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Top-down synthesis of luminescent microplastics and nanoplastics by incorporation of upconverting nanoparticles for environmental assessment

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1 **Top-Down Synthesis of Luminescent Microplastics and Nanoplastics**
2 **by Incorporation of Upconverting Nanoparticles for Environmental**
3 **Assessment**

4

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17

18 **Keywords:** nanoplastic, UCNPs, lanthanide, powdering, milling, top-down synthesis,
19 environmentally relevant, polymer

20

21 **Abstract**

22 The occurrence of micro- and nanoplastics is a major environmental problem. Especially for
23 nanoplastics due to their easy bioavailability and unknown impact on living organisms. The
24 monitoring of these extremely small particles during their ingestion, tissue translocation and
25 transfer through the trophic chain remains very challenging. This study aims to develop an
26 environmentally relevant model of luminescent micro- and nanoplastics. First, lanthanide-
27 based upconverting nanophosphors (20 nm) were incorporated in bulk polyethylene without
28 modification of the polymer structure or morphology. Second, micrometric and nanometric
29 particles were obtained after powdering. Two fractions were obtained with cascade filtration

30 with average sizes of 5 μm and 150 nm and characterized in terms of size distribution,
31 morphology and surface charge. The particles are very polydisperse with an irregular shape
32 and a global negative charge; they exhibit morphological characteristics similar to those
33 formed in the environment. Their luminescent properties upon NIR excitation at 980 nm open
34 the possibility to track them in the tissues of organisms. The powdering method is very simple
35 and compatible with many polymers pure or formulated. As a perspective, the use of
36 weathered materials is possible with the proposed method and will allow the preparation of
37 particles sharing additional properties with environmental micro- and nanoplastics.

38 **Introduction**

39 The scientific community has recently invested crucial effort in evaluating the impact of plastic
40 on ecosystems, and this has allowed us to realize, in addition to the fact that all compartments
41 of the earth were concerned, that the pollution reached the nanoscale. Our experience in
42 engineered nanomaterials enables us to envisage that nanoplastics have unique physical and
43 chemical properties (e.g., size, shape, composition, and reactivity). The very nature of
44 nanoplastics (NPs, sizes are below 1 μm)¹⁻³ being polydisperse, polymorphic, and mainly made
45 of C, H, and O, similar to natural organic matter, further complicates the analysis of NP
46 behavior by traditional colloid science.

47 In recent decades, much attention has been given to the study of the occurrence and potential
48 negative effects of microplastics (MPs, ranging from 1 μm to 5 mm).⁴ MPs were analyzed in
49 organisms ranging from zooplankton⁵ to marine mammals.⁶ Laboratory studies to investigate
50 MP bioaccumulation and immunotoxicity in living organisms reported their biological impact
51 on organisms (see references⁷⁻⁹ for review). However, the fate and role of NPs remain poorly
52 understood because of the lack of suitable analytical methods for monitoring their uptake,
53 transport, and translocation and for understanding their potential adverse effects on living

54 organisms.¹⁰ To date, most of the evaluation studies have been run on model NPs using
55 commercially available polystyrene (PS) nanospheres.^{11, 12} The distinction between model NPs
56 and NPs—the nanoscale fraction of plastic debris generated in the environment—is often
57 omitted even if their physicochemical and biological behavior are drastically different.¹⁰
58 Model NPs are rather easy to handle and characterize but, lacking surface chemistry,
59 polydispersity and an irregular shape similar to the one formed in the environment, they are
60 of limited interest for ecotoxicological study.¹⁰

61 A few recent studies proposed a top-down approach (i.e., producing smaller particles from
62 larger objects)¹³ to prepare more environmentally relevant model NPs. Magri et al. described
63 the use of laser ablation in water to obtain polyethylene terephthalate nanoparticles with an
64 average size of 100 nm and a surface chemical composition similar to plastic exposed to UV
65 light in the environment.¹⁴ Astner et al. produced model MPs and model NPs (in the range
66 360–390 nm) made of polybetaine adipate-coterephthalate and low-density polyethylene by
67 applying a sequence mimicking environmental weathering.¹⁵ El Hadri et al. produced NPs
68 ranging from 20 nm to 1000 nm by applying blade grinding followed by planetary ball milling.¹⁶
69 Model NP production from MPs collected in the environment was also proposed to prepare
70 particles with a surface chemistry even closer to that of native nanoplastics. Their toxicological
71 evaluation demonstrated that their impact on marine organisms was superior to that of PS
72 nanospheres.¹⁷ Even if the plastic particles obtained by the top-down methods reproduce the
73 main physicochemical characteristics of NPs formed in the environment, their tracking
74 remains challenging during biological evaluation tests, making it difficult to monitor their
75 ingestion, transport, and translocation in living organisms.

76 Recent synthesis strategies, such as ¹³C enrichment,¹⁸ rare metals,¹⁹ or simple staining
77 methods with fluorescent dyes,²⁰ were used to obtain label model MPs and NPs. Fluorescence

78 labeling is a widely developed strategy, but most fluorescent tags operate with UV-blue to
79 green excitation light, showing low tissue penetration and toxicity to living organisms and
80 potentially leading to background autofluorescence, thus preventing their use in deep-tissue
81 imaging. Dyes can also suffer from photobleaching with prolonged exposures²¹ or can have
82 solvatochromic properties, making the emission spectra dependent on the polarity of the
83 surrounding environment.²² Another limitation is the potential costaining of the organic
84 matter present in the sample under focus, resulting in false detection. As an alternative to
85 fluorescence labeling, Mitrano et al. proposed the use of heavy metals as tracers and
86 inductively coupled plasma–mass spectrometry (ICP–MS) for indirect plastic monitoring.
87 Polyacrylonitrile particles doped with Pd were accurately assessed in a complex matrix
88 (activated sludge from wastewater treatment plant), and no Pd leaching from particles was
89 observed.¹⁹ This strategy was found to be very efficient but is not well adapted for monitoring
90 MPs and NPs in living organisms because of the use of a destructive analytical technique.

91 The objective of the present study is to synthesize environmentally relevant models of MPs
92 and NPs in terms of polydispersity, shapes and surface properties that could be easily
93 visualized and tracked in the tissues of living organisms. Polyethylene (PE) was selected as a
94 model polymer because it is the most commonly used polymer and the most frequently found
95 polymer in the environment. However, especially at the nanoscale, this material is very
96 stealthy and poorly detectable using nondestructive methods. For easy imaging, lanthanide-
97 based upconverting particles (UCNPs) were incorporated into the polymer matrix. Because of
98 the spectroscopic features of the lanthanide family, these nanomaterials can accumulate
99 near-infrared (NIR) energy and release it as visible emission as a line spectrum typical of the
100 emitting element (thus erbium gives rise to a bright green visible emission).²³ They are
101 commonly used as background-free luminophores in imaging or anti-counterfeiting

102 applications²⁴ or as local sources of light (“nanolamps”)²⁵ in material or biological
103 applications.²⁶⁻²⁹ Moreover, highly photostable (no blinking, no bleaching)³⁰ and requiring
104 lower power density than 2-photon fluorophores, these background-free persistent
105 fluorophores have become an attractive probe for imaging purposes, including thick samples.
106 Furthermore, UCNPs derived from rare element salts (NaREF₄) can be prepared in a very
107 monodispersed manner depending on the choice of rare earth elements and the preparation
108 protocol. We chose to develop a top-down approach for the synthesis of labeled PE by
109 incorporating highly monodispersed, hydrophobic and less than 30 nm UCNPs into bulk PE
110 material and by using powdering to produce labeled nanometric particles with irregular
111 shapes and a variety of sizes. The particles were characterized with many analytical techniques
112 to obtain full information about their physicochemical properties.

113 **Materials and methods**

114 **Chemicals:** PE pellets (High-Density PE, CAS 9002-88-4) were purchased from Sigma Aldrich
115 (Saint Louis, MO, USA). The polymer used was characterized by a melt flow index (MFI) of 12
116 g/10 min, a melting point between 125 and 140 °C and a density of 0.952 g.mL⁻¹ at 25 °C. Rare
117 earth (RE) chloride hydrates, ammonium fluoride, oleic acid (OA) and 1-octadecene (ODE)
118 were purchased from Alfa Aesar and used as received. All other organic solvents were of
119 spectroscopic grade and used as received. Deionized water ($\rho = 18 \text{ M}\Omega \text{ cm}^{-1}$) obtained from an
120 Aquadem apparatus was used in this study.

121 **Upconverting nanoparticle synthesis:** In a three-necked flask, a solution of YCl₃.6H₂O (2.4 g,
122 7.9 mmol), YbCl₃.6H₂O (1.35 g, 3.5 mmol) and ErCl₃.6H₂O (0.088 g, 0.23 mmol) in water (10
123 mL) was added to an OA:ODE mixture (35:190 mL:mL), and water was distilled off under an Ar
124 flush. The resulting cloudy suspension was brought to 160 °C until clear (*ca* 1 h) and then

125 cooled under an Ar flush. NH_4F (2.37 g, 64 mmol, 80 mL, 0.8 M) and NaOH (1.86 g, 46.6 mmol,
126 80 mL, 0.58 M) were dissolved separately in MeOH. These two solutions were added
127 simultaneously using syringe pumps at $0.4 \text{ mL}\cdot\text{min}^{-1}$ to the vigorously stirred $\text{RE}(\text{OA})_3\text{:OA:ODE}$
128 solution. Once the addition was completed, the methanol was removed under an Ar flush at
129 $100 \text{ }^\circ\text{C}$. The reaction mixture, after careful degassing, was then brought to $310 \text{ }^\circ\text{C}$ as fast as
130 possible (*ca* $20 \text{ }^\circ\text{C}\cdot\text{min}^{-1}$) and kept at this temperature for 90 min. After cooling, an equivalent
131 amount of ethanol was added, and particles were collected by centrifugation at 9000 g for 10
132 min. They were purified by repeating twice the following sequence: pellet redissolution in
133 cyclohexane/addition of the same volume of ethanol/centrifugation.

134 **UCNP incorporation into PE** : PE pellets were milled using a RETSCH ZM 200 ultracentrifugal
135 mill. The obtained polymer powder with an average size of $200 \text{ }\mu\text{m}$ was dissolved in boiling o-
136 xylene ($\geq 99.0\%$ (GC grade); Sigma Aldrich, Saint Louis, MO, USA), where UCNPs (10 wt.% PE)
137 were subsequently introduced. The mixture was homogenized by magnetic stirring at $146 \text{ }^\circ\text{C}$
138 for 20 min. Precipitation of the synthesized polymer material was achieved by rapid cooling in
139 an ice bath. UCNPs that were not incorporated into PE were removed by washing the polymer
140 with cyclohexane ($\geq 99.7\%$ (HPLC grade); Sigma Aldrich, Saint Louis, MO, USA). Before future
141 use, the polymer was dried overnight at $40 \text{ }^\circ\text{C}$. As a negative control, a second batch of
142 polymers containing only PE (Blank-PE) was prepared following a similar protocol.

143 **Synthesis of the labelled micro- and nanoparticles:** Bulk materials Blank-PE and Upcon-PE
144 were exposed to a cryogenic grinder (SPEX™ SamplePrep 6775 Freezer/Mill™, Delta Labo,
145 Avignon, France). First, samples were precooled for 5 min, and then grinding was carried out
146 in 10 cycles of 1 min with cooling between cycles for 2 min in liquid nitrogen. The grinding rate
147 was 10 coups per second (CPS). The obtained polymer powder was dispersed in ethanol
148 ($\geq 99.8\%$ (HPLC grade); Sigma Aldrich, Saint Louis, MO, USA) and sonicated for 15 min at room

149 temperature to avoid particle aggregation. Then, particle size separation was performed in 6
150 sequential filtration steps through a metal mesh with a cutoff of 500 μm (Retsch, Haan,
151 Germany) and NITEX nylon mesh (Dominique Dutscher SAS, Brumath, France), with pore sizes
152 of 200 μm , 100 μm , 50 μm , 11 μm , and 1 μm . Wet milling was performed in a 20 mL glass vial
153 filled with approximately 50 g of ZY-E (yttrium stabilized zirconium oxide) beads, 1.0–1.2 mm
154 (Sigmund Lindner GmbH (SiLi), Warmensteinach, Germany), 0.1 to 0.5 g of MPs, and
155 approximately 10 mL of absolute ethanol. The milling process lasted 48, 60, and 120 days
156 under 80 rpm stirring on a horizontal ROLLER 6 digital apparatus (IKA, Staufen, Germany).
157 Eventually, MPs and NPs were separated by the 6 steps of subsequent filtration described
158 above and rinsed with cyclohexane.

159 **Granulometry analysis:** The MP size distribution was determined using a Mastersizer MS3000
160 (Malvern Panalytical, UK). The polymer powder was dispersed in ethanol using a HYDRO MV
161 device with stirring at 2500 rpm. The refractive indices used for ethanol and particles were
162 1.36 and 1.52, respectively, with an absorption index for particles of 0.1. The results are
163 presented as the mean number of particles obtained from 5 measurements.

164 **Differential scanning calorimetry:** DSC was performed with a DSC1 Mettler Toledo apparatus
165 equipped with an HSS8 sensor. Samples were weighed (between 5 and 7 mg) and sealed in
166 40 mL aluminum pans. They were heated from 20 $^{\circ}\text{C}$ to 160 $^{\circ}\text{C}$ at 5 $^{\circ}\text{C}/\text{min}$ with an empty
167 aluminum pan as the reference. Dry nitrogen with a flow rate set at 20 mL/min was used as
168 the purge gas. Each sample was analyzed in triplicate. The first heating cycle was used to erase
169 all processing, thermal, mechanical, crystallization, and shear history of the samples. The
170 melting temperature and crystallinity were obtained from the second heating cycle. The
171 crystallinity was calculated from the expression:

$$172 \quad \text{Crystallinity (\%)} = (\Delta H_m / \Delta H_{mref}) * 100 \quad (1)$$

173 where ΔH_m is the melting enthalpy of the analyzed samples, and ΔH_{mref} is the melting enthalpy
174 of 100% crystalline PE (293 J/g).

175 **Small-angle X-ray scattering (SAXS) analysis:** SAXS measurements were performed on a
176 XEUSS 2.0 laboratory source equipped with a PILATUS 1 M pixel detector (DECTRIS) and an X-
177 ray source provided by a GeniX3D with a fixed wavelength based on Cu K α radiation
178 ($\lambda = 1.54 \text{ \AA}$). The distance between the sample and the detector was fixed at 1216.5 mm, giving
179 a q range starting from 0.005 \AA^{-1} to 0.5 \AA^{-1} , assuming that q is the scattering vector equal to
180 $4\pi/(\lambda \times \sin \theta)$ with a 2θ scattering angle. The distance was calibrated in the small-angle region
181 using silver behenate ($d_{001} = 58.34 \text{ \AA}$). For solid samples, the dry MP powder was placed
182 between two Kapton windows with 1 mm of the optical pathway and placed on the motorized
183 sample holder. For liquid samples, particle dispersion was injected into a capillary tube with a
184 diameter of 1.5 mm and then placed under the beam in the conditions. To remove scattering
185 and absorption from air, a primary vacuum was applied to the entire instrument. To maximize
186 the signal at high angles, 6 acquisitions of 600 seconds were averaged. All scattering curves
187 were corrected for the empty cell contribution or for the solvent, divided by the transmission
188 factor, acquisition time and optical path to obtain SAXS curves in absolute units (cm^{-1}).

189 **Transmission electron microscopy energy-dispersive X-ray spectroscopy:** NP dispersions in
190 ethanol were prepared with polyvinylpyrrolidone (PVP) 0.4% (w/v) to ensure particle adhesion
191 on the top of the formvar/carbon-coated copper grid. One drop of 20 μL of sample solution
192 was placed on the top of the grid. After drying, TEM/EDX analysis was performed using a JEOL
193 cold-FEG JEM-ARM200F operated at 200 kV equipped with a probe Cs corrector reaching a
194 spatial resolution of 0.078 nm. EDX spectra were recorded on a JEOL CENTURIO SDD detector.

195 **Nanoparticle tracking analysis:** NTA analyses were performed at 25 $^\circ\text{C}$ using a NanoSight
196 LM10 (Malvern Instruments, Ltd, UK) instrument equipped with an sCMOS camera. Samples

197 were prepared by NP dispersion in ultrapure Milli-Q water (50 mg/L). Measurement of each
198 sample was performed in triplicate, consisting of three records of 60 s. The results are
199 presented as the mean size of the particles \pm SD. NTA is known to provide accurate
200 measurements even for polydisperse nanoparticles and was thus used without any
201 complementary measurement by dynamic light scattering measurements.

202 **Fluorescence measurement:** Fluorescence spectra were acquired with a Fluorolog-3-2iHR320
203 modular spectrofluorometer (HORIBA Jobin Yvon SAS, Palaiseau, France). The standard light
204 source of the fluorimeter was substituted by a 980 nm continuous-wave laser operating at a
205 power of 1.5 W. Emission was collected between 400 nm and 750 nm.

206 **Two-photon microscopy:** Samples were imaged on a Zeiss LSM-710 two-photon microscope
207 equipped with a Ti: Sapphire Chameleon Vision II laser (Coherent, Santa Clara, California)
208 operating at 980 nm (4% of max power for UCNPs and 50% for μ -Blank-PE and μ -Upcon-PE
209 samples, 140 fs pulses, 80 MHz repetition rate) and a Zeiss Plan-Apochromat 63x/1.40 oil
210 immersion lens. Upconverted light was collected between 490 and 600 nm for the green band
211 and 630 and 710 nm for the red band. Images were processed using ImageJ (Fiji) software.

212 **Zeta-potential measurements:** Zeta-potential measurements for MPs and NPs were carried
213 out at 25 °C on a Zetasizer Nano-ZS (Malvern Instruments, Ltd, UK) equipped with a He-Ne
214 laser (λ = 633 nm) at an angle of 173°. Samples were prepared by dispersion of particles in 10
215 mM NaCl solution to provide a minimum level of conductivity in the samples, following ISO
216 and ASTM standard guides. Before analysis, the pH of every sample was measured. The zeta
217 potential and standard deviation (SD) were obtained from 5 measurements of 11 runs of 10
218 seconds using the Smoluchowski model.

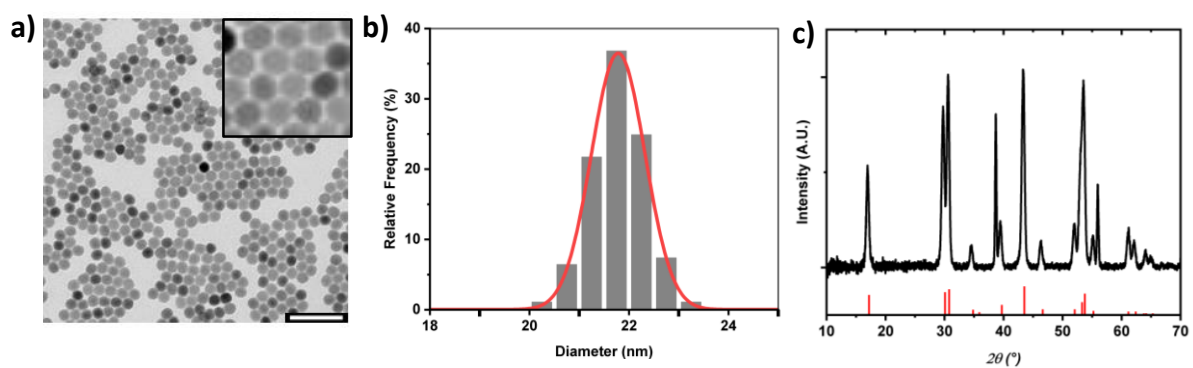
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221 Results

222 Large-scale monodisperse UCNP synthesis

223 Using classical thermal coprecipitation to prepare hydrophobic oleate-coated NaREF₄ UCNPs,
224 we implemented a new protocol to achieve the synthesis of monodisperse 20 nm UCNPs over
225 a multigram scale. This approach allows the use of a single batch of phosphors to be further
226 dispatched in multiple assays, therefore maintaining a homogeneous luminescence response
227 over all samples.^{31, 32} As we noticed that a slow and separate introduction of fluoride and
228 sodium sources via syringe pumps to the rare earth oleate mixture led to a dramatic size
229 reduction,³³ we decided to adapt this strategy to the efficient protocol described by Zhang et
230 al., known to yield 40 nm NaYF₄-based UCNPs.³⁴ We collected regular hexagonal prism-shaped
231 erbium-doped NaYF₄:Yb_{30%} and Er_{2%} UCNPs (NaREF₄, where RE= 2% Er; 30% Yb; 68% Y) with
232 an average size of 21.8±1.3 nm even on an 11.6 mmol scale: up to 3 g of bright green emissive
233 UCNPs could be obtained in this way. XRD analysis was consistent with the pure hexagonal
234 phase (Figure 1).



236 **Figure 1.** Transmission electron microscopy (TEM) and X-ray diffraction characterization of the
237 prepared NaYF₄:Yb_{30%},Er_{2%}. A: TEM image (scale bar is 100 nm). B: Size distribution obtained
238 from analysis of TEM images (Gaussian fit in red). C: XRD diffractogram of the particles (red
239 lines show the characteristic peaks of hexagonal NaYF₄ (JCPDS 00-016-0334)).

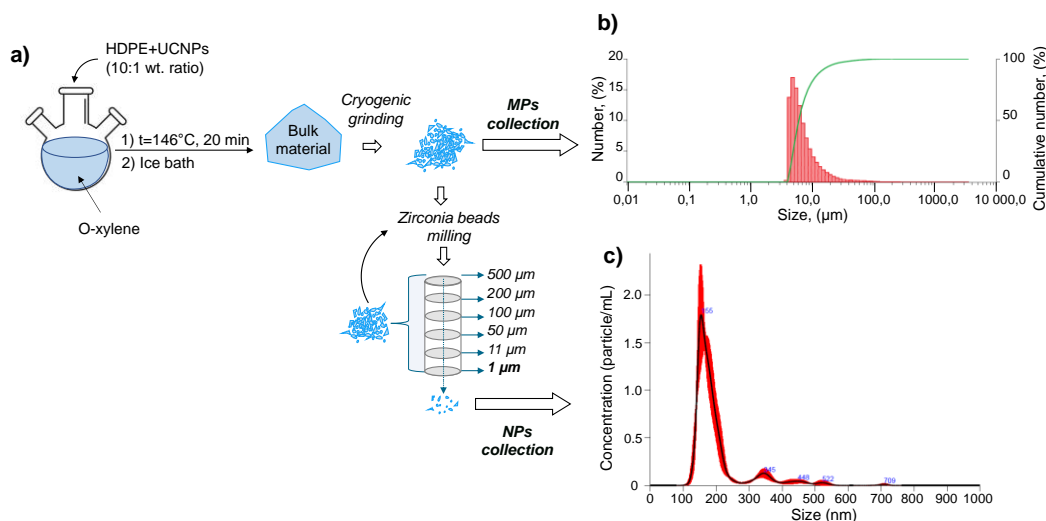
240 Incorporation of UCNP in PE and powdering

241 We chose to incorporate UCNPs into the polymer using a dissolution/precipitation procedure,
242 which allows one to work on smaller quantities than melt mixing (**Figure 2a**). The UCNP:PE
243 ratio was chosen by comparison with a standard nanocomposite formulation involving
244 HDPE.³⁵ Briefly, PE was solubilized in boiling o-xylene in the presence of UCNPs (10% in
245 weight). Cooling the colorless solution at 0 °C led to the quantitative precipitation of the
246 polymer. Excitation of the collected whitish solid with a 976 nm continuous-wave (CW) laser
247 induced the bright green luminescence typical of Er³⁺, indicating that UCNPs were successfully
248 incorporated in bulk PE (Upcon-PE). A similar test was found to be negative for UCNP-free
249 synthesis, Blank-PE.

250 The powdering process consisted of a first step to cryogenic grinding. We selected this method
251 because it is a process where thermally sensitive and elastic substances are generally
252 successfully processed. The effect of several parameters was considered, including precooling
253 time, grinding time, and oscillation frequency. Experiments reveal a nonmonotonous effect of
254 these parameters on abrasion efficiency. At this stage of the powdering process, particles at
255 the nanoscale were not detected by nanoparticle tracking analysis (NTA). Both powders, with
256 or without UCNPs, were named μ -Upcon-PE and μ -Blank-PE, respectively. The size distribution
257 obtained by granulometric analysis showed that approximately 90% of the particles were
258 smaller than 15 μm (**Figure 2b**). The incorporation of the UCNPs did not significantly impact
259 the size distribution of the powder (**Figure S1**).

260 To reach the nanoscale, we proceeded to further size reduction using a low-energy wet-driven
261 milling technique. The method is simple, economical, sustainable and, similar to the first step,
262 provides the advantage of the preservation of the polymer structure.³⁶ It consists of preparing
263 a dispersion of the powder in a solvent, adding zirconium beads and then slowly rolling the
264 solution. Ethanol was chosen as the solvent because synthesis in water, which would be more

265 appropriate for future use in toxicological tests, was not possible. PE is very hydrophobic and
 266 does not wet in water. Furthermore, PE floating in water and contact with zirconium beads
 267 used for milling were not favored. After the two-stage powdering process, the particles were
 268 fractionated by sequential filtration in ethanol (using a 6 mesh cutoff: 500 μm , 200 μm , 100
 269 μm , 50 μm , 11 μm , and 1 μm). This easy-to-implement protocol is aimed at preventing rapid
 270 clogging of the filter mesh during filtration without resorting to a more complex process, such
 271 as tangential flow filtration (TFF).³⁷ Nanoscale particles (nano-Upcon-PE and nanoblank-PE)
 272 were produced with a yield of approximately 6%. The distribution of the particles by
 273 nanoparticle tracking analysis (NTA) showed that approximately 90% of the particles were
 274 smaller than 201 nm for nanoblank-PE and 117 nm for nano-Upcon-PE (**Figure 2c** or **Figure**
 275 **S12** for details). The incorporation of UCNPs led to smaller particles; we have no explanation
 276 for this result.



277

278 **Figure 2.** a) General scheme of upconverting nanoparticles (UCNPs) incorporated into
 279 polyethylene (PE) followed by a two-step powdering process to prepare micro- and
 280 nanometric particles. b) Granulometric analysis of the micrometric fraction obtained after
 281 cryogenic grinding, expressed as a percentage and cumulative percentage as a function of size.
 282 c) Nanoparticle tracking analysis (NTA) of the nanometric fraction obtained after wet milling
 283 and cascade filtration.

284 **Characterization of labelled micro- and nanoplastics**

285 ***Chemical integrity of PE***

286 Attenuated total reflection Fourier transform infrared (ATR-FTIR) analysis of the samples (on
287 bulk, micrometric and nanometric particles) was performed to determine if any PE
288 degradation occurred during synthesis or powdering. An important side reaction could be PE
289 oxidation leading, in particular, to the formation of carbonyl moieties, which is easily detected
290 by an intense absorption band in the region of 1650–1850 cm⁻¹.³⁸ FTIR spectra were obtained
291 after each processing step and compared to the spectra of raw PE pellets. No absorption band
292 due to the presence of oxidation was detected (**Figure S3**). This result indicates that synthesis
293 and grinding processes do not cause any chemical modification of PE.

294 ***Effectiveness of UCNPs incorporation in PE***

295 During the synthesis of the bulk material, the dissolution/precipitation procedure does not
296 allow control of the UCNP rate of incorporation. This amount was measured by two distinct
297 techniques: differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA). DSC
298 was used to accurately determine the PE crystallinity at each stage of the preparation. The
299 melting enthalpy (ΔH_m), onset temperature (T_{onset}), and exothermic peak temperature (T_{peak})
300 of crystallization were obtained from the second heating cycle (Table 1). If a polymer is mixed
301 with a material that does not melt in the same temperature range, DSC is a way to record in
302 what proportion it is added.³⁵ We selected the second cycle because this measurement does
303 not record the history of the material, such as the way it was cooled (however, the use of the
304 enthalpy recorded at the first cycle gave the same proportion of incorporated UCNPs, data
305 not shown).

306 The melting points were similar regardless of the stage of the process; this result confirms the
307 absence of PE chemical modification after the incorporation of UCNPs or powdering. Before
308 engagement in the dissolution/precipitation step, the PE raw pellet and raw powder had

309 similar melting enthalpies. After cryogenic grinding, the enthalpy of μ -Blank-PE was slightly
 310 superior, that is, the polymer was slightly more crystalline at this stage (85% crystallinity
 311 compared to 83% initially). Knowing that UCNPs are thermodynamically stable in the
 312 hexagonal phase up to 700 °C,³⁹ their presence did not contribute to the enthalpy recorded
 313 between 20 °C and 160 °C. This result led us to a UCNP-content in μ Upcon-PE of 6.4 ± 1.2 wt.%.
 314 This rate was confirmed by thermogravimetric analysis, which gave a value of 5.7% (Table S1).

315 **Table 1.** DSC analysis of raw PE pellets, μ -Blank-PE and μ -Upcon-PE. The melting enthalpy,
 316 onset temperature and exothermic peak temperature were obtained from the second heating
 317 cycle (average values \pm SD). The corresponding degrees of crystallinity (X_c) were calculated as
 318 the ratio between ΔH_m of the sample and the reference ΔH_{mref} value of a 100% crystalline form
 319 of PE (293 J g^{-1}).^[37,38]

320

Sample	ΔH_m , (J g^{-1})	T_{onset} , ($^{\circ}\text{C}$)	T_m , ($^{\circ}\text{C}$)	X_c , (%)
PE raw pellet	-243.7 ± 1.4	122.4 ± 0.1	128.3 ± 0.4	83.2 ± 0.5
PE raw powder	-244.2 ± 3.4	122.8 ± 0.2	128.5 ± 0.3	83.3 ± 1.2
μ -Blank-PE	-250.6 ± 2.9	122.2 ± 0.1	128.9 ± 0.2	85.5 ± 1.0
μ -Upcon-PE	-234.5 ± 1.5	122.4 ± 0.3	129.2 ± 0.3	

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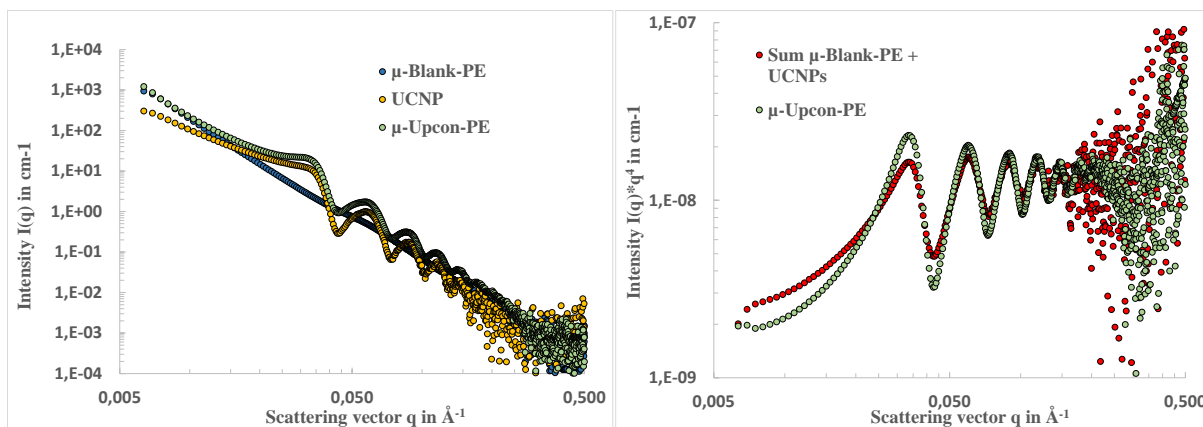
322 ***Study of UCNPs dispersion within PE***

323 The way UCNPs were incorporated in PE was investigated in detail with particles at the
 324 microscale by small-angle X-ray scattering (SAXS). Details of the applied models for SAXS are
 325 given in the Supporting Information. The SAXS curve of μ -Blank-PE (**Figure 3a** and **Figure S4**)
 326 shows a constant decay with a small inflection of approximately 0.05 \AA^{-1} , reflecting the
 327 correlation distance between crystalline parts present in the PE matrix. The SAXS curve of μ -
 328 Upcon-PE follows the same decay as the SAXS curve of μ -Blank-PE plus the contribution of
 329 UCNPs (**Figure 2a**). The first result confirms that the UCNPs do not alter the organization of

330 PE. The contribution of UCNPs was investigated to depict whether the UCNPs were either
331 adsorbed on the surface or only partially incorporated into PE. To discriminate the last case, a
332 simulated SAXS curve was obtained by a linear combination of the two contributions and
333 compared to the experimental curve (**Figure 3b**). Data were plotted in $\text{Log } I(q) \times q^4$ as a function
334 of $\text{Log } q$ to magnify the differences. No linear combination of the SAXS curves of PE and UCNPs
335 was found to fit the experimental SAXS data of Upcon-PE. The difference observed between
336 the curves suggests that another term contributes to the SAXS curves. In the case where
337 UCNPs are distributed in the PE matrix, it is necessary to consider the scattering cross terms,
338 which reflect the interactions between atoms of UCNPs and PE. A simplified model was
339 applied to describe μ -Upcon-PE, and a two-component model was applied to describe the
340 organization at large distances (see “Applied models for SAXS analysis” and **Figure S5** in the
341 Supporting Information for more details). The states of the surfaces of μ -Blank-PE and μ -
342 Upcon-PE were found to be similar, indicating that the incorporation of UCNPs does not
343 induce any modification of the surface particle properties. This inference suggests that UCNPs
344 are not adsorbed onto the surface of PE but rather fully incorporated inside the PE matrix.
345 This result is interesting since PE is not prone to easily accept the incorporation of other
346 materials, and fabrication of PE composites generally requires a more complex process than a
347 simple dissolution/precipitation procedure such as the one proposed here.^{40, 41} The effective
348 incorporation of UCNPs into PE prevents UCNPs from leaching and enables their detectability
349 regardless of the treatments or experiments performed (washing, purification, use in vivo,
350 etc.).

351 a)

b)



352

353 **Figure 3.** Small-angle X-ray scattering (SAXS) analysis of a) μ -Blank-PE (blue curve), μ -Upcon-
 354 PE (green curve) and UCNPs alone (yellow curve), where $\text{Log } I(q)$ is plotted as a function of $\text{Log } q$;
 355 b) μ -Upcon-PE spectrum and a simulated spectrum of a linear combination of the scattering
 356 curves of μ -Blank-PE and UCNPs, considered two independent contributions; $\text{Log } I(q) \times q^4$ is
 357 plotted as a function of $\text{Log } q$.

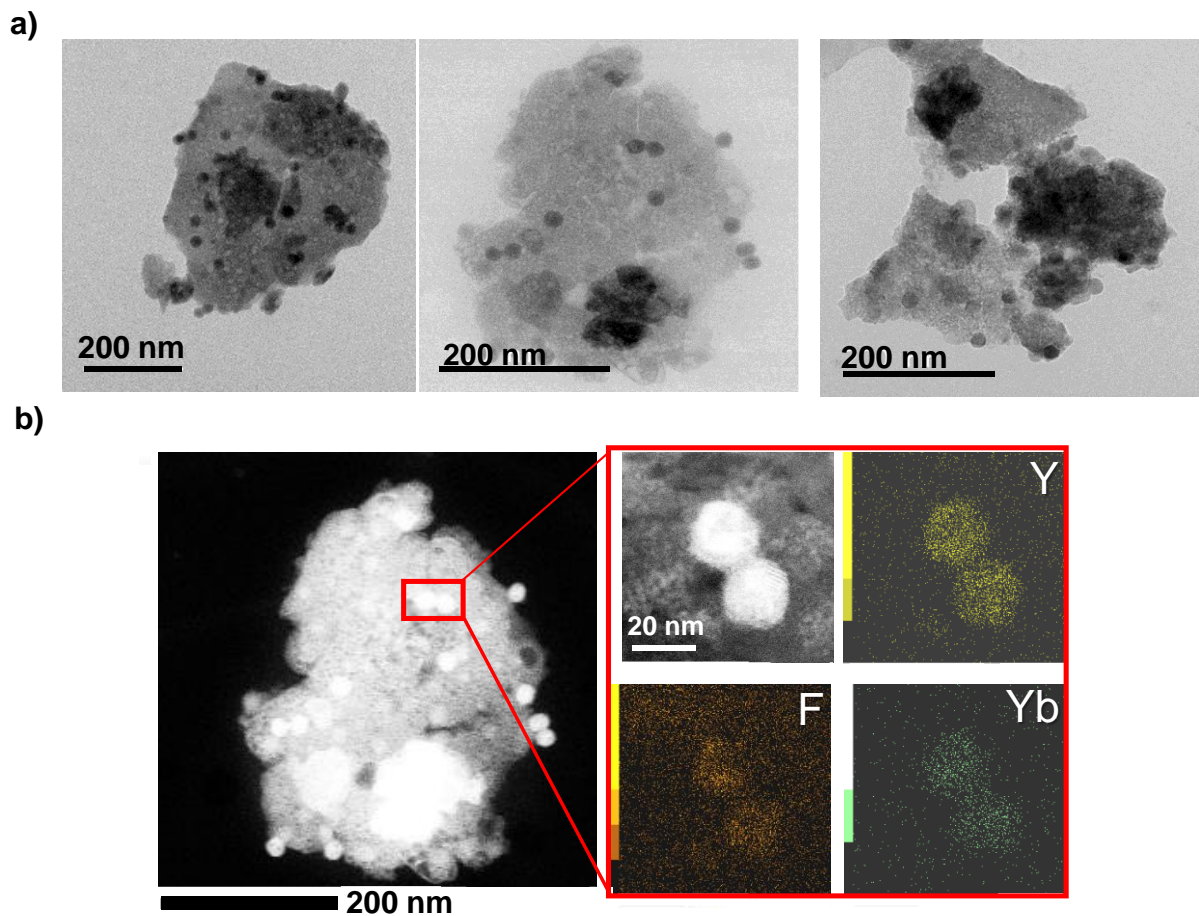
358

359 Colloidal properties of labelled nanoplastics

360 *Electron microscopy*

361 As discussed earlier, NTA allowed us to conclude that the particles prepared at the nanoscale
 362 were very polydisperse and mostly smaller than 200 nm. The shape of the particles was
 363 assessed by electron microscopy (TEM) and showed that the particles were indeed
 364 polydisperse and polymorphic (**Figure 4a**). The incorporation of UCNPs did not modify their
 365 shapes (data not shown). The heterogeneous shapes obtained by this top-down process are
 366 in accordance with a recent study.⁴² The darker dots in the particles were supposed to be
 367 UCNPs because they were expected to be highly dense, and their size was approximately 20
 368 nm (Figure 4a). The images showed that the UCNPs were rather well dispersed within PE
 369 particles. The identification of UCNPs was confirmed by high-resolution TEM coupled to
 370 energy-dispersive X-ray elemental analysis (TEM-EDX), as the dark dots showed the
 371 homogeneous distribution of F, Y and Yb (see **Figure 4 b** and **Figure S6** for more details). This

372 result showed that the chemical integrity of UCNPs was maintained during the powdering
373 process, as well as their sizes and shapes.



374

375 **Figure 4.** a) Transmission electron microscopy (TEM) bright-field images of nano-Upcon-PE.
376 The three images illustrate that the particles were polydisperse and polymorphic. b) High-
377 angle annular dark-field (HAADF) TEM of a single nano-Upcon-PE, along with energy-
378 dispersive X-ray characterization of yttrium (Y), fluorine (F) and ytterbium (Yb).
379

380 ***Zeta potential***

381 The surface charge of the produced micro- and nanometric particles was obtained by zeta-
382 potential (ζ -potential) measurements. At the micro- and nanoscales, the particles were found
383 to be negatively charged (Table S2). The presence of incorporated UCNPs did not significantly
384 modify the particle charges. The zeta potentials for the micro- and nanometric particles were
385 approximately -70 mV and -60 mV, respectively. These values were in accordance with a

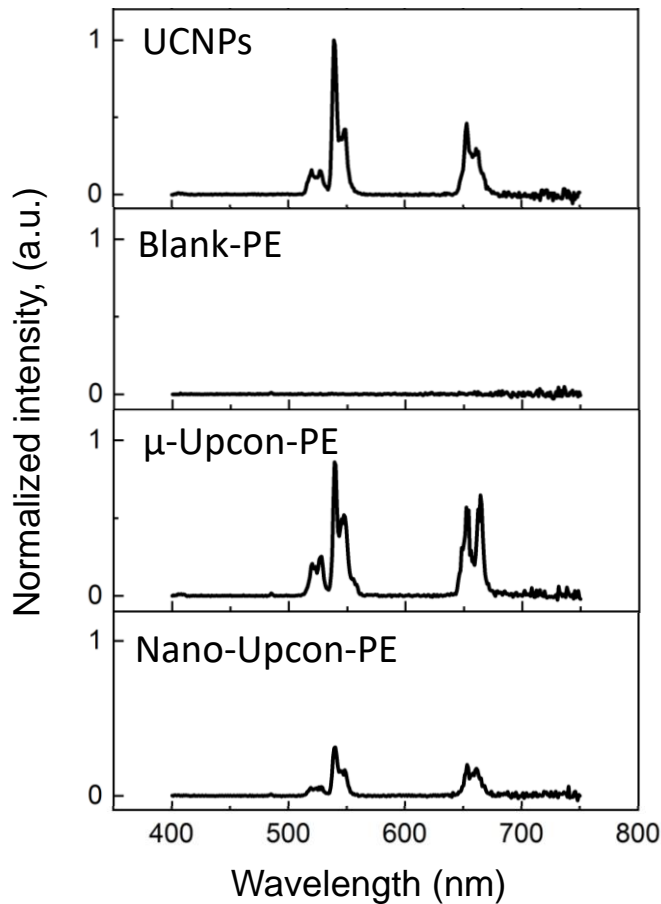
386 recent study using the top-down process; all the model NPs were negatively charged, and
387 those in PE had a zeta potential of -38 ± 2.4 mV at a similar pH.⁴²

388 It is expected that most NP surfaces would bear functional groups such as carboxylic acids or
389 hydroxyl groups resulting from the oxidation of the polymer; consequently, at neutral pH, they
390 are expected to be negatively charged. The model NPs prepared are also negatively charged.
391 This parameter is important, as the surface charge of nanoparticles dramatically affects their
392 behavior in the environment or in vivo.⁴³

393 **Emission properties of labeled nanoplastics**

394 The emission properties of the UCNPs once incorporated in PE were investigated.
395 Fluorescence measurements ($\lambda_{exc.} = 980$ nm) of the dispersed particles in ethanol showed no
396 emission for Blank-PE, while Upcon-PE showed three emission peaks at approximately 525,
397 550 and 655 nm, attributed to the Er³⁺ transitions $^2H_{11/2} \rightarrow ^4I_{15/2}$, $^4S_{3/2} \rightarrow ^4I_{15/2}$ and $^4F_{9/2} \rightarrow$
398 $^4I_{15/2}$.^[33] These specific emission peaks were observed with μ -Upcon-PE and nano-Upcon-PE
399 **(Figure 5)**.

400 To check whether the UCNPs maintained their functionality once encapsulated in PE, the
401 emission properties of all the prepared particles were investigated under upconversion
402 conditions. Under continuous-wave laser irradiation at 980 nm, the pristine Er-UCNPs
403 displayed typical green visible luminescence. The recorded spectrum of the dispersed UCNPs
404 in cyclohexane showed three emission peaks at approximately 525, 550 (green) and 655 nm
405 (red), attributed to the Er³⁺ transitions $^2H_{11/2} \rightarrow ^4I_{15/2}$, $^4S_{3/2} \rightarrow ^4I_{15/2}$ and $^4F_{9/2} \rightarrow ^4I_{15/2}$,
406 respectively.^[33] **(Figure 5)**. As expected, blank PE particles dispersed in ethanol did not show
407 any signal in this spectral range upon excitation under similar conditions. However, Upcon-PE
408 (micro- and nanoscale) displayed an emission spectrum similar to that of UCNPs: the
409 upconversion property was not lost in the PE incorporation/grinding and milling process.



411

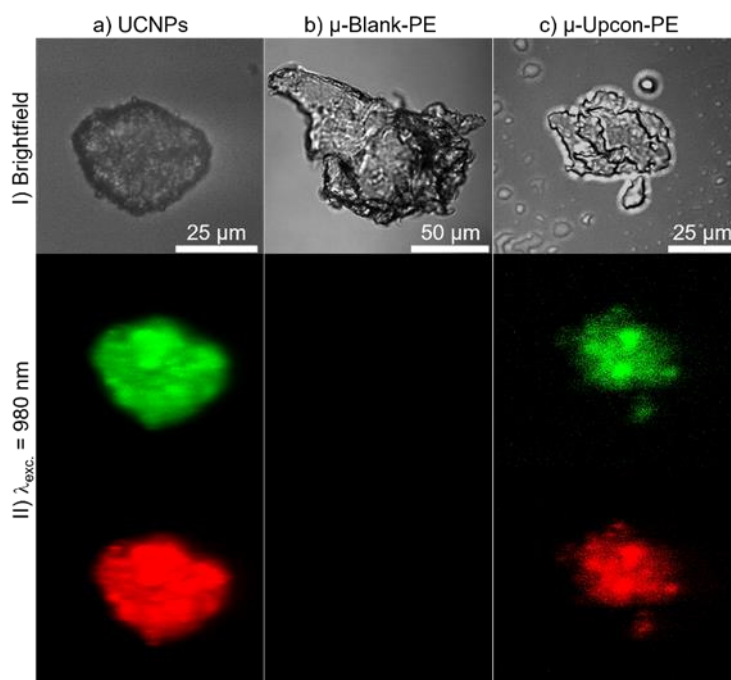
412 **Figure 5.** Upconversion emission spectra of UCNPs, Blank-PE, and Upcon-PE micro- and
 413 nanoparticles; normalized fluorescence intensity as a function of the wavelength ($\lambda_{exc.} = 980$
 414 nm). Acronyms: lanthanide-based upconverting nanophosphors (UCNPs), polyethylene (PE),
 415 raw PE (Blank-PE), UCNP-doped PE particles prepared at the microscale (μ -Upcon-PE), UCNP-
 416 doped PE particles prepared at the nanoscale (nano-Upcon-PE).

417

418 The suitability of these labeled PE particles for imaging purposes was then examined. UCNPs
 419 are known to be able to produce an upconversion spectrum under a large range of laser
 420 fluences; therefore, a standard 2-photon emission microscope setup was employed. To
 421 achieve our goal, the particles were spread over a microscope blade and examined under
 422 standard 2-photon microscopy conditions using NIR pulsed laser light. Images showed Z-
 423 projection in a maximum intensity. Under these conditions, control particles (μ -Blank-PE)
 424 remained nonemissive, while particles of μ -Upcon-PE gave rise to green (G) and red (R)

425 upconverted light that could be collected between 490 and 600 nm and 630 and 710 nm,
426 respectively. The absence of any emission from the blank PE particle indicates that no 2-
427 photon processes occur in pure PE and justifies the use of UCNPs as a background-free
428 luminescent tag for plastic particles. The band ratio G/R was found differ from that recorded
429 at lower fluence. It is known that the power dependence of the emission lines is rather
430 nonlinear; therefore, the band ratio typically observed at very low fluence, such as with a CW
431 980 nm laser, can be different when using a more intense pulse laser, which is commonly used
432 to trigger 2-photon fluorescence emission. This spectral alteration is also a signature of the
433 presence of UCNPs and can be used to identify tagged particles in more complex
434 environments.

435



436

437 Figure 6. I) Bright-field image of particle aggregates: a) UCNPs; b) μ -Blank-PE; c) μ UCNPs-PE.
438 II) Green and red emission under an NIR irradiation observer for a) UCNPs and c) μ UCNPs-PE;
439 b) μ -Blank-PE remains nonemissive.

440

441

442 **Conclusion**

443 This top-down synthesis allows the production of model plastic particles within the micro- and
444 nanometric range, which present heterogeneity in size and shape that is absent for most of
445 the particles formed by the bottom-up approaches. This method does not allow us to control
446 the amount of UCNPs incorporated in the polymer, as the parameters of the precipitation
447 process are difficult to control, but the UCNP content reaches 6% by weight. Using a series of
448 physicochemical characterizations, we demonstrated that the UCNPs were incorporated
449 within the polymer, not localized at its surface (SAXS), and well dispersed (TEM-EDX). The
450 powdering process does not alter the chemical structure or the morphology of the polymer
451 (ATR-FTIR and DSC). The mixture of micro- and nanometric objects formed can easily be
452 separated by filtration; thus, a range of sizes can be selected for a given application. The
453 nanometric particles were formed in a small proportion (6% yield), but the remaining
454 micrometric particles can be engaged in another long process of wet milling for further
455 production of smaller particles.

456 The incorporation of the UCNPs did not alter their luminescent properties, and the labeled PE
457 particles could be easily monitored with a standard two-photon microscope. This feature
458 offers the possibility to easily detect these model NPs in complex organic matrices, while the
459 detection of plastic itself is always difficult.

460 The proposed method is compatible with a large variety of polymers, while the UCNP rate of
461 incorporation in the polymer matrix is not easily controllable and certainly depends on the
462 polarity and affinity of the UCNPs for the polymer. However, this method also offers the
463 possibility to use formulated polymers, such as commercialized plastic, to produce plastic
464 particles even closer to those present in the environment. This process is also compatible with
465 weathered polymers.

466 The prepared particles are very good environmentally relevant models because of their
467 polydispersity and heterogeneity. These two properties are very scarcely considered in actual
468 toxicological evaluation studies because most of the particles used are produced with a
469 bottom-up method.¹⁰ Because of the extreme photostable and NIR-activable luminescent
470 properties of the prepared particles, strategies based on their use can pave the way for a
471 deeper understanding of the fate of plastic particles in the trophic chain.

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Supporting Information

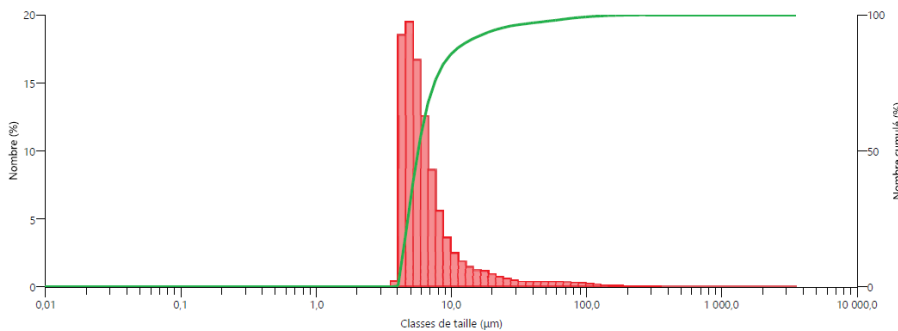
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610 Top-Down Synthesis of Luminescent Microplastics and Nanoplastics by Incorporation of 611 Upconverting Nanoparticles for Environmental Assessment

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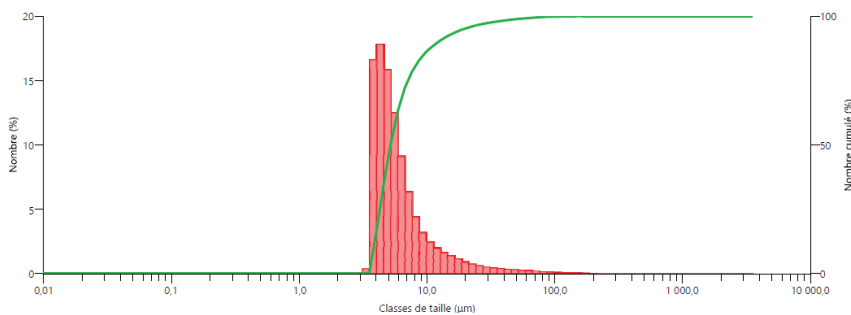
613 Nadiia Yakovenko^a, Baptiste Amouroux^a, Magali Albignac^a, Fabrice Collin^a, Clément Roux^a,
614 Anne-Françoise Mingotaud^a, Pierre Roblin^c, Christophe Coudret^{a*}, Alexandra Ter-Halle^{a*}
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616 a)



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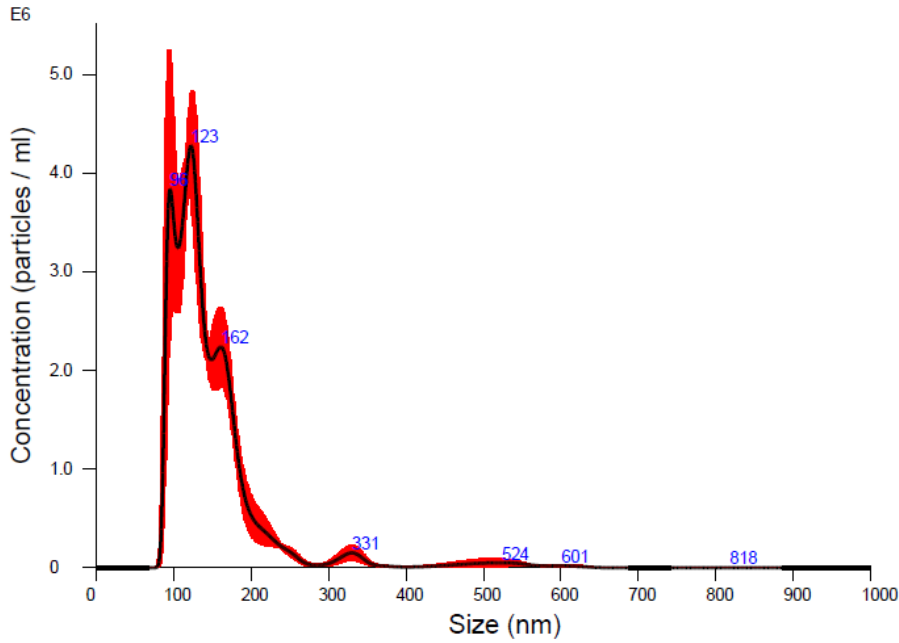
618 b)



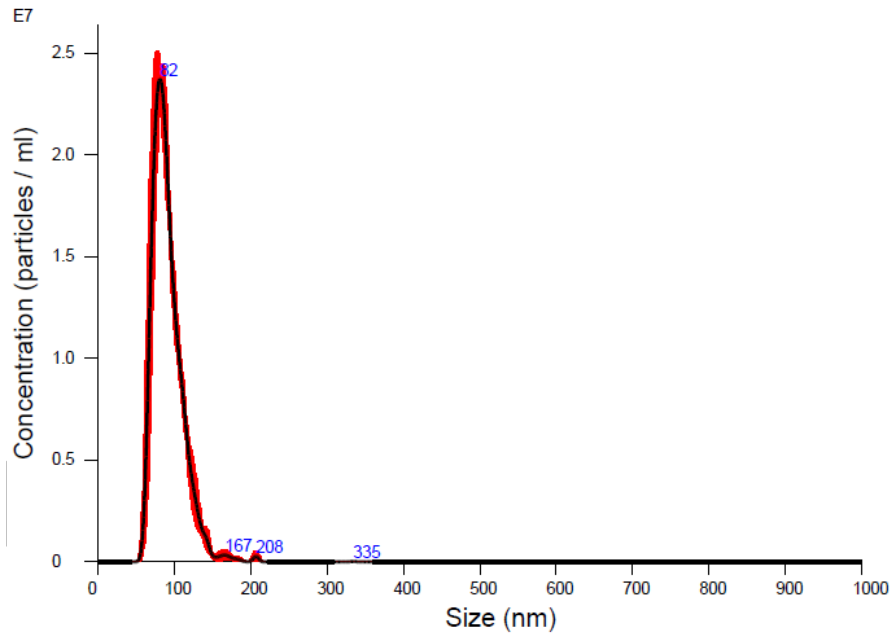
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620 **Figure S1.** Granulometric analysis after cryogenic grinding, expressed as a percentage and
621 cumulative percentage as a function of the size of a) the blank, where $D_n(10)= 4.31 \mu\text{m}$,
622 $D_n(50)=5.69 \mu\text{m}$ and $D_n(90)=13 \mu\text{m}$ and b) the UCNP-doped particle, where $D_n(10)= 3.82 \mu\text{m}$,
623 $D_n(50)=5.18 \mu\text{m}$ and $D_n(90)=12.3 \mu\text{m}$. The distribution of the particles is not significantly
624 different with or without incorporation of UCNPs. The parameter $D_n(x)$ is the value in the size
625 distribution, up to and including which $x\%$ of the total number of particles in the sample is
626 included. Acronyms: upconverting nanoparticles (UCNPs).

627



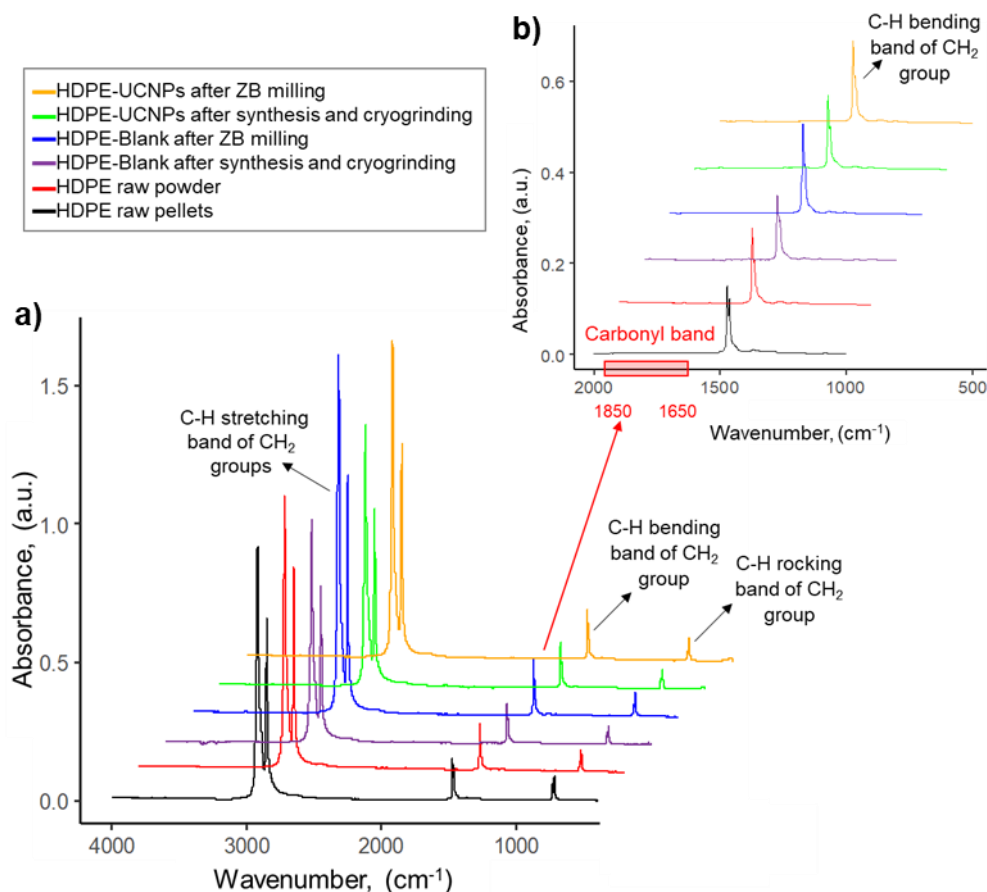
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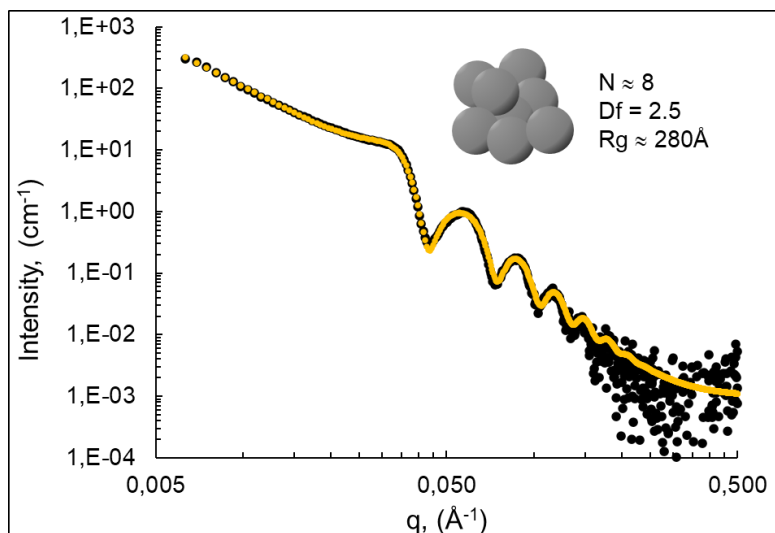
629

630 **Figure S2.** Size distributions of Blank-Upcon-PE and nano-Upcon-PE obtained by nanoparticle
 631 tracking analysis (NTA). Data are presented as the average concentration per size over three
 632 measurements. For Blank-Upcon-PE, the values obtained were $D_n(10)=96.9$ nm,
 633 $D_n(50)=130.9$ nm and $D_n(90)=201.4$ nm, and b) for nano-Upcon-PE, $D_n(10)=70.8$ nm,
 634 $D_n(50)=87.9$ nm and $D_n(90)=117.3$ nm. The distribution of the particles is not significantly
 635 different with or without incorporation of UCNPs. The parameter $D_n(x)$ is the value in the size
 636 distribution, up to and including which $x\%$ of the total number of particles in the sample is
 637 included. Acronyms: upconverting nanoparticles (UCNPs).

638



639
 640 **Figure S3.** Attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR) of
 641 raw PE and at different stages of particle synthesis were recorded using a Thermo Nicolet 6700
 642 spectrometer equipped with a diamond crystal attenuated total reflectance accessory and a
 643 deuterated triglycine sulfate (DTGS) detector. Background and sample spectra were recorded
 644 as the average of 16 scans in the spectral range of 650–4000 cm^{-1} at a resolution of 4 cm^{-1} .
 645 Recorded data were corrected to obtain transmission-like spectra using the ATR Thermo
 646 correction (the refractive index considered as 1.5). The carbonyl signal for all analyzed samples
 647 was integrated in the 1650–1850 cm^{-1} region. a) FTIR spectra of raw PE material and Blank-PE
 648 and Upcon-PE samples recorded after every processing step of MP synthesis (synthesis of bulk
 649 material, cryogenic grinding, ZB milling); b) zoom around the carbonyl region, showing the
 650 absence of a carbonyl band, which signifies that no oxidation occurs for PE during the synthesis
 651 of MPs.
 652



653

654 **Figure S4:** SAXS spectrum of UCNPs in solution. The experimental SAXS curve is plotted in Log
 655 $I(q)$ as a function of Log q (black dots). The scattering curve can be described as the scattering
 656 of an aggregate object containing hard spheres. The fitting curve corresponding to the
 657 modeled SAXS curve is plotted in red dots for a model compatible with the SAXS data that was
 658 found to have the following physical parameters: number of nanospheres per cluster $N = 8$,
 659 fractal dimension $Df = 2.5$ and gyration radius $Rg = 280 \pm 10 \text{ Å}$.

660

661 **Applied models for SAXS analysis**

662 The SAXS curves can be described by the following equation written with the SASView
663 program (SasView, <http://www.sasview.org/>):

664
665
$$I(q)_{global} = I(q)_{powerlaw} + I(q)_{sphere} * S(q)_{hardsphere} \quad (1)$$

666

667
$$I(q)_{global} = Aq^{-P} + \frac{B}{V} \left[\frac{3V(\Delta\rho)(\sin(qr) - qrcos(qr))}{(qr)^3} \right]^2 * S(q)$$

668

669 where A and B are scaling factors, and the sphere is described by the volume V, radius r and
670 contrast $\Delta\rho$. The nanospheres interact with each other, and the conditions where $S(q) = 1$ at
671 small angles were not verified. The form factor S(q) is described here with a “hard sphere
672 interaction model” described by Percus-Yevick (J.K. Percus, J. Yevick, J. Phys. Rev., 1958, 110,
673 1).

674 When UCNPs are distributed in the PE matrix, the scattering cross terms that reflect the
675 interactions between the atoms of UCNPs and PE must be considered. However, we can apply
676 the simplified model proposed in Equation (1) to describe Upcon-PE and a two-component
677 model to describe the organization of PE MPs at large distances as follows:

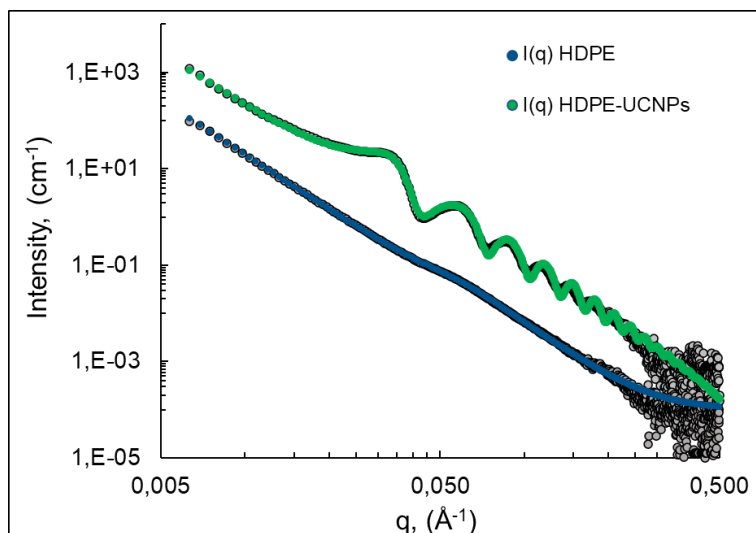
678
679
$$I(q) = Aq^{-P} + \frac{C}{(1+|q_0-q|\xi)^m} \quad (3)$$

680

681 where the second term represents a Lorentzian function to describe the interaction between
682 the crystalline clusters inside the plastic matrix. q_0 is the position of the peak corresponding
683 to the averaged distance separating the clusters ($q_0 = 0.05 \text{ \AA}^{-1} \Leftrightarrow d = 125 \text{ \AA}$).

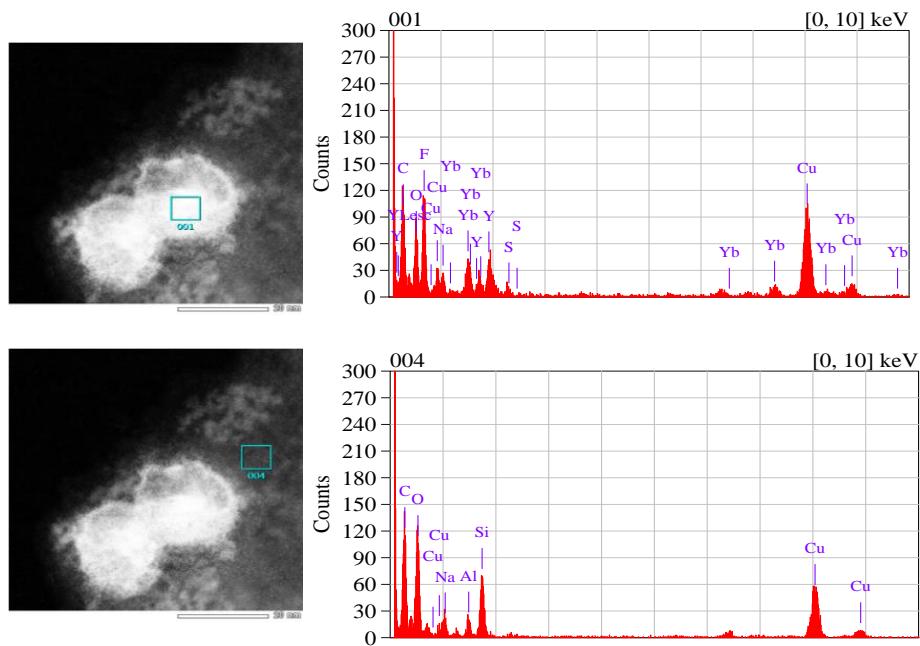
684
685 The most interesting term is the exponential parameter P in Eq. (1) and (3), which describes
686 the state of the particle surface. For both curves, this value is close to 3.7, corresponding to a
687 slightly rough surface. The absence of a difference in the value of P suggests that the presence
688 of UCNPs does not modify the state of the PE MP surface. Thus, these results suggest that
689 UCNPs are absorbed inside the PE matrix.

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Figure S5. SAXS spectra of Blank-PE and Upcon-PE, along with superimposed fitting curves (blue and green, respectively). The experimental SAXS curves are plotted in Log $I(q)$ as a function of Log q .



699
 700 **Figure S6.** TEM image and energy-dispersive X-ray spectra of Upcon-PE nanoparticles. The box
 701 indicates where the measurement was taken on UCNPs (top) and on the PE matrix (bottom).
 702 Fluorine, yttrium, and ytterbium were detected in UCNPs but were not present in the PE
 703 matrix.
 704

705

706 **Table S1.** The thermal stability of the samples was analyzed using thermogravimetric analysis
707 (TGA). The apparatus was a TGA/SDTA 851 Mettler Toledo device. Approximately 15 mg of
708 each sample was placed in 150 μ L aluminum pans with a pierced cover. The analysis was
709 performed from 30 to 900 $^{\circ}$ C at a heating rate of 10 $^{\circ}$ C/min under a nitrogen atmosphere with
710 a rated flow of 40 mL/min. For each sample, a blank (analysis with empty crucible) was carried
711 out under the same conditions as for the sample. All samples were analyzed in duplicate.

712

Sample	Mass, (mg)	T _{peak} , ($^{\circ}$ C)	Mass loss, (%)	Residue, (%)
PE raw powder	15.31	483	100.8	-0.8
Blank-PE	15.45	483	100.7	-0.7
Upcon-PE	15.84	481	94.3	5.7

713

714 **Table S2.** The average zeta potentials were measured in 10 mM NaCl at a native pH of 7.8 for
715 the micrometric particles and at pH 7.5 for the nanometric particles.
716

	Zeta potential (mV)
μ -Blank-PE	-71 ± 7
μ -Upcon-PE	-73 ± 8
nano-Blank-PE	-56 ± 11
nano-Upcon-PE	-62 ± 10

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