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Poly[(9,9-dioctyl-fluorenyl-2,7-diyl)-co-fluorenone]-based orange fluorescence probe for cellular imaging

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ABSTRACT

A novel orange fluorescence probe based on efficient fluorescence resonance energy transfer from poly[(9,9-dioctylfluorenyl-2,7-diyl)-co-fluorenone] nanoparticles to Rhodamine B dye molecules was developed. This novel probe exhibited a larger Stokes shift, enhanced photostability and longer lifetime comparing to Rhodamine B dye molecules. Meanwhile, it demonstrated the characteristics that especially useful in live cell or in vivo studies, such as narrow full width at half-maximum emission, excitation bands in the visible range and the sensitivity of its fluorescence to temperature. Confocal fluorescence images of the fluorescent probe proved that it could effectively label HeLa cells.

1. Introduction

The past several years have witnessed the rapid development of semiconducting conjugated polymer nanoparticles (CPNs) in the interdisciplinary fields of materials chemistry, biology and medicine.[1,2] As these CPNs exhibit exceptional fluorescence, brightness, good photostability, fast radiative rate, non-blinking behavior, facile surface functionalization and low cytotoxicity.[3–7] CPNs can be prepared using reprecipitation method,[8,9] which has proven to be an effective way to prepare small-sized CPNs for both in vivo and in vitro biological applications. Works about blue, green, orange, deep red, and near-infrared CPNs for bioimaging have been published.[10,11] Wu et al. have successfully developed green, yellow, deep red, and near-infrared CPNs with narrow emission for bioimaging.[12] However, there is a significant drawback to orange CPNs at present: most of the reported semiconducting polymers used to form CPNs have broad emission bands.[13–15] Most biological applications demand that multiple targets be detected simultaneously, thus spectral multiplexing requires that the probes possess narrow emissions. The broad emission spectra from orange CPNs significantly limit their usefulness in practical applications. Therefore, there is an urgent need to develop new types of orange CPNs with narrow spectral width to satisfy the booming development in biological applications.

To address this issue, we describe here the encapsulation of Rhodamine B (Rh B) dye molecules within poly[(9,9-dioctylfluorenyl-2,7-diyl)-co-fluorenone] (PFFO) via cascade Förster resonance energy transfer (FRET)[16] in an effort to achieve orange CPNs with narrow emission. The encapsulation occurs during particle self-assembly. PFFO was initially performed for better comprehension of the degradation of polyfluorene-type semiconducting materials[17] and found to show an efficient yellowish photoluminescence.[18] Rh B dye molecules have long been known for its narrow emission, high quantum yield, good brightness, excitable in the visible range, cheap price, broad working range.[19] Now, there are a few flies in the ointment. The limited photostability[20] and toxicity[21] make it impractical for biological applications.

Fortunately, we found that the broad emission spectrum of PFFO provides favorable spectral overlap with the Rh B dye molecules. Thus,

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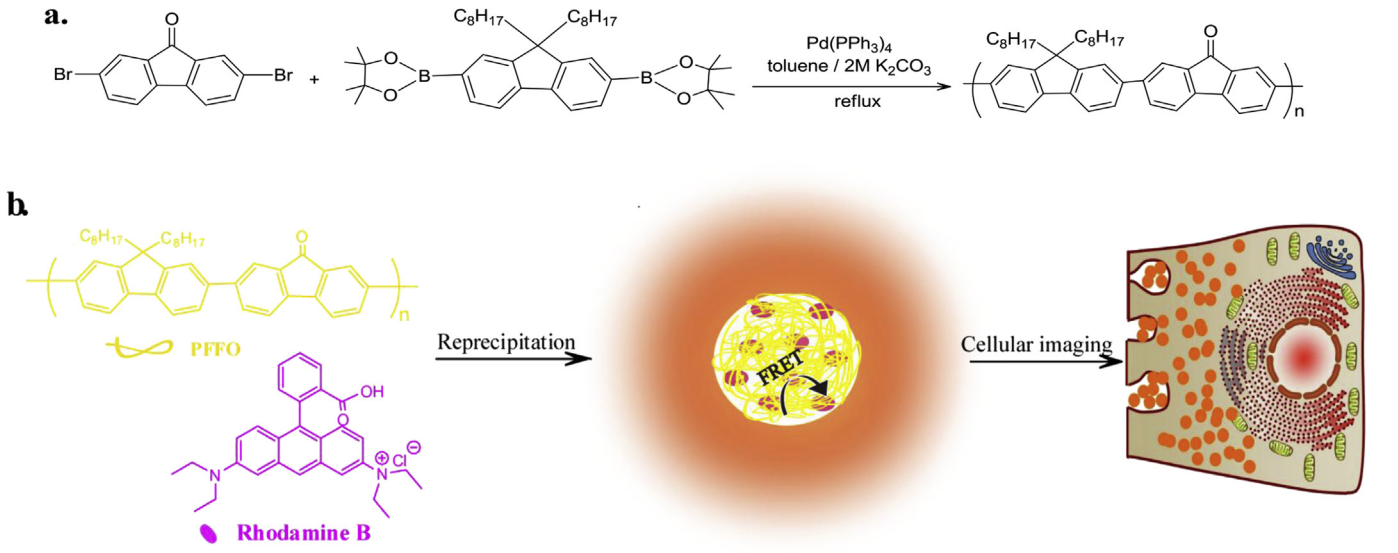
we codoped Rh B dye molecules within the matrix of PFFO CPNs, combining the intrinsic fluorescence properties of the coordination matrix with the characteristics of the encapsulated species.

2. Results and discussion

In this work, the CPNs based on PFFO as matrix with doping of Rh B dye molecules were prepared as shown in Scheme 1. At first, the PFFO was designed and synthesized by Suzuki coupling reaction. Then, the Rh B/PFFO CPNs were acquired by reprecipitation method, and they exhibited a hydrodynamic diameter of 24 nm based on dynamic light scattering (DLS) measurements (Fig. 2b). Efficient FRET from PFFO to Rh B dye molecules was obtained to red-shift both the absorption and emission of PFFO CPNs while acquiring narrow emission (Fig. 1). Confocal fluorescence images of the Rh B/PFFO CPNs proved that they could effectively label HeLa cells. We also applied the CPNs to photostability, lifetime, and temperature sensing experiments and demonstrate their advantages over conventional Rh B dye molecules. We believe that this work should demonstrate the promising applicability of these narrow emission orange CPNs in a wide range of cellular imaging studies.

In Fig. 2a, it is can be found that Rh B/PFFO CPNs show the dominant absorption peaks (~370 nm and ~455 nm)[23,24] of PFFO and the absorption peak of the Rh B (~554 nm). The peaks are very close to the 488 nm and 532 nm excitation sources that are commonly equipped in most fluorescence instruments (e.g., confocal laser scanning microscopy and flow cytometric method), respectively. In order to characterize the stability of Rh B/PFFO CPNs, the absorption spectrum of Rh B/PFFO CPNs in aqueous suspensions was measured before and after centrifugal filtration 30 min with a speed of 10,000 RPM (Fig. 2a). No obvious changes was observed, this indicates that the Rh B dye molecules were encapsulated into CPNs perfectly.

The fluorescence quantum yield (QY) of the Rh B/PFFO CPNs was compared with Rh B dye molecules. The excitation wavelength is 405 nm. As shown in Table 1, it is noted that the QY of Rh B/PFFO CPNs is 0.11, with only a slight drop relative to that of Rh B dye molecules. Further investigation suggests that the Rh B dye molecules have a small Stokes shift (~26 nm) (Table 1). It is generally known that small Stokes shift has limited the applications in high-sensitivity imaging, long-term monitoring, and high-throughput assays. However, the Rh B/PFFO CPNs exhibit large Stokes shift,



Scheme 1. a. Synthesis of PFFO by Suzuki coupling polymerization; b. Preparation of Rh B/PFFO CPNs and their application for cellular imaging.

2.1. Spectroscopic properties of Rh B/PFFO CPNs

A detailed investigation was conducted on spectroscopic properties of Rh B/PFFO CPNs. The emission peak of the Rh B/PFFO CPNs remains unaltered in comparison with that of original Rh B emission (Fig. 1), thereby allowing us to create Rh B/PFFO CPNs that retain the narrow emission characteristics of Rh B dye molecules. This narrow emission was attributed to the high light-harvesting capability of PFFO and the efficient energy transfer from polymer to Rh B dye molecules. More importantly, the full width at half maximum (FHWM) of the emission peak of Rh B/PFFO CPNs (~40 nm) is much narrower relative to that of conventional orange CPNs, such as poly[2-methoxy-5-(2-ethylhexyloxy)-1,4-(1-cyanovinylene-1,4-phenyl-ene)] (CN-PPV) CPNs, poly[9,9-(dioctyl-fluorenyl-2,7-diyl)-co-1,4-benzo-{2,1′-3}-selenadiazole] (PFBS) and poly[2-methoxy-5-((2-ethylhexyl)oxy)-*p*-phenylenevinylene] (MEH-PPV),[13–15] indicating an important feature for multiplexed assays.[22]

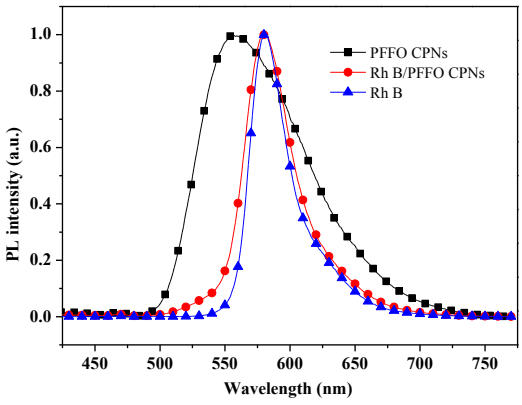


Fig. 1. The fluorescence spectrum of PFFO CPNs in aqueous suspensions, Rh B/PFFO CPNs in aqueous suspensions, and RhB dye molecules in H_2O.