Labeling of HeLa cells using ZrO$_2$:Yb$^{3+}$-Er$^{3+}$ nanoparticles with upconversion emission

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Abstract. This work reports the synthesis, structural characterization, and optical properties of ZrO$_2$∶Yb$^{3+}$-Er$^{3+}$ (2–1 mol%) nanocrystals. The nanoparticles were coated with 3-aminopropyl triethoxysilane (APTES) and further modified with biomolecules, such as Biotin-Anti-rabbit (mouse IgG) and rabbit antibody-AntiKi-67, through a conjugation method. The conjugation was successfully confirmed by Fourier transform infrared, zeta potential, and dynamic light scattering. The internalization of the conjugated nanoparticles in human cervical cancer (HeLa) cells was followed by two-photon confocal microscopy. The ZrO$_2$∶Yb$^{3+}$-Er$^{3+}$ nanocrystals exhibited strong red emission under 970-nm excitation. Moreover, the luminescence change due to the addition of APTES molecules and biomolecules on the nanocrystals was also studied. These results demonstrate that ZrO$_2$∶Yb$^{3+}$-Er$^{3+}$ nanocrystals can be successfully functionalized with biomolecules to develop platforms for bio-labeling and bioimaging.

Keywords: photoluminescence; HeLa cells; Raman spectroscopy; zeta potential; dynamic light scattering; two-photon confocal microscopy.

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1 Introduction

Lanthanide-doped nanomaterials are promising platforms for bioapplications due to their ability to convert low-energy near-infrared (NIR) radiation into higher-energy visible luminescence through a process called upconversion (UPC). There are several potential benefits for the use of nanocrystals with UPC emission in biological applications, such as no damage to tissues; anti-Stokes emission; long lifetimes; photostability; increased contrast in biological specimens due to the absence of autofluorescence upon excitation with IR light; and simultaneous detection of multiple targeted analytes. Other advantages of the UPC emission are the reduction of photo-bleaching and scattering in tissues, which avoid the use of complicated and high-cost femtosecond lasers and photomultiplier tubes.

For biomedical applications, such as cancer detection, bio-labeling, and bioimaging, luminescent nanoparticles preferably have to form a stable colloidal solution under physiological conditions. However, common nanomaterials with strong UPC emission, such as Yb$^{3+}$:Er$^{3+}$ co-doped Y$_2$O$_2$S, Yb$^{3+}$:Ho$^{3+}$ co-doped Y$_2$O$_2$S, and Yb$^{3+}$:Er$^{3+}$:Tm$^{3+}$ doped NaYF$_4$, are hydrophobic. Some efforts have been made to convert hydrophobic UPC nanoparticles into hydrophilic ones using techniques such as polymer capping, surface silanization, and surface ligand oxidation. Recent methods also include ligand exchange in NaGdF$_4$:Ho$^{3+}$:Yb$^{3+}$ and NaYF$_4$:Yb$^{3+}$:Er$^{3+}$ phosphors.

Cancer detection in early stages is a priority for many medical groups around the world. In 2012, according to World Health Organization, cervical cancer was one of the most prevalent cancer types in the world. To detect and diagnose cancer, there are several biomarkers for example, the Ki-67 protein is expressed in all phases of the cell division cycle, but its expression level is strongly downregulated in the resting G0 phase. This characteristic makes the Ki-67 protein an excellent biomarker for cell proliferation. This biomolecule can be used as a prognostic marker in many types of cancers. Moreover, it has been demonstrated that cervical human cancer (HeLa) cells can be labeled using doped or undoped nanomaterials, such as NaYF$_4$:Yb$^{3+}$:Er$^{3+}$, NaYF$_4$:Yb$^{3+}$:Er$^{3+}$@CaF$_2$ core@shell, NaGdF$_4$:Yb$^{3+}$:Er$^{3+}$/Silica/Au, CaF$_2$ and carbon nanoparticles. These nanomaterials were internalized in HeLa cells observing visible light from the nanoparticles under IR excitation. Though these platforms are efficient for labeling HeLa cells, they still show several problems related to the complexity of their fabrication. For example, the synthesis of NaGdF$_4$:Yb$^{3+}$, Er$^{3+}$ nanoparticles have some drawbacks for biomedical applications; therefore, gold or silica need to be used to render them with biocompatible properties. In addition, one of the problems with carbon nanoparticles is the fact that they need to be excited with near-UV light, which can damage tissues around the cancer cells.

Rare earth doped zirconia (ZrO$_2$) nanophosphors present efficient emission in the visible region when they are under IR excitation. The ZrO$_2$ low phonon energy (470 cm$^{-1}$) increases the number and the probability of radiative transitions...
in rare earth doped ZrO2. Strong UPC emission has been obtained by doping ZrO2 with different pairs of rare earths, such as Yb3+-Er3+, Yb3+–Ho3+, Er3+, and Yb3+–Tm3+. Furthermore, ZrO2 nanoparticles can be synthesized by low-cost methods, such as sol-gel sol-emulsion-get spray pyrolysis and precipitation. Interestingly, ZrO2 is a nontoxic material; it has been used as a biocompatible dental material to make pigments.

Due to all of those reasons, ZrO2 is an excellent candidate for developing novel biolabeling and bioimaging platforms. In this work, ZrO2: Yb3+-Er3+ nanocrystals were chemically conjugated with an antiKi-67 protein by a novel method using (3-aminopropyl)triethoxysilane (APTES) and conjugated Biotin molecules as ligands. To the best of our knowledge, there are no reports about the use of luminescent ZrO2: Yb3+, Er3+ nanocrystals to label HeLa cells. Furthermore, the effect of the ligands on the luminescent properties of these nanoparticles was studied. In addition, the internalization of the conjugated nanoparticles in HeLa cells was followed by looking at their strong red luminescence using two-photon confocal microscopy. The results show the successful uptake of conjugated ZrO2: Yb3+-Er3+ nanoparticles in HeLa cells. We envision that this is a promising method for labeling different types of cancer cells for biosensing and bioimaging purposes.

2 Experimental

2.1 Preparation of ZrO2: Yb3+-Er3+ Nanoparticles

ZrO2: Yb3+-Er3+ nanoparticles were prepared following a precipitation process previously reported with some modifications. ZrOCl2·8H2O and YbCl3·6H2O (99.9%) were purchased from Aldrich, and ErCl3·6H2O (99.99%) was acquired from RE Acton. Ammonium hydroxide (NH4OH) at 30 vol% was supplied by Karal. In a typical experiment, Yb3+-Er3+ co-doped ZrO2 with a molar ratio of 2:1 for Yb3+:Er3+ was prepared by dissolving 2.633 g of ZrOCl2, 0.2362 g of YbCl3·6H2O (2 mol % of YbO2), and 0.1351 g of ErCl3·6H2O (1 mol % of Er2O3) in 50 ml of a mixture of H2O/EOH (1:1 wt%). After 15 min under stirring, the non-ionic surfactant Pluronic F127 was introduced in the mixture at a molar ratio of F127/ZrO2 = 0.0082. Afterward, 30 ml of NH4OH was added to precipitate the salts. The resulted suspensions were transferred into a sealed autoclave and a hydrothermal treatment was carried out at 80°C for 12 h. After this, the autoclave was allowed to cool down for 30 min and the solutions were washed twice with absolute ethanol and water in a centrifuge at 4000 rpm for 10 min. Subsequently, the powders were put in a ceramic crucible and dried at 80°C for 12 h. Finally, all samples were annealed at 1000°C using a heating rate of 5°C/min.

2.2 Conjugation and Functionalization of ZrO2: Yb3+-Er3+ Nanoparticles

The conjugation of ZrO2: Yb3+-Er3+ nanoparticles with the Ki-67 protein was carried out by following a previously reported method with some modifications. This process was performed as follows: 0.1 g of Yb3+-Er3+ doped ZrO2 nanoparticles were stirred with 490 μl of APTES for 24 hours. This bifunctional compound has amine- and alkoxysilane groups. The alkoxysilane reacts with the OH moieties on the ZrO2: Yb3+-Er3+ nanoparticles surface, leaving the amino groups exposed for further functionalization. The samples were washed once with ethanol and water to eliminate the excess of residues and centrifuged at 6000 rpm for 10 min. The samples were dried at 40°C for 12 h. The Yb3+-Er3+ co-doped ZrO2 nanoparticles coated with APTES were dispersed in 670 μl of phosphate buffered saline (PBS) 1× (pH = 7.4) and then 200 μl of 1:500 Biotin-Anti-rabbit (mouse IgG) from BIOCARE was added to the suspension to bind the carboxylic acid groups of the IgG with the amino groups exposed in the nanoparticles; this suspension was kept at 4°C for 12 h. After that, the nanoparticles were washed with distilled water and centrifuged at 6000 rpm for 10 min to remove the supernatant. The conjugated material was kept at 37°C for 12 h. Subsequently, 300 μl of PBS 1× and 10 μl of antigen Ki-67-rabbit antibody from BIOCARE were added to the nanoparticles and stored for another 12 h at 4°C. Finally, the conjugated ZrO2: Yb3+-Er3+ nanoparticles were washed with distilled water and centrifuged at 6000 rpm for 10 min. The final material was dispersed and stored in distilled water.

2.3 Structural Characterization (X-Ray Diffraction, Raman, HRTEM, SEM, Fourier Transform Infrared)

X-ray diffraction (XRD) patterns were obtained using a SIEMENS D-5005 equipment using a Cu tube with Kα radiation at 1.5405 Å, scanning in the 2θ to 80 deg 2θ range with increments of 0.02 deg and a sweep time of 2 s. Raman patterns were obtained using a Renishaw Raman System (inVia Raman Microscope), which uses a 785-nm laser and a 50× objective. The nanoparticles were suspended in isopropyl alcohol at room temperature and dispersed with ultrasonication. Afterward, the solution of nanoparticles was dropped on a 3-mm-diameter lacey carbon copper grids to obtain the HRTEM images in an FEI Titan 80-300 with an accelerating voltage set to 300 kV. In addition, the nanoparticle micrographs were obtained by an SEM Hitachi SU8010 at 30.0 kV. The Fourier transform infrared (FTIR) spectra were obtained using a Perkin-Elmer spectrophotometer with a deuterated triglycine sulfate detector and a spectral resolution of 4 cm⁻¹. The samples were prepared using the KBr pellet method and the spectra were obtained in the range of 1000 to 4000 cm⁻¹.

2.4 Photoluminescence Characterization

Photoluminescence characterization was performed using a continuous wave semiconductor laser diode with an excitation power of 350 mW and centered at 970 nm. The luminescence emission was analyzed with a Spectrograph Spectra Pro 2300i and a R955 photomultiplier tube from Hamamatsu. The system was PC controlled with Spectra Sense software. The samples were supported in 1 mm capillary tubes in order to guarantee the same quantity of excited material. Special care was taken to maintain the alignment of the setup in order to compare the intensities between different characterized samples. All measurements were performed at room temperature.

2.5 Incubation and Confocal Microscopy

HeLa cells were grown at a density of 5 × 10⁴ cells/mL in six-well culture plates with coverslips at the bottom and incubated in 3 ml of RPMI-1640 cell media for 24 h at 37°C under 5% CO2. After this, the cell media was replaced by 3 ml of ZrO2: Yb3+-Er3+ nanoparticles, ZrO2: Yb3+-Er3+-APTES, and ZrO2: Yb3+-Er3+-APTES-Biotin-Anti-rabbit/rabbit antibody-AntiKi-67
with a concentration of 100 μg/mL and was incubated for 6 h. Finally, the cell-plated coverslips corresponding to each sample were washed twice with PBS buffer (1 mM, pH 7.4) and stained with nuclei-staining NucBlue® Live solution for 15 min. All the cell-plated coverslips were fixed with a solution of 4% formaldehyde. The fixed and stained coverslips were placed in microscope slides and analyzed under a two-photon Olympus FV1000 MPE SIM laser scanning confocal microscope.

### 2.6 Zeta Potential and Dynamic Light Scattering Measurements

Dynamic light scattering (DLS) and zeta potential measurements were carried out using a Malvern Instrument Zetasizer Nano (red laser 633 nm). The samples were dispersed in PBS (1 mM, pH = 7.4) with a concentration of 1 mg/mL. The DLS and zeta potential were analyzed at 25°C.

### 3 Results and Discussion

#### 3.1 Crystalline Structure and Morphology

The XRD pattern of the ZrO$_2$·Yb$^{3+}$·Er$^{3+}$ nanopowder is shown in Fig. 1(a). This plot shows peaks corresponding to (1,0,1), (0,1,1), (2,1,1), and (1,1,2) planes, respectively. All the peaks are associated with the tetragonal phase of zirconia, according to the JCPDS 37-1413 card.\footnote{Fig. 1(a)} The ZrO$_2$·Yb$^{3+}$·Er$^{3+}$ nanopowder obtained by the precipitation method was analyzed by Raman spectroscopy, see Fig. 1(b). The peaks at 626, 552, 525, 445, 336, 260, 238, and 185 cm$^{-1}$ represent the spectrum. The peaks located at 445 and 626 cm$^{-1}$ as well as the shoulders located at 185 and 260 cm$^{-1}$ are in agreement with the tetragonal phase of zirconia.\footnote{Fig. 1(b)} The nanocrystal sizes were determined by TEM and a representative micrograph is presented in Fig. 2(a). The nanocrystals have an average size of 20 nm and spherical shape. Besides, Fig. 2(b) is an SEM image, which shows well-dispersed nanocrystals, and this was caused by the introduction of PF127 during the synthesis process.\footnote{Fig. 2(b)} The size and dispersion of the co-doped ZrO$_2$·Yb$^{3+}$·Er$^{3+}$ nanocrystals was controlled from the nucleation process due to the presence of ammonia, water/ethanol, and surfactant Pluronic PF127.\footnote{Fig. 2(b)} To promote the efficient internalization in HeLa cells, it is important to have particles in the nanoscale size regime. In addition, the colloidal stability of the nanoparticles is also significant to avoid the formation of aggregates, which may prevent the effective interaction between the nanoparticles and the cell surface.

#### 3.2 FTIR, Zeta-Potential, and DLS

Figure 3 shows the FTIR spectra of ZrO$_2$·Yb$^{3+}$·Er$^{3+}$ nanocrystals and ZrO$_2$·Yb$^{3+}$·Er$^{3+}$ prepared with APTES, Biotin-Anti-rabbit (mouse IgG), and rabbit antibody-AntiKi-67, respectively. These spectra provide information regarding functional groups and impurities on the surface of nanoparticles. They also corroborated that the process of functionalization and conjugation was successfully achieved. Figure 3(a) shows the FTIR spectra of nonfunctionalized nanoparticles ZrO$_2$·Yb$^{3+}$·Er$^{3+}$. It depicts small peaks associated with OH groups in the range of 3000 to 3600 cm$^{-1}$. Moreover, a peak is also observed at 450 cm$^{-1}$, which is related to the Zr–O stretching vibrations.\footnote{Fig. 3(a)} The spectrum in Fig. 3(b) shows a broadening of the bands centered at 3600 and 564 cm$^{-1}$ due to the presence of Si–OH and Si–O–Si bonds, respectively.\footnote{Fig. 3(b)} Other peaks at ~2923 and 2351 cm$^{-1}$ are related to C–H bonds and CO$_2$ impurities, respectively. The band located in the range of 3000 to 3400 cm$^{-1}$ is associated with amine groups.\footnote{Fig. 3(b)} The CO$_2$ impurities adsorbed in the surface of the nanoparticles can come from the synthesis and/or the environment during the
measurement process, which was probably caused by the granular characteristic of the nanopowder. The OH groups were introduced during the hydrolysis and condensation process where the M-OH (M = Zr, Er, and Yb) bond was formed due to the excess of hydroxyls in solution. According to the FTIR spectra in Figs. 3(a) and 3(b), the contamination produced by those hydroxyl groups is very low. Biotin-Anti-rabbit (mouse IgG) protein is conjugated to the nanoparticles containing APTES by forming an amide bond between the free amino groups located at the surface of ZrO$_2$:Yb$^{3+}$-Er$^{3+}$/APTES and the carboxylic acid groups exposed in the IgG protein. The ZrO$_2$:Yb$^{3+}$-Er$^{3+}$/APTES/Biotin FTIR spectrum is shown in Fig. 3(c); the spectrum illustrates a new band associated with the amide bond at 1770 cm$^{-1}$. Moreover, a peak centered at 658 cm$^{-1}$ is also associated with Biotin according to literature [65,66]. These data further confirm the functionalization of nanoparticles. The next step is to analyze the process of conjugation with the antigen Ki-67-rabbit antibody (ZrO$_2$:Yb$^{3+}$-Er$^{3+}$/APTES/Biotin/AntiKi-67), see Fig. 4. The bands related to Biotin are still observed, and there is a general decrease of the peaks related to impurities such as CO$_2$ and OH radicals. However, it is observed that there is a widening of the 658 cm$^{-1}$ band when AntiKi-67 is added. Based on this information, it is expected that AntiKi-67 is readily available to interact with HeLa cells.

The conjugation of the nanoparticles was also analyzed by zeta potential and DLS measurements, see Table 1. The zeta potential changed from negative to positive when the ZrO$_2$:Yb$^{3+}$-Er$^{3+}$ nanoparticles surface is modified with APTES, which is an indication that the amino groups are covering the nanoparticles surface. Moreover, the zeta potential was shifted from positive to negative after Biotin and antigen Ki-67 proteins were chemically attached to the surface of the material, suggesting the presence of carboxylate groups. A value of $-36$ mV obtained in ZrO$_2$:Yb$^{3+}$-Er$^{3+}$/APTES/Biotin/AntiKi-67 also indicates that nanoparticles can be stable in PBS due to their high electrostatic repulsion, which is suitable for bioapplications. A high negative value also suggests a high adsorption of nanoparticles on the nucleus of HeLa cells. Moreover, DLS measurements showed that the hydrodynamic diameter of the nanoparticles increased when the different molecules were added; the average sizes for ZrO$_2$:Yb$^{3+}$-Er$^{3+}$, ZrO$_2$:Yb$^{3+}$-Er$^{3+}$/APTES, and ZrO$_2$:Yb$^{3+}$-Er$^{3+}$/APTES/Biotin/AntiKi-67 were 748, 1232, and 4694 nm, see Table 1. The size of the nanoparticles in ZrO$_2$:Yb$^{3+}$-Er$^{3+}$ does not coincide with the one measured with TEM, probably due to the agglomeration of nanoparticles that were dispersed in PBS.

Figure 5 shows a schematic representation for the functionalization and conjugation of the nanoparticles. The OH moieties produced after ZrO$_2$:Yb$^{3+}$-Er$^{3+}$ synthesis react with the alkoxysilane groups of APTES to afford a silica shell on the nanoparticles leaving the amine groups exposed on the surface of the material. In the next step of the reaction, the Biotin-Anti-rabbit (mouse IgG) molecule is conjugated to the amino groups by using the COOH moieties of the IgG. At this point, the Anti-rabbit can interact with the biomolecule AntiKi-67-rabbit antibody.

### 3.3 Luminescent Properties

The mechanism of UPC emission in Er$^{3+}$:Yb$^{3+}$ co-doped ZrO$_2$ is well established in the literature. Figure 6 shows a strong red emission band with peaks at 653 and 657 nm as well as a weak green band after excitation at 970 nm. Green and red emission bands are assigned to $2_{11/2}$ + $4_{S_{1/2}}$ → $4_{15/2}$ and $4_{S_{9/2}}$ → $4_{11/2}$ transitions of the Er$^{3+}$ ion, and they are caused by the successive absorption of two photons after energy transfer from Yb$^{3+}$ ion. According to previous works, the emission is predominantly red because OH groups have a vibrational energy (3000 to 4000 cm$^{-1}$) which produces nonradiative relaxations from the mixed level $2_{11/2}$ + $4_{S_{1/2}}$ toward the $4_{S_{9/2}}$ level. In our case, the presence of OH moieties in all samples is corroborated by the FTIR spectra in Fig. 3. The inset in Fig. 3 shows that the integrated emission corresponds to the red band.

Moreover, it is observed that the red emission of the samples ZrO$_2$:Yb$^{3+}$-Er$^{3+}$/APTES (Z-A) and ZrO$_2$:Yb$^{3+}$-Er$^{3+}$/APTES/Biotin (Z-A-B) decreases progressively with respect to the sample of reference without conjugated (ZrO$_2$:Yb$^{3+}$-Er$^{3+}$) (Z). Nevertheless, the emission was improved when the

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**Table 1** Nanoparticle characterization using dynamic light scattering (DLS) and zeta potential for ZrO$_2$:Yb$^{3+}$-Er$^{3+}$, ZrO$_2$:Yb$^{3+}$-Er$^{3+}$-[3-aminopropyl]triethoxysilane (APTES), and ZrO$_2$:Yb$^{3+}$-Er$^{3+}$/APTES/Biotin-AntiKi-67 nanocrystals.

<table>
<thead>
<tr>
<th>ZrO$_2$:Yb$^{3+}$-Er$^{3+}$</th>
<th>ZrO$_2$:Yb$^{3+}$-Er$^{3+}$/APTES</th>
<th>ZrO$_2$:Yb$^{3+}$-Er$^{3+}$/APTES/Biotin-AntiKi-67</th>
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<tbody>
<tr>
<td>DLS (d, nm)</td>
<td>748</td>
<td>1232</td>
</tr>
<tr>
<td>Zeta potential (mV)</td>
<td>$-36.8$</td>
<td>$+10.8$</td>
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<tr>
<td></td>
<td></td>
<td>$-36.0$</td>
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nanoparticles were conjugated with AntiKi-67 (APTES-Biotin-AntiKi-67) (Z-A-B-K). The integrated red emission diminished with the addition of APTES and Biotin molecules because other contaminants, such as CO₂, C—H, and amine groups, appeared and the presence of hydroxyls increased. These elements may act as quenching centers of luminescence and also create defects, which behave as traps for luminescence.\(^7\) It is important to point out that the sample with APTES-Biotin had the highest levels of impurities [see Fig. 3(c)]; therefore, it showed the lowest luminescence. In contrast, the sample with APTES-Biotin-AntiKi-67 had the lowest amount of contaminants (OH, CO₂, and C—H) [see Fig. 3(d)]; therefore, it presented the highest red emission, see Fig. 5.

3.4 Imaging of ZrO₂:Yb³⁺-Er³⁺ Nanocrystals Incubated in HeLa Cells

Figure 6 shows the images obtained by the two-photon confocal microscope after HeLa cells were incubated with the different materials synthesized in this work. Figure 6(a) shows that the ZrO₂:Yb³⁺-Er³⁺ nanoparticles are situated out of the cell, probably due to the negative charge on the surface of the nanoparticles, which limits the internalization in HeLa cells. Figure 6(b) depicts ZrO₂:Yb³⁺-Er³⁺/APTES nanoparticles located on the cytoplasm of HeLa cells; these nanoparticles have no AntiKi-67, but they have APTES on their surface; this indicates that the positive charge on the surface of the nanoparticles enhances the internalization in HeLa cells. Figure 6(c) is an image of HeLa cells with nanoparticles conjugated with AntiKi-67; it is observed that 6 h of incubation is sufficient to reach the cytoplasm of HeLa cells. It is observed that there are a greater number of particles within the cell and near the nucleus. The most accepted theory is that nanoparticles are internalized via endosome-mediated transport or through ribosome exchanges. In general, the red emission from nanoparticles is strong in all images, which demonstrates the efficient luminescence generated by the nanoparticles synthesized in this work. It is worth noting that there was no autofluorescence from the cells after exciting the UPC nanoparticles with 970 nm. Furthermore, these images denote different sizes of emission points; this is probably induced by the nanoparticle agglomeration.
Compared to other methods for the conjugation of nanoparticles, our technique avoids the use of other elements, such as carbon and citrate, which are relatively toxic. Moreover, it uses biomolecules (antigen and antibody) to lead our nanoparticle toward a targeted organelle; to the best of our knowledge, this kind of molecule has not been used on luminescent nanoparticles. Finally, further research is needed not only to improve the distribution and internalization of nanoparticles, but also to label specific organelles inside the HeLa cells. Those studies are in progress, and they will be presented in a subsequent work.

4 Conclusions

In summary, we conjugated Yb$^{3+}$:Er$^{3+}$ co-doped ZrO$_2$ nanoparticles using Biotin-Anti-rabbit (mouse IgG) and rabbit antibody-AntiKi-67 biomolecules. The successful conjugation was confirmed by FTIR, zeta potential, and DLS. The nanoparticles internalized in HeLa cells demonstrated a strong red luminescence and were observed using a two-photon confocal microscope. The photoluminescence spectra indicated that the UPC red emission of Er$^{3+}$ ions is affected by the molecules located on the nanocrystals’ surface. An enhancement of the red emission was obtained in the nanoparticles with the conjugation with AntiKi-67. This was mainly caused by an enormous reduction of impurities compared to the rest of the samples. Our results indicate that the method of conjugation depicted in this work can be a promising alternative to afford stable colloidal dispersions of nanoparticles in water and efficiently label cancer cells.

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