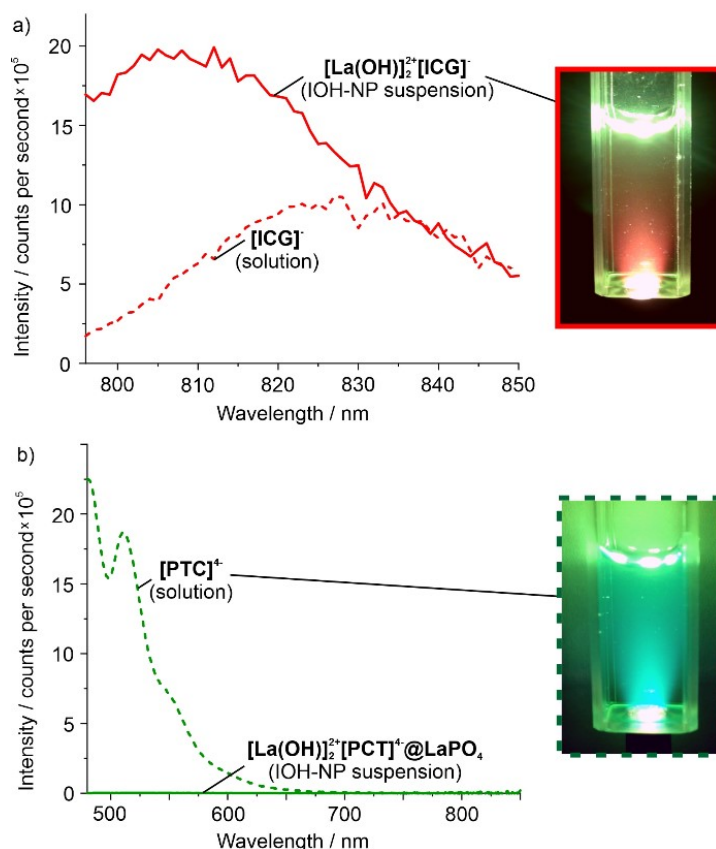


Figure S12. Emission of a) $[\text{La}(\text{OH})]_2^{2+}[\text{PTC}]^{4-}@LaPO_4$ IOH-NP suspensions (with photo) and of freely dissolved ICG and b) $[\text{La}(\text{OH})]_2^{2+}[\text{PTC}]^{4-}@LaPO_4$ IOH-NP suspensions and of freely dissolved PTC (with photo). All samples in distilled water; dissolution in 0.1 M phosphate solution; excitation of ICG at 781 nm and of PTC at 465 nm). Photos with blue-light-emitting LED for excitation.

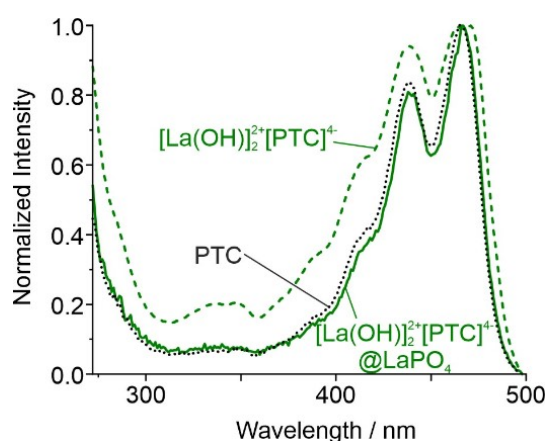


Figure S13. Excitation spectra of the $[\text{La}(\text{OH})]_2^{2+}[\text{PTC}]^{4-}@LaPO_4$ IOH-NPs (aqueous suspension) with $[\text{La}(\text{OH})]_2^{2+}[\text{PTC}]^{4-}$ IOH-NPs (aqueous suspension) and aqueous solution of $\text{Na}_4(\text{PTC})$ as references.

6. In-vitro Studies

Cell cultures. The immortalized and adherent mouse alveolar macrophage cell line, MH-S (CRL-2019, ATCC) was cultivated at 37 °C in a humidified atmosphere of 5% CO₂ in complete RPMI medium supplemented with 10% fetal calf serum (FCS) and 0.05 mM 2-mercaptoethanol.

Incubation with IOH-NPs. MH-S cells were plated on a 35 mm μ -dish equipped with a polymer coverslip bottom (IBIDI), in a concentration of \sim 15.000 cells/cm² in 1 mL cell culture medium. Cells were allowed to attach overnight. On the next day, the macrophages were supplemented with 1 mL of medium, containing 50 μ g/mL of the [La(OH)]²⁺[ICG]⁻² and [La(OH)]²⁺₂[PTC]⁴⁻ IOH-NPs.

Microscopy and image analysis. A Leica SP5 confocal laser-scanning microscope was used for imaging. Time-resolved imaging was performed after 30 min and 5 h of incubation with the IOH-NPs. Fluorescence micrographs of a living macrophages were recorded over 15 and 20 hours of incubation with the IOH-NPs. [La(OH)]²⁺[ICG]⁻² IOH-NPs were excited at 633 nm and the emission recorded at 643-800 nm. [La(OH)]²⁺₂[PTC]⁴⁻ IOH-NPs were excited at 514 nm and the emission recorded at 524-574 nm (*see main paper: Figure 7*). Confocal images were processed using ImageJ (available by ftp at [zippy.nimh.nih.gov](ftp://zippy.nimh.nih.gov) or at <http://rsb.info.nih.gov/nih-imageJ>, developed by W. Rasband, National Institutes of Health, U.S.).