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Enhanced single-particle brightness and photostability of semiconductor polymer dots by enzymatic oxygen scavenging system

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ABSTRACT

Semiconductor polymer dots (Pdots) are emerging as an excellent fluorescent probe in biology and medicine. However, the photostability of Pdots can't meet the requirements of long term single-particle imaging and tracking applications. Here we describe the enhanced single-particle brightness and photostability of Pdots by using an efficient enzymatic oxygen scavenging system (OSS). Pdots with particle diameters of 21 nm and 43 nm (PFBT21 and PFBT43) were prepared by a nanoprecipitation method. Single-particle imaging and photobleaching were performed to investigate the effect of OSS on the per-particle brightness and photostability of Pdots. Our results indicate that the single-particle brightness of the PFBT21 Pdots in OSS was enhanced nearly two times as compare to the PFBT21 Pdots in water. The photobleaching percentages of PFBT21 and PFBT43 in OSS were determined to be 29% and 33%, respectively. These values are decreased by 2–3 times as compared to those of the same Pdots in water, indicating the significantly improved photostability of Pdots by OSS. This study provides a promising approach for enhancing photostability of Pdots in long term single-particle tracking.

1. Introduction

Single-particle fluorescence techniques have been widely used in biomedical engineering and biophotonics for investigation of cellular processes such as molecular transport, membrane dynamics, protein folding, and enzyme kinetics [1–8]. Optical super-resolution imaging modalities have been able to resolve subcellular structures and dynamic movements at the accuracy of the nanometer scale [9,10]. Bright and photostable probes are essential to achieve precise localization and high resolution in these optical imaging and tracking techniques. Commonly, fluorescent proteins [11,12], small-molecule dyes [13], and inorganic quantum dots (Qdots) [14], have been used as fluorescence probes in super-resolution imaging and single-particle fluorescence techniques. In many cases, however, these probes are not bright and stable enough for photon-starved applications such as long-term imaging and single-particle tracking experiments. The fluorescent dyes and proteins suffer from poor photostability, and the intrinsic toxicity of Qdots restrict their widespread applications in biology.

In past years, we and other groups demonstrated semiconductor polymer dots (Pdots) as bright probes for biology and medicine [15–20]. The large absorption sections, high quantum yield, and efficient energy transfer properties make them suitable for biological imaging and sensing applications. In a pioneer single-particle tracking study, the high fluorescence brightness of Pdots yielded a theoretical particle tracking uncertainty of less than 1 nm on the basis of the trajectories of fixed and freely diffusing particles [21]. We recently investigated the size-dependent single-particle brightness and photobleaching behavior of Pdots and found that the large Pdots (>20 nm) commonly displayed relatively fast photobleaching components and small Pdots (10 nm) exhibited intensity blinking behaviors [22]. The fast photobleaching significantly diminish the photon number generated from the Pdots, thus limit their application for long-term imaging and tracking studies. Photobleaching is observed in most fluorescent semiconductor polymers in both solution and solid state. One primary pathway for photobleaching is the photooxidation of semiconductor polymer by molecular oxygen [23]. Under light excitation, the formation of singlet oxygen, peroxides, and other reactive oxygen species (ROS) can lead to oxidative damage to the semiconductor polymer backbone. Moreover, the photo-oxidized defects or photogenerated hole polarons can act as highly efficient fluorescence quenchers that significantly exacerbate the photobleaching processes. For small-molecule, intra-molecular

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triplet energy transfer mechanism is a general approach for improving performance and photostability of organic fluorophores. By restoring the triplet state to ground state through intramolecular triplet energy transfer, the methods improved the photostability of cyanine fluorophores [24]. In a recent report, photobleaching of fluorescence Pdots was reduced by doping the Pdots with hydrophobic antifade additives that improve the photostability via mechanisms including quenching of the relevant excited electronic states (e.g., triplet fluorophores and singlet oxygen) and scavenging of ROS [25]. However, fast photobleaching components were still observed in the ensemble (bulk) and single-particle photobleaching measurements.

Here, we describe the enhancement of single-particle brightness and photostability of Pdots by an enzymatic oxygen scavenging system (OSS). The enzymatic OSS was based on the rapid reaction of the glucose and the glucose oxidase that consumed the molecule oxygen (O_2) around the Pdots. Under the same excitation and collection conditions, the single-particle fluorescence brightness of Pdots by using the OSS exhibit a two-fold increase as compared to that of the Pdots in typical air-saturated solutions. Moreover, we performed single-particle photobleaching statistics to estimate the photostability of Pdots in the presence of the OSS. The photostability of Pdots was significantly improved as compared to the Pdots without the OSS. Furthermore, the fast bleaching component that restricts the applications of Pdots in long term single-particle tracking were suppressed by the rapid and efficient enzymatic oxygen scavenging system.

2. Material and methods

2.1. Materials

The semiconductor polymer used in this study is poly [(9, 9-dioctylfluorenyl-2, 7-diyl)-co-(1, 4-benzo-{2, 1′, 3}-thiadiazole)] (PFBT, MW = 115,000, polydispersity = 3.2), purchased from ADS Dyes, Inc. (Quebec, Canada). The solvent tetrahydrofuran (THF, anhydrous, 99.9%), the functional polymer poly (styrene co maleic anhydride) (PSMA, average MW = ~1700), glucose (MW = 180.16), glucose oxidase (GOx) (MW = 160 KDa) were purchased from Sigma-Aldrich. All chemical were used without further purification and all experiments were performed at room temperature.

2.2. Preparation and characterization of semiconductor polymer dots

The PFBT Pdots were prepared by a nanoprecipitation procedure modified from the previous reports [26,27]. Briefly, the semiconductor polymer PFBT and functional polymer PSMA were dissolved in THF to obtain a concentration of 1 mg mL^{-1} primitive solution, respectively. As the precursor solution, the PFBT and PSMA primitive solutions were mixed and diluted to 100 μg mL^{-1} and 20 μg mL^{-1}, respectively. A 3 mL quantity of the precursor solution was quickly injected into 10 mL deionized water under vigorous ultrasonic. The particle size and morphology of the PFBT Pdots were characterized by dynamic light scattering (DLS) and transmission electron microscopy (TEM). DLS measurements were performed

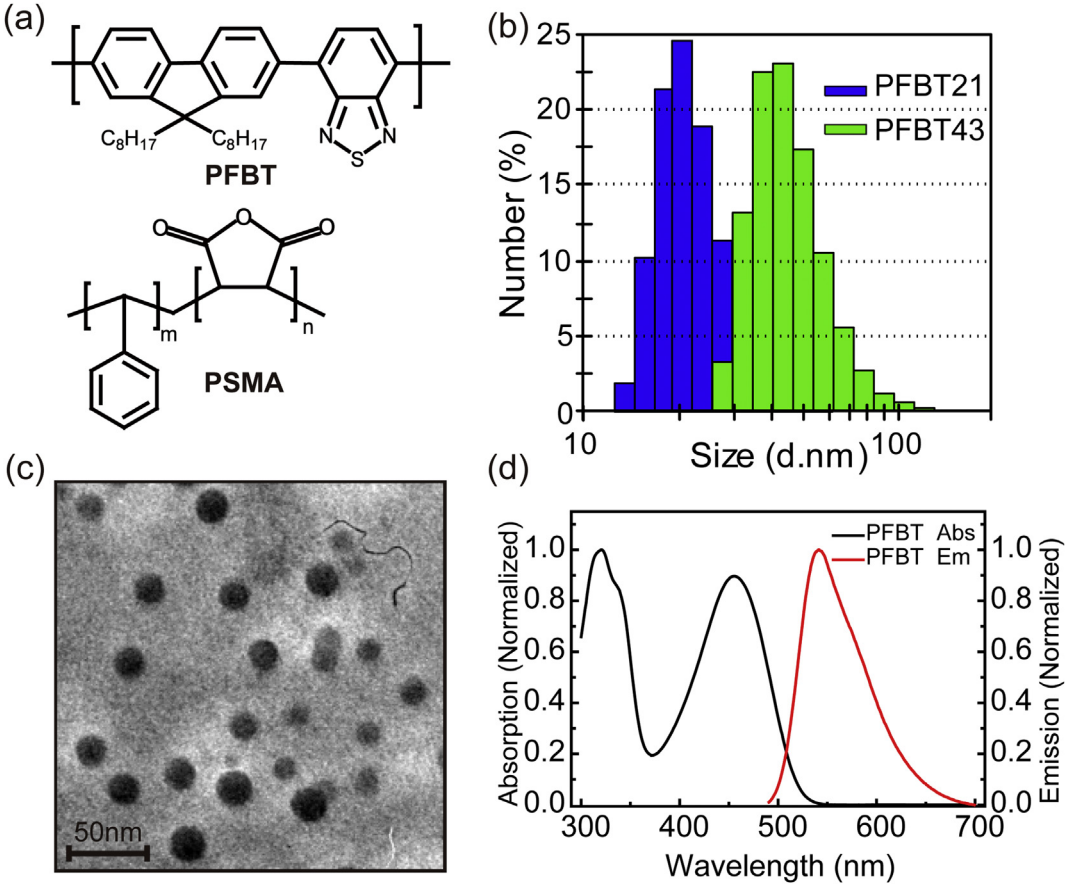

Fig. 1. (a) Chemical structure of semiconductor polymer PFBT and functional polymer PSMA. (b) Dynamic light scattering (DLS) results of PFBT Pdots. (c) Typical transmission electron microscopy (TEM) image of the Pdots. (d) The UV/VIS absorption spectrum and the fluorescence spectrum of the PFBT Pdots.