Improved Fluorescence and Brightness of Near-infrared and Shortwave Infrared Emitting Polymer Dots for Bioimaging Applications

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Abstract

Development of near-infrared (NIR) and shortwave infrared (SWIR) emitting fluorophores is central to the fluorescence-based bioimaging. While conjugated polymer nanoparticles (polymer dots) are one of the promising fluorophores for this application, obtaining polymer dots that show bright fluorescence, especially in the SWIR wavelength region, has been challenging. Here, we report generalized approach to obtain bright polymer dots through a systematic characterization of photophysical properties of NIR and SWIR emitting polymer dots. Detailed photophysical characterization of a series of polymer dots fabricated using polycarbazole (PCz)-based conjugated polymers that adopt bent and twisted conformation reveals that the fluorescence brightness of the PCz-based polymer dots is determined by subtle balance between fluorescence quenching due to polymer chain interaction inside the particles and the twisting between the donor and acceptor moieties of the conjugated polymers inside the particles. Our results provide important insight into the rational design of highly fluorescing SWIR-emitting polymer dots.

Keywords: conjugated polymers, nanoparticles, intramolecular twist, spatial packing, ultrasmall, NIR-II, SWIR, second near infrared

Introduction

Recent development in advanced fluorescence microscopy techniques has been enabling capture and quantification of biological processes at cellular to tissue, organ and whole organism level. A high optical transparency of biological specimens in near-infrared (NIR, 650-950 nm) and shortwave infrared (SWIR, 1,000-1,700 nm) due to their smaller absorption and scattering of light makes these wavelength regions particularly suitable for bioimaging.¹⁻⁴ Development of new fluorophores that emit NIR and SWIR fluorescence is currently one of the main focuses for successful fluorescence-based bioimaging applications since a limited choice of fluorophores, in particular in the SWIR wavelength region,⁵⁻⁷ has been a major obstacle to applying fluorescence imaging to a wide range of biological specimens. In association with this, new SWIR fluorescent probes for multimodal imaging and therapy has also been developed in recent years.⁸⁻⁹

Bright fluorescence, small size, and low cytotoxicity are some of the most important requirements for the fluorophores. Different types of fluorophores have been developed toward this goal, including small molecule-based fluorophores,¹⁰⁻¹⁴ single-walled carbon nanotubes,¹⁵⁻¹⁸ semiconductor quantum dots,¹⁹⁻²¹ and rare earth-doped nanoparticles.²²⁻²⁵ Among them, conjugated polymer nanoparticles (polymer dots) are promising fluorophores because fluorescence properties and size of polymer dots can be controlled by a proper molecular design and an optimization of fabrication procedure.²⁶⁻²⁸ Further, polymer dots in general have a low cytotoxicity. Due to these features, polymer dots are becoming increasingly common in recent years.²⁹⁻³⁶

A general issue in the development of polymer dots is that fluorescence quenching sites (i.e., energy trap sites) are generated through either interchain or intrachain interactions (e.g., π - π

stacking) inside polymer dot particles, which results in a drop of fluorescence quantum yields (i.e., fluorescence brightness).³⁷⁻⁴¹ This issue becomes more problematic in NIR and SWIR emitting conjugated polymers since their extended π -conjugation system often needs large and flat-shaped molecules, leading to increased inter- and intra-chain interactions. To prevent the generation of fluorescence quenching sites, polymer dots have often been fabricated by mixing conjugated and non-conjugated polymers that enables the physical separation of conjugated polymer chains inside the particles.⁴² This approach effectively maintains the high fluorescence quantum yields inside the particles although fluorescence brightness of the particles significantly drops because of the small fraction of the light-emitting molecules inside the particles. Polymer dots using aggregate-induced emission (AIE) have also been reported to obtain particles with high fluorescence quantum yields.⁴³ While relatively large fluorescence quantum yields have been reported using this approach, incorporating AIE moieties in conjugated polymers is essential that restricts flexibility of the molecular design.

Recently, we reported a new strategy to obtain brighter polymer dots using NIR emitting polycarbazole (PCz)-based donor-acceptor (DA)-type conjugated polymers.⁴⁴ Our previous study suggested that the generation of fluorescence quenching sites inside the particles can be suppressed by using conjugated polymers that have bent and twisted conformation.⁴⁴ Based on this finding, here we report systematic photophysical characterization of NIR and SWIR emitting polymer dots by synthesizing a series of PCz-based DA-type conjugated polymers that adopt bent and twisted conformation. By comparing photophysical properties of polymer dots obtained using polyfluorene (PF)-based planar conjugated polymers, we demonstrated that the fluorescence brightness of these polymer dots are controlled by subtle balance between the

fluorescence quenching due to polymer chain interactions inside the particles and the twisting between the donor and acceptor moieties of the conjugated polymers inside the particles.

Experimental Section

Synthesis of the conjugated polymers:

The synthesis of **BT**, **BT2**, **DTBT**, **DTBT2**, **PSN**, and **PSeN** are reported elsewhere.⁴⁵⁻⁴⁷ **DPP**, **isoindigo**, **NDI**, and **BBT** were newly synthesized by the following general procedure (Scheme 1): A solution of 3,6-di-*tert*-butyl-1,8-diethynyl-9-hexadecyl-9*H*-carbazole⁴⁸ (0.1134 g 0.2000 mmol) and dibromo-comonomer (0.2000 mmol), namely, 3,6-bis(5-bromothiophen-2-yl)-2,5-dioctyl-2,5-dihydropyrrolo[3,4-*c*]pyrrole-1,4-dione⁴⁹, (*E*)-6,6'-dibromo-1,1'-dioctyl-[3,3'-biindolinylidene]-2,2'-dione⁵⁰, 4,9-dibromo-2,7-dioctylbenzo[*lmn*][3,8]phenanthroline-1,3,6,8(2*H*,7*H*)-tetraone⁵¹, and 4,7-bis[5-bromo-4-(2-butyloctyl)thiophen-2-yl]benzo[1,2-*c*:4,5-*c'*]bis[1,2,5]thiadiazole⁵², respectively, in 3 ml toluene and 1 ml *i*Pr₂NH was degassed with nitrogen for 15 min. PdCl₂(PPh₃)₂ (5.7 mg, 0.008 mmol) and CuI (1.5 mg, 0.008 mmol) were added. The reaction mixture was further degassed and then heated to 80 °C for 24 h. After cooling to room temperature, the mixture was poured into methanol. The precipitate was collected and washed with methanol and hexane. The precipitate was further subjected to Soxhlet washing with methanol to remove the low molecular fractions.

DPP: Yield 78%; ¹H NMR (300 MHz, CDCl₃): δ 9.11 (br, Ar-H), 8.20 (br, Ar-H), 7.80 (br, Ar-H), 7.51 (br, Ar-H), 5.34 (br, Ar-H), 4.16 (br, CH₂), 1.97 (br, CH₂), 1.55 (br, CH₂), 1.35-1.16 (br, CH₂, CH₃), 0.91 ppm (br, CH₃); IR (neat): ν = 2954, 2920, 2852, 2357, 2186, 1662, 1550, 1481,

1466, 1440, 1407, 1364, 1275, 1261, 1234, 1186, 1094, 1071, 1023, 913, 870, 814, 747, 667, 637 cm⁻¹; GPC (eluent: THF): $M_n = 4.2 \text{ kg mol}^{-1}$, $M_w/M_n = 1.9$.

isoindigo: Yield 75%; ¹H NMR (300 MHz, CDCl₃): δ 8.95 (br, Ar-H), 8.23 (br, Ar-H), 8.07 (br, Ar-H), 7.62 (br, Ar-H), 4.25 (br, CH₂), 4.00-1.10 (br, CH₂, CH₃), 0.76 ppm (br, CH₃); IR (neat): $\nu = 3005, 2961, 2922, 2853, 2357, 2193, 1696, 1609, 1550, 1476, 1441, 1366, 1275, 1261, 1191, 1108, 1072, 1027, 913, 873, 849, 825, 668, 635 \text{ cm}^{-1}$; GPC (eluent: THF): $M_n = 5.5 \text{ kg mol}^{-1}$, $M_w/M_n = 1.6$.

NDI: Yield 80%; ¹H NMR (300 MHz, CDCl₃): δ 8.95 (br, Ar-H), 8.22 (br, Ar-H), 8.06 (br, Ar-H), 4.24 (br, CH₂), 2.11 (br, CH₂), 2.07 (br, CH₂), 2.92-1.25 (br, CH₂), 1.16 (s, CH₃), 0.76 ppm (s, CH₃); IR (neat): $\nu = 2954, 2918, 2851, 2366, 2328, 2173, 1703, 1664, 1562, 1483, 1443, 1396, 1363, 1313, 1224, 1181, 1103, 1026, 922, 875, 793, 722, 668, 636 \text{ cm}^{-1}$; GPC (eluent: THF): $M_n = 4.6 \text{ kg mol}^{-1}$, $M_w/M_n = 1.7$.

BBT: Yield 50%; ¹H NMR (300 MHz, CDCl₃): δ 8.12 (br, Ar-H), 7.72 (br, Ar-H), 5.49 (br, Ar-H), 2.92 (br, CH₂), 1.99 (br, CH), 1.74-0.78 ppm (br, CH₂, CH₃); IR (neat): $\nu = 2956, 2924, 2854, 2361, 1450, 1427, 1259, 1186, 914, 869, 804, 741, 670 \text{ cm}^{-1}$; GPC (eluent: THF): $M_n = 5.5 \text{ kg mol}^{-1}$, $M_w/M_n = 1.5$.

Scheme 1. Synthesis of DPP, isoindigo, NDI, and BBT.

Fabrication method of the polymer dots:

Conjugated polymer nanoparticles of **BT**, **BT2**, **DTBT**, and **DTBT2** were fabricated by a reprecipitation method described elsewhere.⁴⁴ Polymer nanoparticles of **NDI**, **isoindigo**, **BBT**, **PSN**, and **PSeN** were made from THF solutions prepared directly before precipitation. A small amount of polymer material was dissolved in THF and briefly sonicated in an ultrasonic bath (Elmasonic P60H, Elma Schmidbauer GmbH), further filtered through a 0.1- μm membrane syringe filter (Anatop 10, Whatman). Polymer concentrations were adjusted to keep the absorption values of 0.1 at the maximum of their charge-transfer absorption bands. 2 ml of the THF/polymer solution was then rapidly injected into the 12 ml of MilliQ water (Millipore, Milli-Q Reference). The mixed solution was kept under sonication in an ultrasonic bath at 277 K for 60 min. THF was removed from the mixed solution by evaporation under vacuum for 30 min,

further nanoparticle colloidal solutions were concentrated to the final volume of 200–250 μ l by a continuous flow of compressed dry air. The resulting polymer dot suspension was filtered through a 0.1- μ m membrane filter.

Experimental details for ensemble steady-state and time-resolved experiments:

Steady-state absorption measurements were performed by a U-3900 Spectrometer (Hitachi High-Technologies) to collect absorption spectra of **BT**, **BT2**, **DTBT**, **DTBT2**, and **DPP**, and by a Cary 5000 (Agilent) UV-Vis-NIR spectrophotometer to collect absorption spectra of **NDI**, **isoindigo**, **BBT**, **PSN**, and **PSeN**. A Fluoromax-4 spectrofluorometer (Horiba Scientific) was used to collect fluorescence spectra of **BT**, **BT2**, **DTBT**, **DTBT2**, and **DPP**, and a Cary Eclipse 1000 (Agilent) fluorescence spectrophotometer was used to collect fluorescence spectra of **NDI** and **isoindigo**. Fluorescence spectra of **BBT**, **PSN**, and **PSeN** were recorded with an Isoplan SCT320 spectrograph (Princeton Instruments) equipped with liquid nitrogen cooled IR camera PyLoN 1024/1.7 (Princeton Instruments). Samples were kept in CVH100 Cuvette Holder (Thorlabs) equipped with long-pass edge filter FELH0800 (Thorlabs), collimating optic and fiber optic adapter, allowing the cuvette holder to be directly connected to spectrograph. Samples were excited with 785 nm CW laser diode module LDM785 (Thorlabs).

The fluorescence quantum yields (QY) of **BT**, **BT2**, **DTBT**, and **DTBT2** polymers were determined using Rhodamine 101 (Acros Organics), 4-(dicyanomethylene)-2-methyl-6-(4-dimethylaminostyryl)-4H-pyran (DCM, Sigma Aldrich) as references. The QY of **DPP**, **isoindigo**, and **NDI** were determined using oxazine 170 perchlorate dye (Ox-170, Sigma Aldrich) and 1,19,3,3,39,39-hexamethylindotricarbocyanine dye iodide (HITCI, Sigma Aldrich)

as references. The QY of **BBT**, **PSN**, and **PSeN** were determined using IR-E1050 (Nirmidas Biotech) as references.

Fluorescence decays of **BT**, **BT2**, **DTBT**, and **DTBT2** were measured using a home-built time-correlated single-photon counting (TCSPC) setup. Samples were excited at 531 nm using an LDH-P-FA-530L picosecond pulsed diode laser (PicoQuant). Fluorescence from the samples was focused on a monochromator (9030DS, Scientech) to obtain spectral separation and detected with a microchannel plate photomultiplier tube (MCP-PMT R3809U-63, Hamamatsu Photonics K.K.) in the photon counting mode. The signal from MCP-PMT was amplified by a high-speed amplifier (C5594-12, Hamamatsu Photonics K.K.). Fluorescence decays of **DPP**, **isoindigo**, and **NDI** were measured using a home-built single-photon counting (TCSPC) microscope setup based on an Olympus IX 71 platform.⁹ Measurements were performed in flow cells assembled from two glass coverslips sealed together with melted parafilm stripes. Sample illumination and fluorescence collection were performed through a water-immersion 60x objective lens (UPlanSApo, 60×, NA 1.20, Olympus). Samples were excited at 634 nm using a LDH-D-C-640 picosecond pulsed diode laser (PicoQuant).

Fluorescence decays of **BBT**, **PSN**, and **PSeN** were recorded with 785 nm excitation (Spectra Physic, Mai Tai Ti:Sapphire laser) using a water-immersion 60x objective (LUMFL N 60x/ 1.10 W, Olympus). The sync signal for the TCSPC measurement was generated by introducing a small fraction of the excitation beam to a silicon photodiode (TDA-200, Picoquant). The fluorescence signal from samples was collected by a silicon single-photon avalanche photodiode (τ -SPAD-50, PicoQuant,) after passing a long-pass emission filter, BLP01-532R (Semrock) for **BT**, **BT2**, **DTBT**, and **DTBT2**, BLP01-647R (Semrock) for **DPP**, **isoindigo**, and **NDI**, and RET 792 LP (Chroma) for **BBT**, **PSN**, and **PSeN**, respectively. All the TCSPC measurements were

conducted using a TCSPC module (HydraHarp 400, PicoQuant). The setup was controlled by SymPhoTime64 software (PicoQuant). The fluorescence decay curves were fitted to multi-exponential decaying functions with deconvolution of the instrument response function using FluoFit software (PicoQuant).

Dynamic light scattering experiment:

DLS measurements were conducted with dynamic light scattering Malvern Zetasizer NanoZS. The zeta-potential measurements were carried out in a polycarbonate folded capillary cell with gold-plated electrodes (DTS1070, Malvern Instruments). The Zetasizer NanoZS software used in this study calculated the zeta potentials by determining the electrophoretic mobility of the polymer dots.

Transmission electron microscopy experiment:

Transmission electron microscopy (TEM) images of the polymer dots were recorded using either Tecnai G2 Spirit TWIN (FEI) or Titan 80-300 kV CT (FEI) electron microscopes. The TEM grids were treated with plasma before sample preparation. The samples were prepared by drop-casting colloidal solutions of the polymer dots onto an ultrathin carbon on Cu grids (Q225-CMA, EMS; FCF-200-Cu, EMS; 668-400-CU, Ted Pella, Inc.) and drying them under vacuum by keeping in desiccator for 1 h before the measurement.

Results and Discussion

For a systematic investigation, we designed and synthesized a series of PCz-based DA-type conjugated polymers with different acceptor moieties (**BT**, **DTBT**, **DPP**, **isoindigo**, **NDI**, and

BBT, Figure 1).⁴⁶ To investigate the effect of side chains, we also designed and synthesized PCz-based polymers with different alkyl side chains on the Cz moiety (tertiary butyl for **BT** and **DTBT** and octyl for **BT2** and **DTBT2**, Figure 1). These polymers adopt twisted conformation due to the steric hindrance between the donor and acceptor moieties caused by their bent shape.⁵³ As reference compounds, we used PF-based DA-type conjugated polymers that have linear and planar shape (**PSN** and **PSeN**, Figure 1).⁴⁷

Figure 1. Chemical structures of the conjugated polymer molecules used in this study.

These conjugated polymer molecules dissolved in tetrahydrofuran (THF) showed broad and featureless steady-state absorption spectra that are typical for intramolecular charge-transfer (CT) absorption (Figure 2a, Table S1). Peak absorption wavelength of the PCz-based polymers ranges between 482 – 835 nm, depending on the energy gap between the ground and lowest excited states of the CT states. These conjugated polymer molecules showed fluorescence from the CT

states in either NIR (**BT**, **BT2**, **DTBT**, **DTBT2**, **DPP**, **isoindigo**, and **NDI**) or SWIR (**BBT**, **PSN**, and **PSeN**) wavelength regions (Figure 2b, Table S1).

Figure 2. Steady-state absorption and fluorescence spectra of the conjugated polymers. (a) Absorption and (b) fluorescence spectra of the conjugated polymer molecules dissolved in THF. (c) Absorption and (d) fluorescence spectra of the polymer dots dispersed in water.

Polymer dots of these conjugated polymers were fabricated using a reprecipitation protocol that we reported previously.⁴⁴ This protocol enabled us to fabricate polymer dots with a diameter of a few nanometers. Transmission electron microscopy (TEM) as well as dynamic light scattering

(DLS) experiments on the obtained polymer dots showed that the polymer dots fabricated using the series of conjugated polymers have similar sizes except **DPP** (Figure S1 and S2, Table 1). Differences in the particle sizes observed in the TEM and DLS experiments are attributed to hydration of the particles as well as size distribution of the particles (see Supporting Note). The fabricated polymer dots dispersed in water displayed relatively large negative zeta potentials, demonstrating a high colloidal stability of these polymer dots (Table 1). Most of the polymer dots showed slight bathochromic shift of the absorption (**BT**, **BT2**, **DTBT**, **DTBT2**, **DPP**, **isoindigo**, **NDI**, and **PSeN**) and fluorescence (**BT**, **DTBT**, **DTBT2**, **DPP**, **isoindigo**, and **BBT**) spectra (Figure 2c, 2d, and Table 1). The absence of large bathochromic shifts upon the particle formation observed in most of the PCz-based polymer dots indicates effective suppression of the intra- and inter-chain interactions. On the other hand, the fluorescence spectrum of the PF-based polymer dots (**PSN**) exhibited a large bathochromic shift (116 nm) upon the particle formation, indicating a strong interaction between the polymer chains inside the particles (Table 1).

Table 1. Spectroscopic and physical properties of the polymer dots in water

Sample	λ_{abs} (nm)	λ_{flu} (nm)	QY (%)	τ_{flu} (ns)	k_r (s ⁻¹)	k_{nr} (s ⁻¹)	d (nm)	d_h (nm)	ζ (mV)
BT	497	631	16	2.9	5.5×10^7	2.9×10^8	3.0	38	-54
BT2	497	603	5	2.0	2.5×10^7	4.8×10^8	5.3	73	-43
DTBT	501	659	20	3.6	5.6×10^7	2.2×10^8	4.4	44	-51
DTBT2	520	682	6	2.9	2.1×10^7	3.2×10^8	4.4	97	-43
DPP	593	653	4	2.6	1.5×10^7	3.7×10^8	27	61	-77
isoindigo	477/560	710	0.5	0.68	7.5×10^6	1.5×10^9	3.3	43	-51
NDI	622	733	2.3	2.5	9.2×10^6	3.9×10^8	5.0	29	-73
BBT	835	1035	0.29	0.38	7.6×10^6	2.6×10^9	3.6	32	-40
PSN	736	1040	0.31	0.83	3.7×10^8	1.2×10^9	3.5	80	-61
PSeN	812	934	0.016	1.1	1.5×10^5	9.1×10^8	3.0	51	-29

λ_{abs} : peak absorption wavelength, λ_{flu} : peak fluorescence wavelength, QY: fluorescence quantum yield, τ_{flu} : average fluorescence lifetime, k_r : radiative deactivation rate constant, k_{nr} : non-radiative deactivation rate constant, d : diameter, d_h : hydrodynamic diameter, ζ : zeta potential.

The conjugated polymers in THF showed a general trend of decreased fluorescence quantum yield (QY) for the molecules that emit fluorescence at longer wavelengths (Figure 3, Table S1).

This trend is interpreted by an energy gap law that predicts a larger non-radiative rate constant of the excited state deactivation when energy gap between the ground and excited state becomes smaller (i.e., fluorescence at longer wavelength).⁵⁴ A drop in the QY upon the formation of the polymer dots was observed for most of the conjugated polymers (**BT**, **BT2**, **DTBT**, **DTBT2**, **DPP**, **BBT**, **PSN**, and **PSeN**) except **isoindigo** and **NDI** that showed slight increase in the QY (Figure 3, Table 1). The decrease in the QY upon the particle formation suggests the generation

of energy trap (i.e., fluorescence quenching) sites inside the particles due to the intra- and inter-chain interactions.⁵⁵⁻⁵⁶ This is consistent with the bathochromic shifts of the steady-state absorption and fluorescence spectra of the conjugated polymers upon the particle formation (Figure 2). Importantly, most of the PCz-based polymer dots (**BT**, **BT2**, **DTBT**, **DTBT2**, and **BBT**) displayed relatively small reduction in the QY (up to six fold reduction) upon the particle formation. Much larger reduction in the QY is usually observed in many polymer dots.⁵⁷ Indeed, a large reduction in the QY (25-87 fold reduction) was observed in the PF-based polymer dots (**PSN** and **PSeN**). These results demonstrate that the reduction of the QY can be effectively suppressed by fabricating polymer dots using conjugated polymers that have bent and twisted conformation. While this was suggested in our previous study on **BT** and **DTBT**, the comparison between the PCz-based and PF-based polymer dots and the systematic characterization of the series of the PCz-based conjugated polymers provide strong evidences that this approach can be generalized to a wider range of conjugated polymer molecules that show fluorescence in NIR to SWIR wavelength regions. It should also be mentioned that molar extinction coefficients (ϵ) of the Cz-based conjugated polymers are higher than those previously reported for donor-acceptor-donor-type molecules.^{10, 44, 58-59} Since the fluorescence brightness of fluorescent probes is determined by both ϵ and QY, the large ϵ of the Cz-based conjugated polymers and therefore the large ϵ of the Cz-based polymer dots provides an additional advantage in fluorescence imaging experiments.

Figure 3. Fluorescence quantum yields of the PCz-based (blue) and PF-based (red) conjugated polymers in THF (open circles) and the polymer dots dispersed in water (filled circles) plotted against their peak fluorescence wavelengths. The arrows highlight the change in the fluorescence quantum yield and peak fluorescence wavelength upon the formation of the polymer dots.

We next investigated factors affecting the fluorescence brightness (i.e., QY) by conducting time-resolved fluorescence spectroscopy experiments. Fluorescence decay curves of the conjugated polymer molecules in THF and the polymer dots dispersed in water were measured using time-correlated single-photon counting method (Figure S3 and S4). We calculated radiative (k_r) and non-radiative (k_{nr}) deactivation rate constants using the QY of the conjugated polymers and polymer dots and mean fluorescence lifetimes determined by the time-resolved fluorescence measurement (Figure 4). We found that both k_r and k_{nr} obtained from the PCz-based conjugated polymers change upon the particle formation (Figure 4). A comparison between the data obtained from the PCz-based conjugated polymer molecules in THF and the polymer dots dispersed in water revealed that the changes in k_r and the changes in QY upon the particle

formation are positively correlated in most of the molecules (**BT**, **BT2**, **DTBT**, **DTBT2**, **DPP**, **NDI**, and **BBT**, Figure 3 and Figure 4a) whereas the changes in k_{nr} and the changes in QY are negatively correlated in all the molecules (**BT**, **BT2**, **DTBT**, **DTBT2**, **DPP**, **isoindigo**, **NDI**, and **BBT**, Figure 3 and Figure 4b). In stark contrast to the PCz-based conjugated polymers, the PF-based conjugated polymers (**PSN** and **PSeN**) did not show significant change in k_{nr} upon the particle formation although we observed the large reduction of QY (Figure 3 and Figure 4b). Only k_r showed large changes (i.e., reduction) when the PF-based conjugated polymers (**PSN** and **PSeN**) formed the polymer dots (Figure 4a). These results indicate that photophysical mechanisms that regulate fluorescence brightness of the PCz-based and PF-based polymer dots are distinct.

A drop of QY, and therefore a drop of fluorescence brightness, has been commonly observed when polymer dots are fabricated from conjugated polymers.⁵⁷ In many cases, the generation of fluorescence quenching sites inside the particles due to polymer chain interactions, which accelerates non-radiative deactivation,⁵⁵⁻⁵⁶ has been considered as a primary origin of the reduction of QY. The negative correlation between k_{nr} and QY can, therefore, be interpreted by this mechanism. Importantly, the change in the k_{nr} upon the particle formation is relatively small (maximum 3 fold increase) in all the PCz-based polymer dots (**BT**, **BT2**, **DTBT**, **DTBT2**, **DPP**, **isoindigo**, **NDI**, and **BBT**, Table 1). This observation is consistent with the relatively small reduction in QY upon the formation of the particles and provides a firm evidence that the undesired fluorescence quenching is efficiently suppressed in the PCz-based polymer dots.

Figure 4. (a) Radiative (k_r) and (b) non-radiative (k_{nr}) deactivation rate constants of the PCz-based (blue) and PF-based (red) conjugated polymers in THF (open circles) and the polymer dots dispersed in water (filled circles) plotted against their peak fluorescence wavelengths. The arrows highlight the change in k_r , k_{nr} , and peak fluorescence wavelength upon the formation of the polymer dots.

In contrast to the well-studied effect of non-radiative deactivation pathways on the fluorescence brightness of polymer dots, the contribution of radiative process on their fluorescence brightness

has received little attention. The time-resolved fluorescence measurements clearly revealed the positive correlation between the changes in k_r and the changes in QY upon the particle formation in most of the PCz-based polymer dots (Figure 3 and Figure 4a). We previously demonstrated using **BT** and **DTBT** that larger intramolecular twisting between the Cz and acceptor moieties causes smaller k_r due to the reduced oscillator strength.⁵³ Given the structural similarity between the PCz-based conjugated polymers, our data strongly suggest that change in the twist angle between the Cz and acceptor moieties upon the particle formation is the primary reason for the observed change in k_r , which is positively correlated with QY. Since k_r is proportional to the oscillator strength that describes the probability of light absorption,⁶⁰ the intramolecular twist influences the fluorescence brightness of the polymer dots not only through QY but also through the probability of light absorption (i.e., absorption cross section) by the particles.

These findings provide important insight into the development of NIR and SWIR emitting polymer dots with bright fluorescence. Ratios of the QY obtained for the polymer dots dispersed in water and the conjugated polymer molecules dissolved in THF (QY^{Pdots}/QY^{THF}) were plotted against the ratios of k_{nr} obtained for the polymer dots and the conjugated polymer molecules ($k_{nr}^{Pdots}/k_{nr}^{THF}$) in Figure 5a. QY^{Pdots}/QY^{THF} were also plotted against the ratios of k_r obtained for the polymer dots and the conjugated polymer molecules (k_r^{Pdots}/k_r^{THF}) in Figure 5b. These plots clearly show general trends that can be applied to all the PCz-based polymer dots (Figure 5 shaded region). First, the larger the reduction of k_{nr} ($k_{nr}^{Pdots}/k_{nr}^{THF} < 1$) is, the larger the fluorescence enhancement ($QY^{Pdots}/QY^{THF} > 1$) is, and the larger the enhancement of k_{nr} ($k_{nr}^{Pdots}/k_{nr}^{THF} > 1$) is, the larger the reduction of fluorescence intensity ($QY^{Pdots}/QY^{THF} < 1$) is. Second, the larger the enhancement of k_r ($k_r^{Pdots}/k_r^{THF} > 1$) is, the larger the fluorescence

enhancement ($QY^{Pdots}/QY^{THF} > 1$) is, and the larger the reduction of k_r ($k_r^{Pdots}/k_r^{THF} < 1$) is, the larger the reduction of fluorescence intensity ($QY^{Pdots}/QY^{THF} < 1$) is.

Figure 5. Ratios of the QY obtained for the polymer dots dispersed in water and the conjugated polymer molecules dissolved in THF (QY^{Pdots}/QY^{THF}) plotted against (a) the ratios of k_{nr} obtained for the polymer dots and the conjugated polymer molecules ($k_{nr}^{Pdots}/k_{nr}^{THF}$) and (b) the ratios of k_r obtained for the polymer dots and the conjugated polymer molecules (k_r^{Pdots}/k_r^{THF}). The data obtained for the PCz- and PF-based polymer dots are shown in blue and red, respectively.

Important point is that these correlations are not dependent on the fluorescence wavelength of these PCz-based polymer dots. This means that the fluorescence brightness of the PCz-based polymer dots is governed by the two factors (i.e., fluorescence quenching inside the particles due to the chain-chain interactions and intramolecular twisting between the Cz and acceptor moieties) irrespective of the fluorescence wavelength. Therefore, polymer dots with bright fluorescence can be, in principle, obtained by controlling these two factors through proper molecular design and optimization of fabrication protocol. Indeed, the polymer dots fabricated using the conjugated polymers with the same acceptor moiety but different alkyl side chains (**BT** and **BT2**, and **DTBT** and **DTBT2**) showed distinct behavior (Figure 5), which points to the possibility of controlling the two factors by proper molecular design. The fluorescence behavior of **BT** and **DTBT** polymer dots also depends on spatial packing of the polymer chains inside the particles,⁴⁴ which poses another possibility of controlling these factors by fabrication protocol of the polymer dots. The PF-based polymer dots that were fabricated using linear and planar-shaped PF-based conjugated polymer molecules did not follow the general trends that we observed for the PCz-based polymer dots (Figure 5). This result implies that the bent and twisted shape of the PCz-based conjugated polymers is essential for the observed general trends. Unlike PCz-based polymer dots, fluorescence properties of the PF-based polymer dots do not depend on fabrication protocol of the particles (Figure S5), providing another evidence that the bent and twisted shape of the PCz-based conjugated polymers creates a new possibility for engineering fluorescence properties of polymer dots including fluorescence brightness.

Conclusions

In conclusion, we showed in this study that polymer dots that exhibit bright fluorescence in the NIR to SWIR wavelength regions can be obtained by using PCz-based DA-type conjugated polymer molecules that adopt bent and twisted shape (Table S2). Our results demonstrated that the fluorescence brightness of the polymer dots is regulated by the intraparticle fluorescence quenching due to the polymer chain interactions and the twisting between the Cz and acceptor moieties of the conjugated polymers inside the particles, irrespective of the fluorescence wavelength. Our findings provide important insight into a new strategy for the rational design and fabrication of polymer dots that show bright fluorescence in the SWIR wavelength region, which would contribute to the development of SWIR fluorescence-based single-molecule (particle) bioimaging technology.⁶¹⁻⁶²

ASSOCIATED CONTENT

Supporting Information. The supporting Information is available free of charge on the ACS Publications website at DOI:

Results of spectroscopic characterization of the conjugated polymers in THF, results of TEM and dynamic light scattering experiments on the polymer dots, results of spectroscopic characterization of PSN polymer dots fabricated at different conditions, a table summarizing fluorescence properties of SWIR-emitting fluorophores (PDF)

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Notes

The authors declare no competing financial interests.

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