Dyes and Pigments 123 (2015) 218–221

Contents lists available at ScienceDirect

Dyes and Pigments

journal homepage: www.elsevier.com/locate/dyepig

Strong nonlinear optical phosphorescence from water-soluble polymer dots: Towards the application of two-photon bioimaging

Tingchao He [a], Wenbo Hu [b], Huifang Shi [c], Qunfeng Pan [d], Guohong Ma [d], Wei Huang [b, c], Quli Fan [b], Xiaodong Lin [a, *]

[a] College of Physics Science & Technology, Shenzhen University, Shenzhen, 518060, China
[b] Key Laboratory for Organic Electronics & Information Displays (KLOEID) and Institute of Advanced Materials (IAM), Nanjing University of Posts & Telecommunications, Nanjing, 210023, Jiangsu, China
[c] Key Laboratory of Flexible Electronics (KLOFE) & Institute of Advanced Materials (IAM), Jiangsu National Synergistic Innovation Center for Advanced Materials (SICAM), Nanjing Tech University (NanjingTech), 30 South Puzhu Road, Nanjing, 211816, China
[d] Department of Physics, Shanghai University, Shanghai, 200444, China

ARTICLE INFO

Article history:
Received 8 July 2015
Received in revised form
3 August 2015
Accepted 4 August 2015
Available online 12 August 2015

Keywords:
Polymer dots
Z-scan measurement
Two-photon absorption
Two-photon excited phosphorescence
FRET
Bioimaging

ABSTRACT

Multiphoton fluorescence imaging of tissues offers an essential tool for studying biological systems. However, autofluorescence becomes a serious issue in complicated tissue imaging where biological molecules compete with the fluorophore. Such a critical issue can be resolved through the design of phosphorescent molecules with long emission lifetime. In this work, the nonlinear optical properties of small phosphorescent polymer dots (Pdots) with strong red-emitting phosphorescence (620 nm) have been disclosed. An analysis of decay data from fs pump-probe experiment yields a value of 164 ps for the singlet excited state lifetime of the Pdots. Notably, thanks to efficient two-photon excited energy transfer and moderate phosphorescence quantum yield (η = 1.36%), the Pdots exhibit large two-photon absorption action cross-section ($\eta\delta_{max}$ = 15 GM) in aqueous solution. Results of living cell imaging experiments show the potential of these Pdots in two-photon time-resolved phosphorescent microscopy.

1. Introduction

Two-photon absorption (TPA) materials, which can be excited by near-infrared light (700–1100 nm), have found many promising applications, including up-converting lasing, bioimaging, photodynamic therapy and optical limiting [1]. In particular, two-photon excitation has several advantages over conventional one-photon excitation when applied in sensing and bioimaging, such as the decrease of photobleaching and photodamaging, large penetrating depth, and the ability of precise 3D localization. Hence, considerable research efforts have been devoted to the development of excellent two-photon luminescent probes, towards the above-mentioned applications [1–4].

Among the reported or commercial organic luminophores, metal complexes emitting phosphorescence from their triplet excited states (TESs) are uniquely useful for various applications [5–7]. With much longer lifetime of TES and larger Stokes shift, phosphorescence can more effectively reduce interference from excitation light, autofluorescence and self-quenching. Additionally, the phosphorescent metal complexes can be utilized not only as optical sensors for oxygen, but also as photosensitizers for photodynamic therapy [8,9]. Although there were many reported phosphorescent probes based on metal complexes, most of them were achieved through one-photon excitation [8,9]. In fact, heavy-metal complexes are also good candidates as nonlinear optical phosphorescent chromophores, whose TPA can be easily tuned by changing the metal centers or/and ligand structures through their synergistic role [10]. As a new research direction, we demonstrated that the enhancement of nonlinear optical photoluminescence in the metal-organic complexes could be realized through Förster resonance energy transfer (FRET) [11,12]. Based on these results, we wish to realize the amplification of nonlinear optical phosphorescence by utilizing this design strategy.

In the current work, with water-soluble small polymer dots (Pdots) composed of phosphorescent Ir(III) complex and polyfluorene

* Corresponding author. Tel./fax: +86 755 26538735.
E-mail address: linxd@szu.edu.cn (X. Lin).

http://dx.doi.org/10.1016/j.dyepig.2015.08.002
0143-7208/

unit in the main polymer chain as the research target, their nonlinear optical behaviors, including singlet excited state (SES) absorption and TPA under aqueous condition as well as their potential for cell imaging, are investigated in details.

2. Experimental

2.1. General information

UV–Vis absorption and luminescence spectra of the samples were measured by using a Shimadzu UV–Vis spectrophotometer and Fluorolog-3 (Horiba JOBIN YVON), respectively. Transmission electron microscopy (TEM) images were recorded on an HT7700 transmission electron microscope at an accelerating voltage of 100 kV. The TEM specimens were made by dropping some aqueous solution of the Pdots on ultrathin carbon-coated copper grids. The measurements of fs time-resolved transient absorption (TA) were performed to determine the SES lifetime of Pdots, with the help of pump-probe technique. Fs pulses at 800 nm were divided into two beams by a beam splitter. One was used as probe beam while the other traveled through a 1-mm thickness type-I BBO nonlinear crystal to generate pulses at 400 nm used as pump wavelength. The TPA cross-sections (δ) of samples were measured by using Z-scan technique [13]. In the measurements of Z-scan, the excitation intensity was 31 GW/cm^2 for all the measured wavelengths. For both of Z-scan and two-photon excited phosphorescence measurements, the laser pulses from an OPA combined with TOPAS (1000 Hz, 100 fs, Spectra-Physics, Spitfire Pro) with tunable wavelength were used as excitation source. The phosphorescence signals were then detected by a spectrometer (SpectraPro275) coupled to a photomultiplier tube with a lock-in system.

2.2. Two-photon bioimaging

The HeLa cancer cells were first cultured in confocal microscope dishes at 5 × 10^5/mL in complete Dulbecco's modified Eagle's medium (DMEM, Gibco, America) for 24 h at 37 °C. After that, the cultured cells were washed with phosphate buffered saline (PBS) three times and then incubated for 4 h with the medium containing Pdots. Finally, the cells were washed twice with PBS. Two-photon cell imaging was acquired using a confocal laser scanning microscope (CLSM) (Zeiss LSM 410) with imaging software (Fluoview FV500), under the excitation of fs 720 nm.

3. Results and discussion

The chemical structures of Ir(III) complex (acceptor), polyfluorene (donor) and their polymer (Pdots) are shown in Fig. 1, and their synthesis routine was published previously [14]. Shi et al. investigated the one-photon excited FRET induced enhancement of phosphorescence in the Pdots and their applications for ratiometric oxygen sensing and photodynamic cancer therapy [14]. However, their nonlinear optical properties have not been developed, which may be used to explore new applications. It was reported that water-soluble polyfluorene-based conjugated polymers were excellent TPA donors [15]. As the FRET mechanism induced by one-photon absorption was confirmed by Shi et al., two-photon excited FRET also could be expected due to the large TPA properties of donor, even though their application sceneries were different. From TEM image, it was found that the Pdots had ultrasmall particle size of (6 ± 2) nm in aqueous solution (Fig. 2a), which made the cellular uptake of the Pdots easy. In luminescence spectra recorded under both 360 nm (excitation intensity: 0.02 W/cm^2) and 720 nm (excitation intensity: 1 GM/cm^2) excitation, as shown in Fig. 2b, a major emission peak at 425 nm from the polyfluorene main chain was observed,

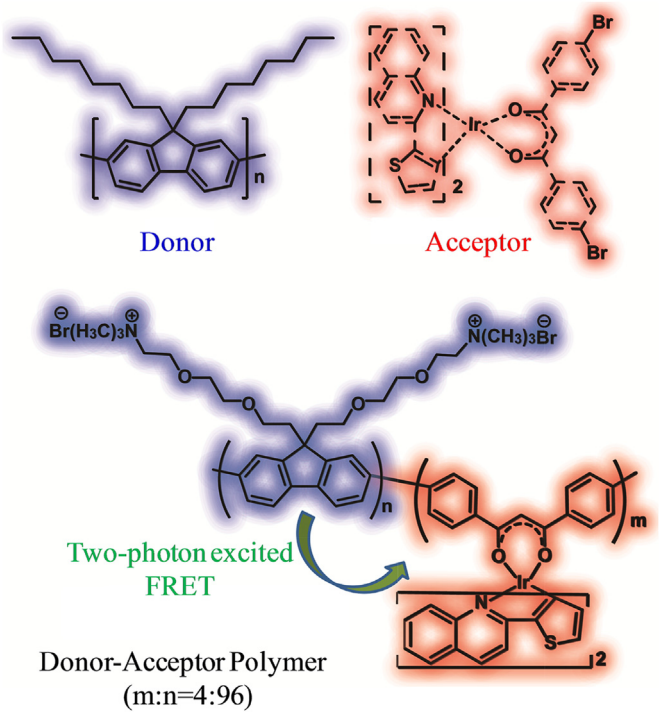

Fig. 1. Chemical structures of polyfluorene (donor), phosphorescent Ir(III) complex (acceptor) and Pdots (donor-acceptor polymer).

accompanied by a red emission peak at 620 nm from phosphorescent Ir(III) complex. Obviously, from the spectral profiles that were normalized at the maximal emission intensity of donor, the red emission intensity of Ir(III) complex in Pdots was relatively higher under 720 nm excitation, implying that there were different cross-section ratios between donor and acceptor under the excitation of 360 nm and 720 nm. As will be discussed later, the large enhancement factor, which was obtained under 720 nm excitation, was

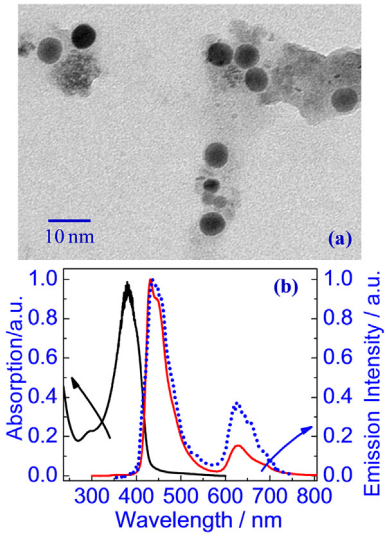

Fig. 2. (a) TEM image of Pdots in aqueous solution. (b) Normalized absorption and luminescence spectra of Pdots in water under the excitation of 360 nm (red solid line) and 720 nm (blue dash line). The luminescence spectra are normalized at the maximal emission intensity of polyfluorene. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)