Electronic Supplementary Material (ESI) for Nanoscale.
This journal is © The Royal Society of Chemistry 2017

ELECTRONIC SUPPLEMENTARY INFORMATION

Tb^{3+}-doped LaF_3 nanocrystals for correlative cathodoluminescence electron microscopy imaging with nanometric resolution in focus ion beam-sectioned biological samples

Kerda Keevend,[1] Michael Stiefel,[2] Anna Lena Neuer,[1] Martin T. Matter,[1] Antonia Neels,[3] Sergio Bertazzo,[4] Inge K. Herrmann[1]*

*inge.herrmann@empa.ch

+41 (0)58 765 71 53

s.bertazzo@ucl.ac.uk

[1]Particles-Biology Interactions, Department of Materials Meet Life, Swiss Federal Laboratories for Materials Science and Technology (Empa), Lerchenfeldstrasse 5, 9014 St. Gallen, Switzerland

[2]Reliability Science and Technology, Department of Materials Meet Life, Swiss Federal Laboratories for Materials Science and Technology (Empa), Uberlandstrasse 129, 8600 Dubendorf, Switzerland

[3]Center for X-ray Analytics, Department of Materials Meet Life, Swiss Federal Laboratories for Materials Science and Technology (Empa), Uberlandstrasse 129, 8600 Dubendorf, Switzerland

[4]Department of Medical Physics and Biomedical Engineering, University College London (UCL), Malet Place Engineering Building, London, WC1E 6BT, United Kingdom

MATERIALS AND METHODS

Reagents

Lanthanum (III) nitrate hexahydrate ($La(NO_3)_3·6H_2O$, 99.999%), terbium (III) nitrate pentahydrate ($Tb(NO_3)_3·5H_2O$, 99.9%), ammonium fluoride (NH_4F, ≥ 99.99%), ethylene glycol ($HOCH_2CH_2OH$, ≥ 99.5%) and polyvinylpyrrolidone (PVP, MW ~55,000) were purchased from Sigma-Aldrich. Poly(ethylene glycol) methyl ether thiol (mPEG-SH, MW 1k) was purchased from Creative PEGWorks. All the reagents were used without further purification. Cell culture media and supplements were purchased from Life Technologies. Used water was double distilled (ddH_2O).

Synthesis of $LaF_3:Tb^{3+}$- PVP/ -PEG nanocrystals

$LaF_3:Tb^{3+}$ nanocrystals capped with polymer were synthesized in ethylene glycol via co-precipitation. The dopant concentration was kept around 10%. Typically, 30 ml of 0.10M $La(NO_3)_3$, 10 ml of 0.03M $Tb(NO_3)_3$ and 0.2 grams of polymer (either PEG or PVP) were mixed under continuous stirring. Then 10 ml of 0.32M NH_4F solution was added to the mixture of RE ions. The reaction was carried out at 150 °C for 3h under stirring. Synthesized nanocrystals were collected by centrifugation and washed several times with ethanol and ddH_2O. Finally, particles were stored in ddH_2O for further use.

Physicochemical characterization

Particles were prepared for TEM by sonicating the initial colloid for 10 minutes in ultrasonication bath. Then, 10 µl of colloid was diluted with ddH_2O. The TEM grids (200 mesh Cu grids with formvar film (Electron Microscopy Science, Lucerna-Chem AG, Lucerne, Switzerland)) were placed on the droplet and soaked for 5 minutes. The grid was air-dried. After drying, the grid was imaged in a JEOL 2000FX transmission electron microscope.

Fluorescence spectroscopy was performed using a Quantaurus-QY C11347-11, Hamamatsu Spectrometer, using an excitation wavelength of 346 nm.

XRD was done on white powders of as-prepared terbium-doped LaF_3 nanocrystals which were mounted on a Stoe Mark II-Imaging Plate Diffractometer System (Stoe & Cie, 2015) equipped with a graphite-monochromator. Data collection was performed at –100˚C using Mo-Kα radiation (λ = 0.71073 Å, beam diameter 0.5 mm). Two-dimensional diffraction

images (30 min per exposure) were obtained at an image plate distance of 200 mm with a continued sample rotation. The resolution was D_{min} - D_{max} 24.00 - 0.82 Å and intensity integration has been performed over the entire image (360°). Crystallite size was calculated using the Scherrer equation

$$D = \frac{0.94\lambda}{\beta cos\theta}$$

where D is the diameter of the particle, λ is the wavelength of the X-ray and β is the full width at half maximum (FWHM) corrected for instrument broadening.

Thermogravimetric analysis (TGA) of powdered samples was performed in air using a NETZSCH TG 209 F1 instrument, heating from 25-500 °C at 10 $K \cdot min^{-1}$.

Fourier-transformed infrared spectroscopy (FTIR) was performed on a Bruker Tensor 27 FT-IR spectrometer for powdered samples.

Hydrodynamic size and concentration measurements were performed using a Nano Sight NS 500 (Malvern) nanoparticle tracking analysis (NTA) instrument. Suspended particles were illuminated with a 532 nm laser beam and the hydrodynamic size was calculated based on the Strokes-Einstein equation. Before the measurement, the NS500 instrument was primed following the user manual. Particles were diluted 1:100 in cell culture media (RPMI-1640, Life Technologies) and vortexed before the measurements. Particle suspensions were analysed using measurement conditions of 30-100 particles/frame and 5 consecutive runs of 200 seconds each were recorded.

Cytotoxicity measurements

Cytotoxicity measurements were carried out according to the protocol from the manufacturer using Promega CytoTox 96® Non-Radioactive Cytotoxicity Assay kit.

Preparation of cellular specimens for CL-FIB-SEM imaging and imaging conditions

Human lung cells (A549) were cultured in Roswell Park Memorial Institute Media (RPMI-1640) containing 10% fetal bovine serum (FBS) at 37°C in 5% CO_2. Cells were sub-cultured every fifth day and grown to 75% confluence. For experiments, cells were seeded at a density of 60 000 cells/cm^2 in cell culture media containing 10% FBS and left to attach for 24 hours. Next, cells were incubated with LaF_3 nanocrystals (100 µg for 100 000 cells) for 24 hours.

Cells were then gently washed with pre-warmed PBS, trypsinized and fixed with 4% methanol-free paraformaldehyde (PFA) overnight in the fridge. Pellets were then washed with ddH2O (3x) and cacodylate buffer (0.1M) (3x) and stained with 2% osmium tetroxide and 1.5% potassium ferricyanide for 1 hour. Pellets were washed with ddH2O and then gradually dehydrated using an ethanol gradient (20%, 40%, 50%, 60 %, 70%, 80%, 90%, 95%, 100% (3x)) and embedded in epoxy resin (EPON 812), according to procedures described in the manufacturer's protocol. Resin blocks were cured in the oven for 48 hours, trimmed with a razor blade and then sectioned in 100 nm sections using an ultramicrotome, where applicable. The thin sections were imaged in a FEI Helios 660 G3 UC FIB/SEM system using a 30 kV electron beam in the transmission mode. Resin-blocks were sputter-coated with gold/platinum and trenches of 50 µm width and several tens of micrometers in depth were cut using a focused gallium ion beam. The focused ion beam was operated at 30 kV and beam currents between 47 nA to 9 nA have been used to cut and polish the cross-sections. Cross-sections were then imaged using an in-lens backscattering and secondary electron detector. Cathodoluminescence images were acquired using a Delmic SPARC detection system. The detector was operated either in spectroscopic mode or in imaging mode, therefore either an Andor Shamrock 193 spectrograph or a PMT were used respectively to image the sample. Cathodoluminescence images are slightly distorted due to a sample shift related to sample charging and discharging during data acquisition and therefore for overlay images, the angles have been changed.

SUPPLEMENTARY FIGURES

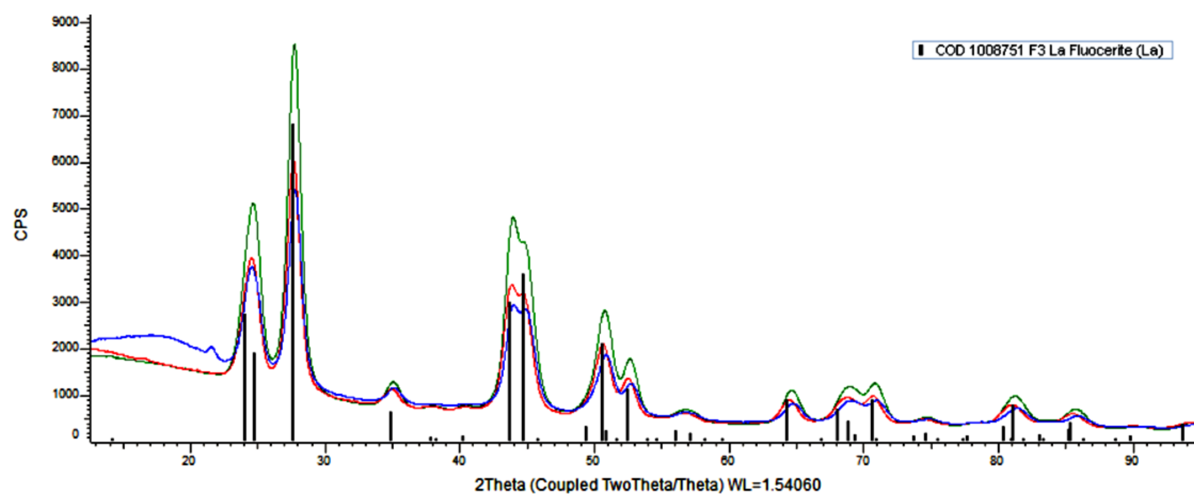

Figure S1. Reference data of hexagonal LaF_3.

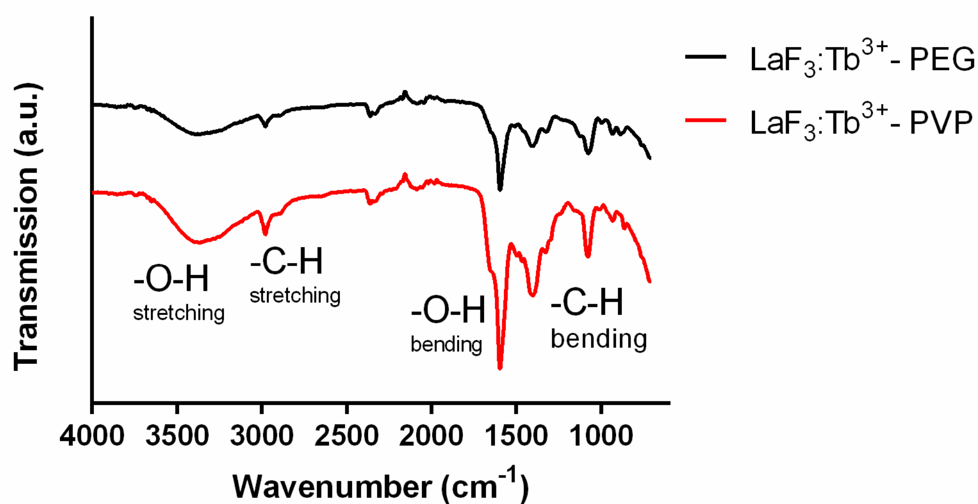

Figure S2. Fourier-transformed infrared spectroscopy (FTIR) spectra for polymer coated as-prepared $LaF_3:Tb^{3+}$ nanocrystals.

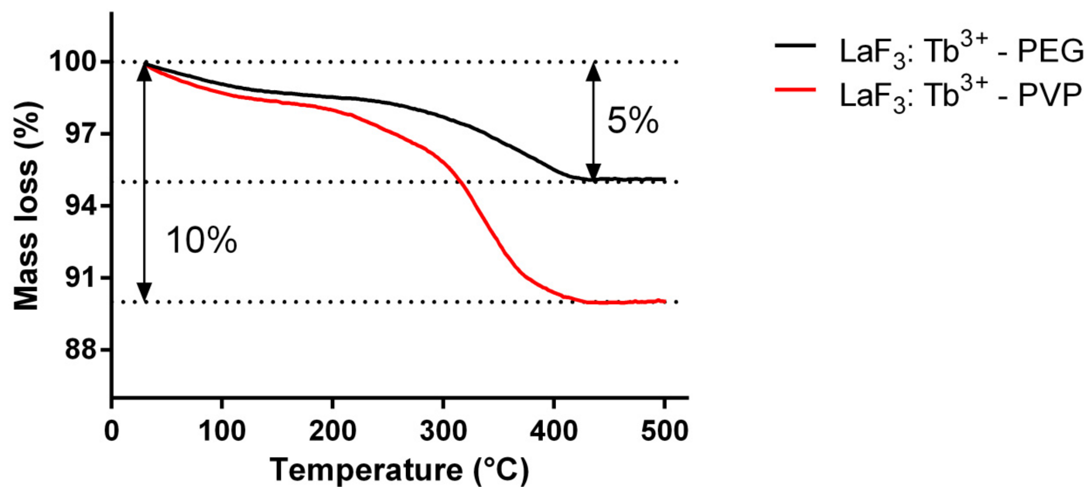

Figure S3. Thermogravimetric analysis of polymer coated as-prepared $LaF_3:Tb^{3+}$ nanocrystals.

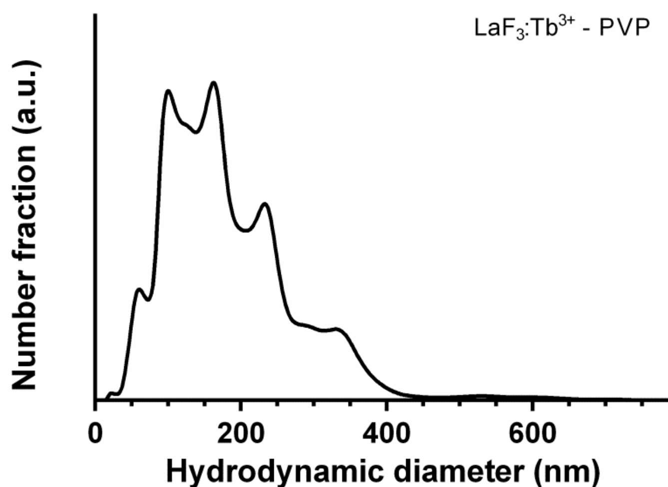

Figure S4. Nanoparticle tracking analysis (NTA) measurements for PVP covered $LaF_3:Tb^{3+}$ nanocrystals.

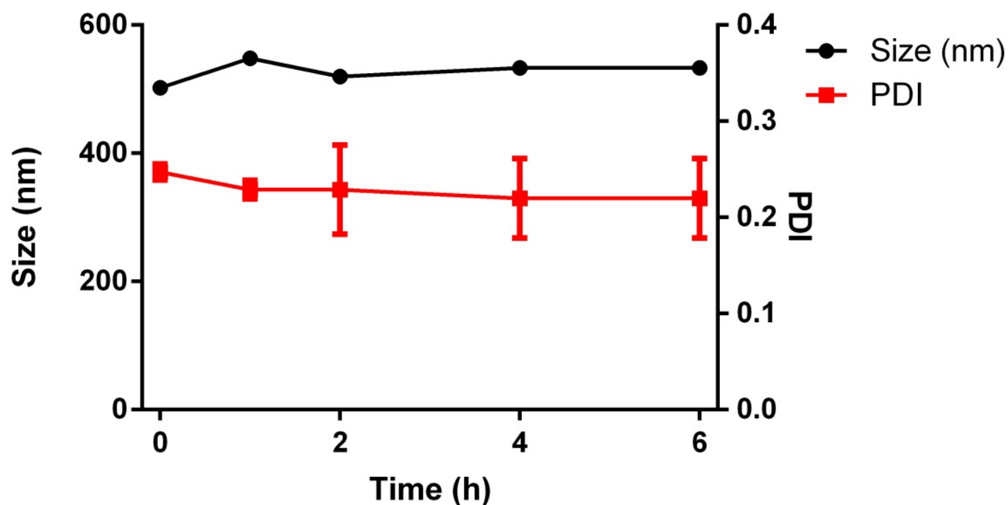

Figure S5. Colloidal stability of PEGylated $LaF_3:Tb^{3+}$ nanocrystals in protein containing media over time.

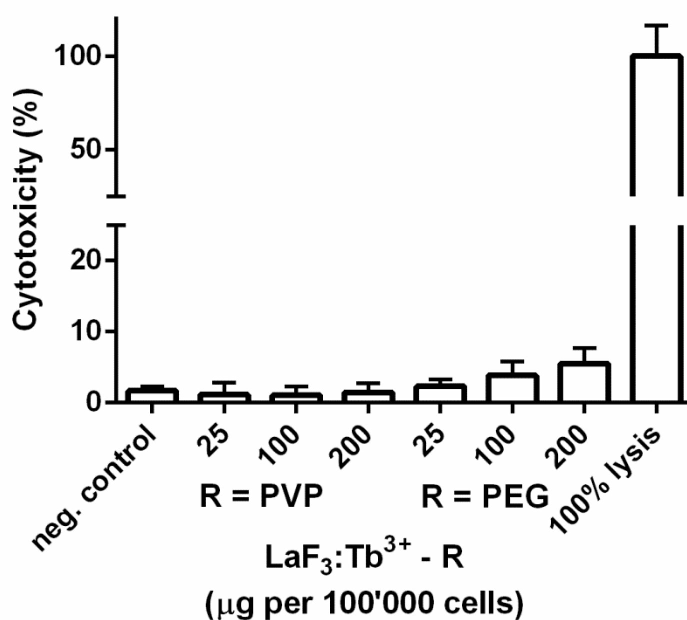

Figure S6. LDH activity measurements in cell culture supernatants after 24 hours of polymer-coated $LaF_3:Tb^{3+}$ nanoparticles to human lung cells (A549).

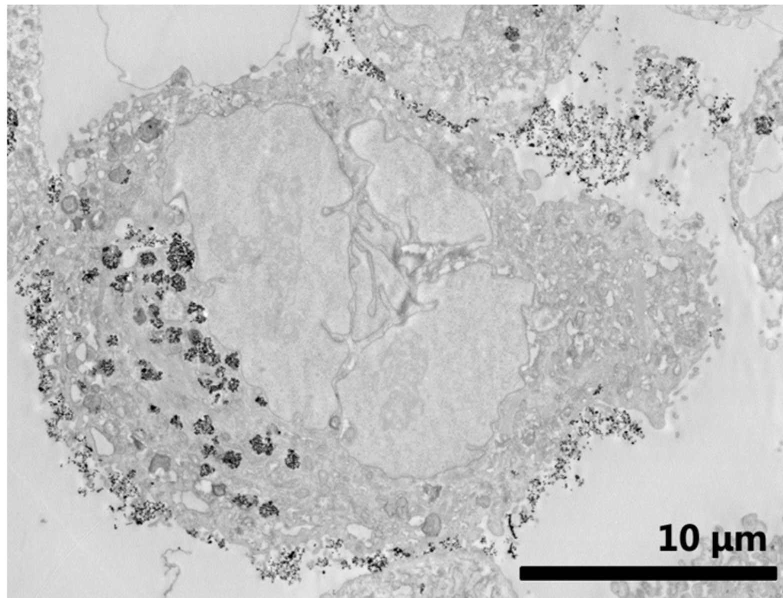

Figure S7. Scanning transmission electron (STEM) micrograph of 100 nm sections of A549 cells showing particles localized in endosomes and in proximity of the outer cell membrane.

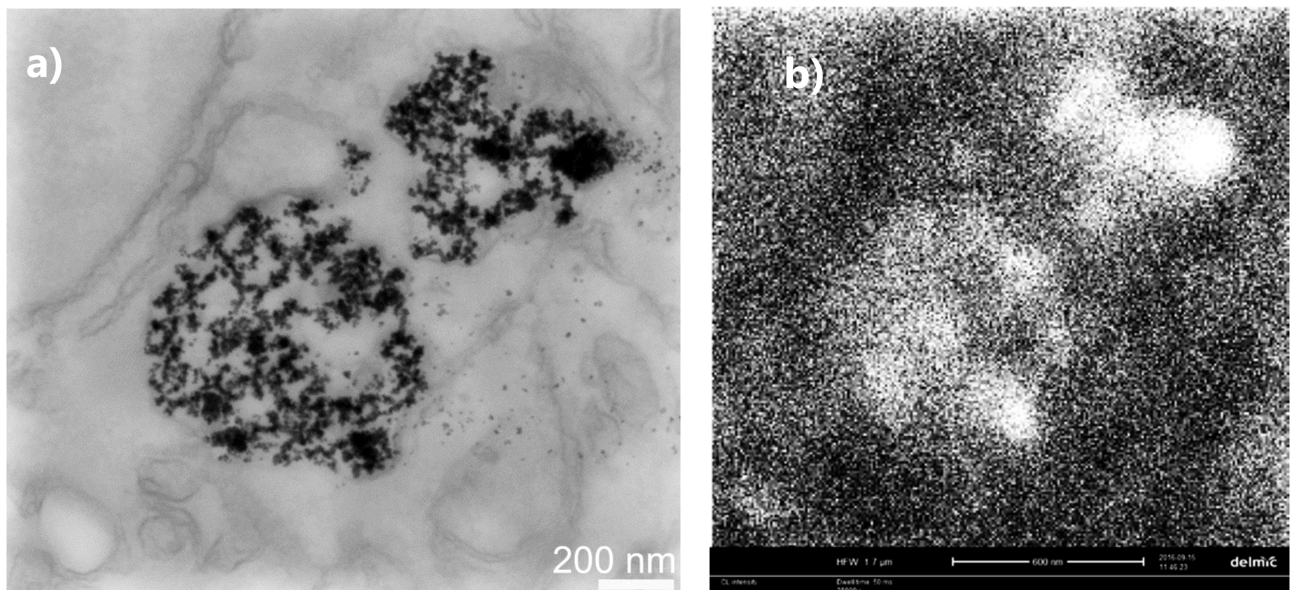

Figure S8. Scanning transmission electron micrograph of 100 nm sections of A549 cells **(a)** and corresponding original cathodoluminescence image of the area with pixel sizes of 2 nm **(b)**.

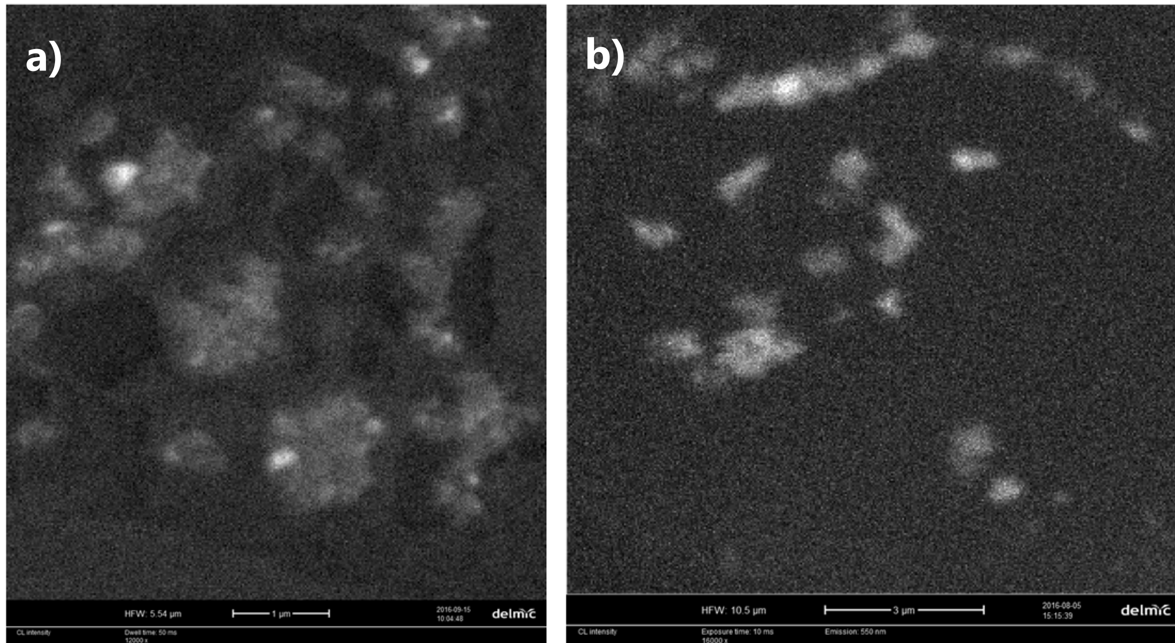

Figure S9. Original cathodoluminescence images of luminescent particles in 100 nm sections of A549 cells **(a)** and cells exposed in a FIB cut **(b)**.

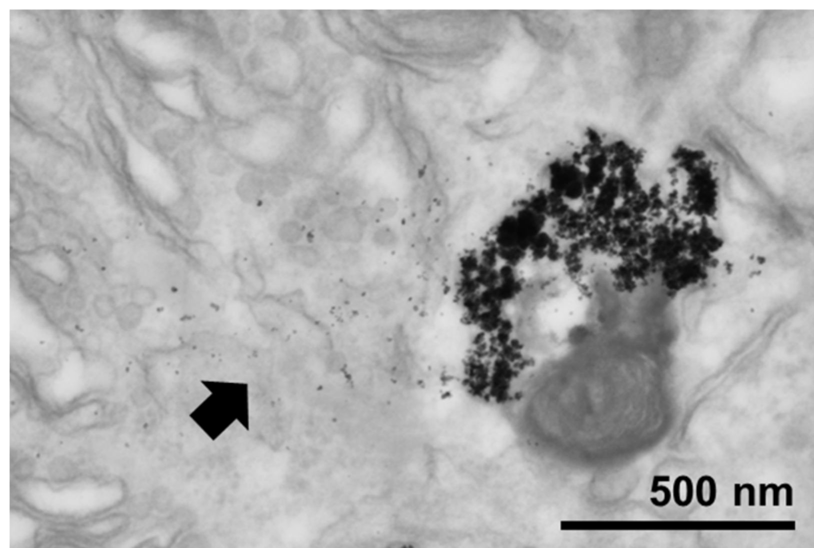

Figure S10. STEM images reveal mechanical artefact caused by the sectioning in the ultra-microtome, the arrow indicates particles outside of vesicular bodies.

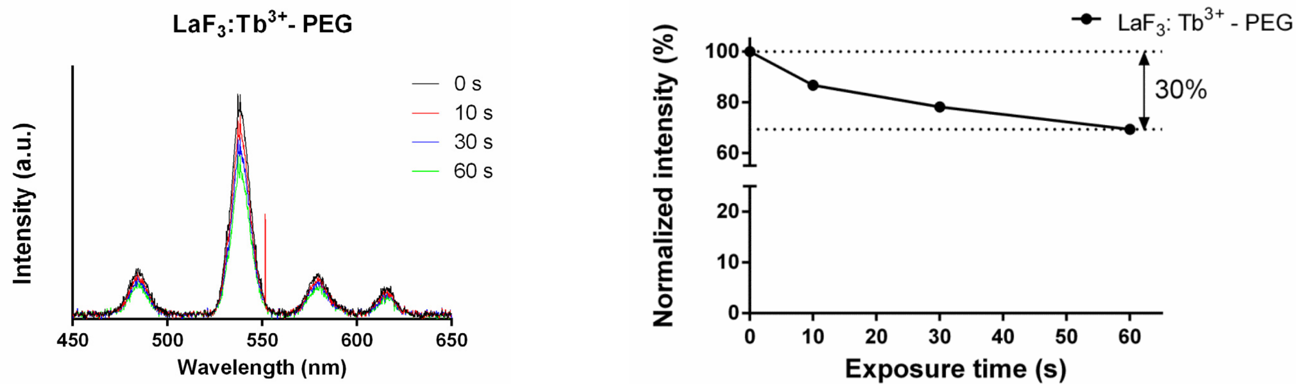

Figure S11: Cathodoluminescence intensity as a function of electron beam (5 kV) exposure time. These measurements have been performed using nanocrystals on TEM grids with a dwell time of 1 µs.