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Highly Bright Eu-complex Doped Semiconducting Polymer Dots with Ultranarrow Emission and Long Luminescence Lifetime for Time-Gated Cellular Imaging**

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Polymer dots (Pdots) have emerged as a new type of nanoprobe that have a wide range of applications in fluorescence imaging and biosensing.^[1] Compared to small fluorescent dyes and Qdots, Pdots possess larger absorption cross-sections, faster emission rate, and better photostability.^[2, 3] These properties have enabled them to be used for single-particle/carrier tracking over long periods of time.^[4, 5] Moreover, the full width at half maximum (FWHM) of the Pdots' emission band can be as narrow as ~40 nm, an important feature for

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multiplexed assays.^[2, 6] In addition, the size of Pdots can be tuned to be less than 10 nm in diameter, which makes them excellent nanoprobe for targeted subcellular labeling experiments.^[7] The applicability of the Pdots' superior optical properties has also been demonstrated for tumor imaging in animals.^[8]

Organic europium complexes (Eu-complexes) are bright, luminescent materials which have been used for applications such as making organic light-emitting diodes^[9] and bioimaging^[10–12]. Eu-complexes have several merits not found in other organic fluorescent dyes: 1) their luminescence emission is usually line-like with FWHM smaller than 10 nm; 2) the emission spectra are less affected by the environment, such as fluctuations in oxygen concentration, compared to emission spectra of other heavy metal complexes;^[13] 3) they have a large Stokes shift; 4) they have long luminescence lifetime.^[14] Various kinds of luminescent nanoparticles have been developed by doping Eu-complexes into a matrix, such as silica or a non-fluorescent polymer.^[15–19] But because of a self-quenching issue among Eu-complexes, the loading ratio of Eu-complexes into the matrix has been kept to low levels to improve the quantum yield (QY).^[15, 20] Therefore, for these types of fluorescent nanoparticles, the total brightness still has been limited.

Our recent study has proved that Pdots can act as excellent host matrices and good energy-transfer donors for small organic dyes and inorganic nanoparticles.^[21–23] Here, we doped Eu-complexes into Pdots made of the semiconducting polymer PVK. Not only did PVK function as a host matrix to disperse the Eu-complex molecules and reduce the Eu-complex molecules' self-quenching, it also acted as an efficient fluorescence energy donor with a large absorption cross-section to transfer energy to the Eu-complexes. The Eu-complex/PVK Pdots had ultra-narrow red fluorescence emission and showed much higher emission brightness than pure Eu-complex nanoparticles. In addition, the long luminescence lifetime of the Eu-complex was carried over to the Eu-complex/PVK Pdots; we demonstrated this long luminescence lifetime was useful in particle differentiation and cell imaging with a greatly improved signal-to-noise (S/N) ratio.

The method to prepare Eu-complex/PVK Pdots was based on nanoprecipitation^[24] as depicted in Scheme 1b. Here, an Eu-complex (Eu15F or EuDNM) was mixed with the semiconducting polymer PVK and block copolymer PS-PEG-COOH in THF. After being injected into water, the polymer chains collapsed to form nanoparticles and embedded Eu-complexes inside the nanoparticles. The sizes of Eu15F/PVK and EuDNM/PVK Pdots were measured by dynamic light scattering (DLS) to be smaller than 20 nm in diameter, which were confirmed by high-resolution transmission electron microscopy (TEM) images (Figure 1a–d).

In this work, PVK was chosen as the host matrix because its emission spectrum overlapped with the excitation spectra of Eu15F and EuDNM (Figure 1e, f). The spectral overlap made it possible for Förster resonance energy transfer (FRET) to take place between the donor PVK and the acceptor Eu-complexes inside the Eu-complex/PVK Pdots. For example, as shown in the emission spectra (Figure 1e), the emission peak of the pure PVK Pdots was centered at 398 nm when the excitation wavelength was 342 nm; when the Eu-complex/PVK Pdots were excited at 342 nm, a red-shifted emission was observed whose peak was centered at ~ 612 nm. In addition, with the increase of emission intensity at 612 nm, the emission intensity at 398 nm (from PVK) dropped significantly even when the ratio of doped Eu-complex to PVK was only 20% (Figure 1g and h). The trend was observed for both Eu15F/PVK and EuDNM/PVK Pdots. Moreover, the emission of the Eu-complex/PVK Pdots was very narrow. For pure PVK Pdots, its broad emission covered the range from 350 nm – 500 nm; for Eu-complex/PVK Pdots, the FWHM of the emission peak (612 nm) was only 8 nm, which is also much narrower than that of most organic dyes and Qdots.

The QY values of the Eu-complex/PVK Pdots were also measured at various Eu-complex doping ratios. First, pure Eu-complex nanoparticles were prepared by injecting Eu-complexes dissolved in THF into water. The measured QYs of pure Eu15F and EuDNM nanoparticles were 7.8% and 5.8%, respectively. For both Eu complexes at the doping ratio of 60 wt% in the PVK host polymer, the QY increased to be as high as 33.5% for Eu15F/PVK Pdots and 11.2% for EuDNM/PVK Pdots. The significant QY enhancement indicated that the self-quenching among Eu-complex molecules inside the Eu-complex/PVK Pdots was greatly reduced. We noted that when the doping ratio of the Eu-complex reached 80 wt %, the QY of Eu-complex/PVK Pdots began to decrease slightly (Figure 1g, 1h), which was possibly due to concentration quenching. The high doping ratio of Eu-complex and extinction coefficient of PVK made it possible for the Eu-complex/PVK Pdots to have a large absorption cross-section (Table 1), which was on the same order of magnitude as that of other reported Pdots.^[24, 25] Because of their high absorption cross-section, doping ratio, and quantum yield, the Eu-complex/PVK Pdots exhibited great single-particle brightness. In the Eu-complex/polymer^[20] nanoparticles or Eu-complex/silica^[16, 17] nanoparticles reported so far in the literature, they may have high quantum yields but the single-particle brightness of these kinds of fluorescent nanoparticles have been low. The doping ratios of Eu-complex inside these nanoparticles were usually kept at a low level (smaller than 5%)^[16, 17], and the host materials (silica or latex polymer) are generally transparent in the region of 300–650 nm; these properties do not help to increase the single-particle absorption cross-section. Therefore, our Pdots provided a new way to decrease the self-quenching of Eu-complex fluorescence and reach high single-particle brightness.

We have recently performed surface-bioconjugation reactions to Pdots successfully and demonstrated the application of Pdot-streptavidin (Pdot-SA) bioconjugates to *in vitro* targeting of specific cellular components and *in vivo* tumor targeting.^[24] Similarly, for this work, we conjugated the Eu-complex/PVK Pdots with streptavidin using the same strategy. The Eu-complex/PVK-streptavidin conjugates successfully labeled the cell surface receptor EpCAM (Scheme 1c). As shown in the flow cytometry results in Figure 2a and 2b, the positively labeled cells (incubated with Eu-complex/PVK Pdot-streptavidin in the presence of the biotinylated primary antibody) were distinctly separated from the negatively labeled cells (incubated with Eu-PVK Pdot-streptavidin in the absence of the biotinylated primary antibody). The specific cellular labeling was also confirmed by fluorescence microscopy because the positively labeled cells emitted very strong red fluorescence (Figure 2c). The flow cytometry results demonstrated that the streptavidin-conjugated Eu-complex/PVK Pdots had high specific labeling efficiency with low nonspecific binding.

From the luminescence lifetime measurements of the Eu-complex/PVK Pdots, we found that these Pdots inherited the long luminescence lifetimes of their corresponding Eu-complexes. As shown in Table 1, the lifetime of Eu15F/PVK and EuDNM/PVK Pdots was around 509 μ s and 202 μ s, respectively, which were significantly longer than the 17 ns lifetime of pure PVK Pdots. It also indicated that there was efficient FRET between the PVK polymer and the Eu-complex inside the Pdots. The unique property of long luminescence lifetime endowed Eu-complex/PVK Pdots with the ability to stand out from other nanoparticles emitting red fluorescence. For example, both Eu15F/PVK Pdots and commercial red fluorescent nanoparticles (R300 NPs) had strong emission at 612 nm (Figure 3a). Due to the spectral overlap, the two types of nanoparticles could not be differentiated by using a band-pass filter when the mixture was imaged under a normal epifluorescence microscope (Figure 3d, lower image). But time-gated fluorescence microscopy allowed them to be easily differentiated (Figure 3d, top image). The delay time, between the termination of excitation illumination and start of fluorescence signal collection, of the time-gated microscope was set to be shorter than the lifetime of Eu15F/PVK Pdots but longer than that of R300 NPs. In this setup, only the Eu15F/PVK Pdots could be imaged because they still emitted photons after

the delay time (Figure 3b); the R300 NPs failed to emit photons after the delay time because of their short lifetime (3.6 ns) (Figure 3c). With this approach, two different types of nanoparticles with overlapping emission spectra were successfully differentiated by exploiting their luminescence lifetime difference.

The long luminescence lifetime of the Eu15F/PVK Pdots also can be applied to improve the S/N ratio in cell imaging. For example, time-gated and ungated microscopy images of MCF-7 cells with internalized Eu15F/PVK Pdots were collected (Figure 3e–i). The background in the time-gated fluorescence cell images (Figure 3g and 3i) was much cleaner than that of the ungated images (Figure 3e and 3h). This was because the lifetime of cellular autofluorescence, generated by molecules such as nicotinamide adenine dinucleotide, riboflavin, and flavin coenzymes, is on the order of nanoseconds,^[26] which can be efficiently filtered out in a time-gated microscope. As a result, the S/N ratio of cell imaging was successfully improved, from 85 to 232.

In summary, we designed and prepared Eu-complex/PVK Pdots, which are a new type of Pdots with ultra-narrow fluorescence emission with 8-nm FWHM; greatly decreased self-quenching of the Eu-complexes; highly improved luminescence QY; and long fluorescence lifetimes. The bioconjugated Eu-complex/Pdots were successfully used to label cellular components. The long lifetimes of the Eu-complex/Pdots helped to distinguish them from other red fluorescence-emitting nanoparticles and improve S/N in imaging. We believe that the Eu-complex/PVK Pdots may also find applications in imaging other specimens, such as biological tissues which usually have high autofluorescence background signals.

Experimental Section

Poly (9-vinylcarbazole) (PVK, MW 50,000 Da) was purchased from Sigma-Aldrich (St. Louis, MO, USA). Polystyrene-grafted poly (ethylene glycol) functionalized with carboxyl groups (PS-PEG-COOH) was purchased from Polymer Source Inc. (Quebec, Canada). Europium (Eu) complexes Eu15F and EuDNM were synthesized in the lab as reported earlier.^[15, 27] 300-nm nanoparticles with red fluorescence (R300 NPs) were purchased from Thermo Scientific (IL, USA). Eu-complex/PVK Pdot preparation, conjugation and flow cytometry experiments are described in more detail in supplementary materials.

The absorption and fluorescence spectra were measured with a DU 720 scanning spectrophotometer (Beckman Coulter, Inc., CA) and a Fluorolog-3 fluorospectrometer (HORIBA JobinYvon, NJ, USA). Fluorescence QYs were collected using an integrating sphere (model C9920-02, Hamamatsu Photonics). The luminescence lifetime data of PVK Pdots and Eu-complex-doped PVK Pdots were obtained with a Lecroy Wave Runner 6100 digital oscilloscope (1 GHz) using the excitation of a third harmonic (355 nm) of a Nd:YAG laser (pulse width of 6 ns).^[27] The lifetime of R300 NPs was measured by using a PicoQuant Fluorescence lifetime system (PicoQuant Photonics North America Inc. Westfield, MA USA).^[6]

A Nikon TE2000 inverted microscope was modified for time-gated fluorescence image collection. Instrumentation details are described in supplementary materials.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

References

1. Wu C, Chiu DT. *Angewandte Chemie International Edition*. 2013; 52:3086.

2. Rong Y, Wu C, Yu J, Zhang X, Ye F, Zeigler M, Gallina ME, Wu IC, Zhang Y, Chan Y-H, Sun W, Uvdal K, Chiu DT. *Acs Nano*. 2013; 7:376. [PubMed: 23282278]
3. Tian Z, Yu J, Wang X, Groff LC, Grimland JL, McNeill JD. *The Journal of Physical Chemistry B*. 2012;4517. [PubMed: 23214470]
4. Yu J, Wu C, Sahu SP, Fernando LP, Szymanski C, McNeill J. *Journal of the American Chemical Society*. 2009; 131:18410. [PubMed: 20028148]
5. Yu J, Wu C, Tian Z, McNeill J. *Nano Letters*. 2012; 12:1300. [PubMed: 22313320]
6. Zhang X, Yu J, Rong Y, Ye F, Chiu DT, Uvdal K. *Chemical Science*. 2013; 4:2143.
7. Yu JB, Wu CF, Zhang XJ, Ye FM, Gallina ME, Rong Y, Wu IC, Sun W, Chan YH, Chiu DT. *Advanced Materials*. 2012; 24:3498. [PubMed: 22684783]
8. Wu CF, Hansen SJ, Hou QO, Yu JB, Zeigler M, Jin YH, Burnham DR, McNeill JD, Olson JM, Chiu DT. *Angewandte Chemie-International Edition*. 2011; 50:3430.
9. Yu J, Zhou L, Zhang H, Zheng Y, Li H, Deng R, Peng Z, Li Z. *Inorganic Chemistry*. 2005; 44:1611. [PubMed: 15733004]
10. Bunzli J-CG. *Chemical Reviews*. 2010; 110:2729. [PubMed: 20151630]
11. Carlos LD, Ferreira RAS, Bermudez VD, Julian-Lopez B, Escribano P. *Chemical Society Reviews*. 2011; 40:536. [PubMed: 21180708]
12. Ma Y, Wang Y. *Coordination Chemistry Reviews*. 2010; 254:972.
13. Eliseeva SV, Bunzli J-CG. *Chemical Society Reviews*. 2010; 39:189. [PubMed: 20023849]
14. Binnemans K. *Chemical Reviews*. 2009; 109:4283. [PubMed: 19650663]
15. Peng H, Stich MIJ, Yu J, Sun L-n, Fischer LH, Wolfbeis OS. *Advanced Materials*. 2009; 22:716. [PubMed: 20217776]
16. Ai K, Zhang B, Lu L. *Angewandte Chemie International Edition*. 2009; 48:304.
17. Ambili Raj DB, Biju S, Reddy MLP. *Journal of Materials Chemistry*. 2009; 19:7976.
18. Philippot C, Bourdolle A, Maury O, Dubois F, Boury B, Brustlein S, Brasselet S, Andraud C, Ibanez A. *Journal of Materials Chemistry*. 2011; 21:18613.
19. Duarte AP, Gressier M, Menu M-J, Dexpert-Ghys J, Caiut JMA, Ribeiro SJL. *The Journal of Physical Chemistry C*. 2011; 116:505.
20. Wartenberg N, Raccurt O, Imbert D, Mazzanti M, Bourgeat-Lami E. *Journal of Materials Chemistry C*. 2013; 1:2061.
21. Jin YH, Ye FM, Zeigler M, Wu CF, Chiu DT. *Acs Nano*. 2011; 5:1468. [PubMed: 21280613]
22. Wu CF, Bull B, Christensen K, McNeill J. *Angewandte Chemie-International Edition*. 2009; 48:2741.
23. Sun W, Hayden S, Jin YH, Rong Y, Yu JB, Ye FM, Chan YH, Zeigler M, Wu CF, Chiu DT. *Nanoscale*. 2012; 4:7246. [PubMed: 23072832]
24. Wu C, Bull B, Szymanski C, Christensen K, McNeill J. *ACS Nano*. 2008; 2:2415. [PubMed: 19206410]
25. Zhang X, Yu J, Wu C, Jin Y, Rong Y, Ye F, Chiu DT. *ACS Nano*. 2012; 6:5429. [PubMed: 22607220]
26. Aubin JE. *Journal of Histochemistry & Cytochemistry*. 1979; 27:36. [PubMed: 220325]
27. Yu JB, Deng RP, Sun LN, Li ZF, Zhang HJ. *Journal of Luminescence*. 2011; 131:328.

Figure 1.

Size and optical property characterization of Eu-complex/PVK Pdots. (a) High-resolution TEM image of Eu15F/PVK Pdots. (b) DLS size distribution histogram of Eu15F/PVK Pdots. (c) High-resolution TEM image of EuDNM/PVK Pdots. (d) DLS size distribution histogram of EuDNM/PVK Pdots. (e) Normalized absorption (abs), excitation (ex), and emission (em) spectra of pure PVK Pdots, Eu15F nanoparticles, and Eu15F/PVK Pdots. All spectra were measured in aqueous solution. (f) Normalized spectra of pure PVK Pdots, EuDNM nanoparticles, and EuDNM/PVK Pdots. (g) The emission spectra of Eu15F/PVK Pdots with doping ratio of Eu15F ranging from 20% to 80 %; the excitation wavelength was 342 nm; QY data and amplified partial emission bands of Pdots are shown in the inset. (h) The emission spectra of EuDNM/PVK Pdots with doping ratio of EuDNM ranging from

20% to 80%; the excitation wavelength was 342 nm; QY data of Pdots are shown in the inset.

Figure 2.

Immunofluorescence labeling of MCF-7 cells with Eu-complex/PVK Pdots and flow cytometry measurements. (a) Flow cytometry measurements of cells labeled with Eu15F/PVK Pdot-SA; (b) Flow cytometry measurements of cells labeled with EuDNM/PVK Pdot-SA. In both (a) and (b), the gray curve shows the negative control and the dark curve is for the positive sample. (c) Bright-field image taken by optical microscopy of MCF-7 cells labeled with Eu15F/PVK Pdots. (d) Fluorescence image of MCF-7 cells labeled with Eu15F/PVK Pdots. In both (c) and (d), scale bar is 20 μm .

Figure 3.

The application of long luminescence lifetime of Eu-complex/PVK Pdots in particle differentiation and cell imaging. (a) Emission spectra of Eu15F/PVK Pdots and R300 nanoparticles (excited at 342 nm). (b), (c) and (d) panels show the time-gated and ungated single particle images of Eu15F/PVK Pdots, R300 nanoparticles, and mixtures of Eu15F/PVK Pdots and R300 nanoparticles, respectively; scale bar represents 5 μm . (e) ungated fluorescence microscopy image, (f) bright field microscopy image, and (g) time-gated fluorescence microscopy image of unconjugated Eu15F/PVK Pdots inside MCF-7 cells; scale bar represents 50 μm . (h, i) The interactive 3D surface plot of ungated image (e) and gated image (g).

Scheme 1.

Schematic of (a) chemical structures of Eu15F, EuDNM, PS-PEG-COOH, and PVK, (b) the preparation procedure of Eu-complex/PVK Pdots, and (c) the procedure for labeling cells by using Eu-complex/PVK Pdot-streptavidin bioconjugates.

Table 1

Summarized Photophysical Properties of Eu-complex/PVK Pdots.

Pdots	Size (nm)	σ^a ($\times 10^{-13}$ cm ²)	η^b (%)	τ^c		
				A ₁	τ_1 (μ s)	τ_2 (μ s)
Eu15F/PVK (40wt%)	15	0.65	31.5	0.09	103	509
EuDNNM/PVK (60wt%)	18	1.20 (0.70 ^d)	11.2	0.02	38	202

Note:

^a absorption cross-section for single particle;

^b absolute luminescence QY ($\pm 0.5\%$);

^c luminescence lifetime;

^d absorption cross-section calculated for single particle with size of 15 nm; size refers to the hydrodynamic diameter measured by DLS.