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Brilliant BODIPY-fluorene Copolymers With Dispersed Absorption and Emission Maxima

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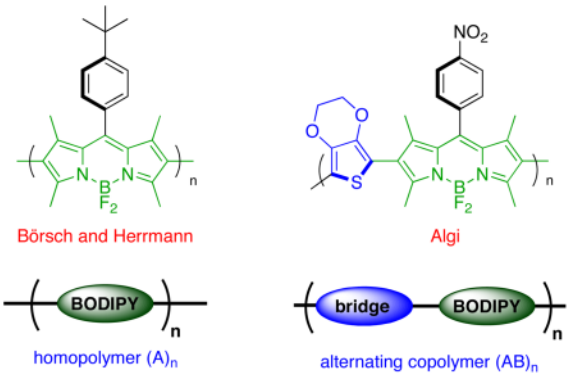
Abstract

Four systems **1a** – **d** were prepared to investigate the optical properties of copolymers comprised of polyfluorene doped with BODIPY-based fluors. The underlying hypothesis was energy harvested via the strong absorptivity of the major component, fluorine, would be primarily emitted from the BODIPY parts at much higher wavelengths. Optimization of the polymerization process as a function of the mol % of BODIPY, indicated that the brightest polymers were formed when approximately 4 fluorene units were co-polymerized with every BODIPY precursor. These polymers were cast into nanoparticles of *ca* 40 nm diameter. Treatment of clone 9 rat liver cells with suspensions of these particles resulted in uptake without encapsulation in lysosomes, or organelle targeting.

A relatively new field of interest in the chemistry of BODIPY (4,4-difluoro-4-bora-3a,4a-diaza-*s*-indacene) dyes[1–3] is to polymerize them to concentrate multiple fluor units in single molecules,[4–7] hence to form bright particles for materials or for imaging biological systems. Polymers of this kind tend to have desirable properties for those applications because they concentrate several fluorescent groups in one molecule. Brightness-per-fluorophore in *homo*polymeric-dye systems is not necessarily greater than the free dye, and scope for manipulation of spectroscopic properties is limited; consequently it is logical to research similar polymers from two or more components. This strategy allows donor fragments that absorb light of a desirable wavelength to collect and transfer energy to fluorophores that then fluoresce. In this paradigm, donor:acceptor ratios may be adjusted to optimize the brightness-per-fluorophore (*note* brightness-per-fluorophore = absorption cross-section of the donor parts × quantum yield of the fluorophore in the polymer).



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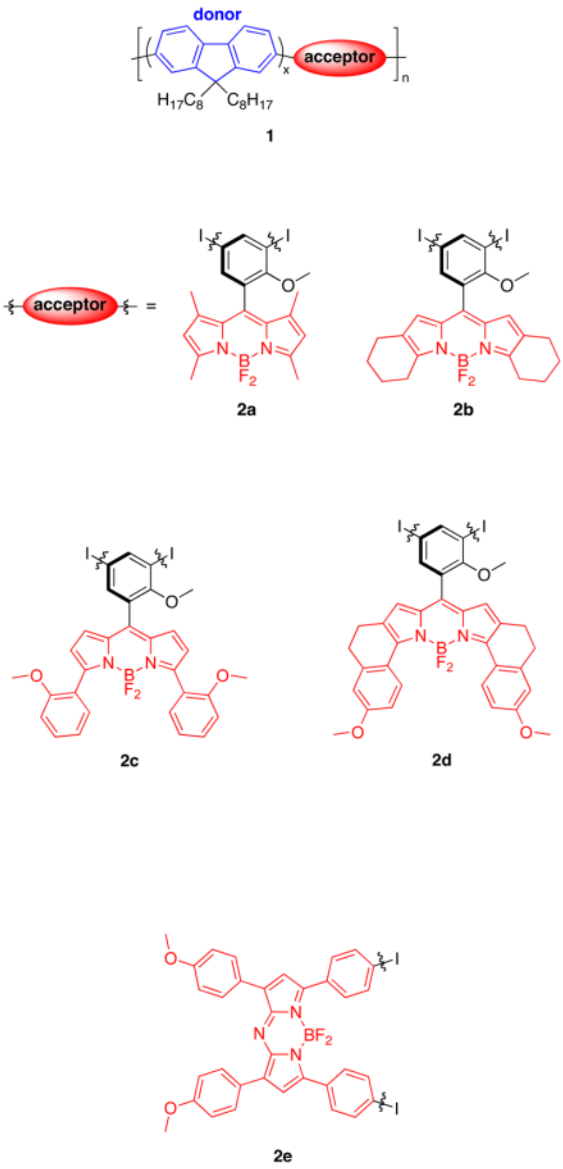
Supporting Information Available. Procedures and characterization data for all the new compounds, details of the live-cell imaging experiments, and other tables and figures mentioned in manuscript. This material is available free of charge via the Internet at http://pubs.acs.org.

To the best of our knowledge this has not been attempted; all the BODIPY-containing polymers reported to date are homopolymeric or BODIPY-bridge-BODIPY-bridge... (*ie* ABAB) composites.[5–15] There are none of the type $(donor)_m$-BODIPY-$(donor)_n$-BODIPY-.... in which the acceptor fluorophores is "doped" into the donor-polymer backbone. This is significant because donor-rich polymers could have extremely high UV absorption cross sections, and accommodate fast, efficient, energy transfer to the BODIPY fluorophores, affording bright probes with excellent separations of absorption and emission wavelengths. Significantly, direct covalent linkage of the donor and acceptor fragments facilitates energy transfer *through-bonds* hence the requirement for overlap of the donor emission with the acceptor absorption (as in through-space energy transfer) no longer applies.

This communication describes donor-rich polymers **1** designed to maximize energy transfer to BODIPY or related acceptor fragments. Fluorene donor parts were chosen because of their photostabilities, large molar absorptivities, high two-photon cross-sections, and ease of syntheses.[16–18] Violet-blue emissions from polyfluorenes are *not* ideal for cellular or *in vivo* imaging, but we hypothesized that appropriately designed BODIPY-doped polyfluorene polymers could be excited at the donor parts, and would emit brightly at much longer wavelengths characteristic of the acceptor parts. Another design attribute of these systems is that variations of the acceptors would enable tuning of fluorescence outputs. Data described here shows that in fact such polymers could be made and optimized for emission via the acceptor. Further, representative systems were cast into particles of around 50 nm that were shown to permeate into Clone 9 rat liver cells where they could be imaged.



Scheme 1 outlines the synthetic route that was used to obtain the probes **1** featured in this study. It relies on Suzuki-based polymerizations of two fluorene components and the diiodides **2a–e**; the latter **2a–d** are all novel compounds, and their syntheses were based on related materials that had previously been prepared but without the two aryl iodide groups or, in some cases, without *meso*-substituents entirely (see supporting), while AzaBODIPY **2e** was previously prepared by our group.[23] Those acceptors have emissions that are dispersed at intervals of about 30 to 40 nm, from 520 to 700 nm (Table 1). BODIPY **2b** has an extremely high molar absorptivity and an exceptional quantum yield, and **2c** shows an unusually large Stokes' shift (*ie* 1565 cm^{-1}). Acceptor-precursors **2a–d** have *ortho*-methoxy-substituents on their *meso*-aryl groups to minimize radiationless decay via rotation. The polymers were purified by precipitation from the medium used for the Suzuki couplings (toluene) by addition to methanol. Low molecular weight impurities were subsequently removed via multiple acetone washes, each over an extended period.

Experiments were performed to optimize the brightness of **1a** when excited at the fluorene $\lambda_{max/abs}$ by varying the fluorene:BODIPY ratios used in the polymerization. These studies (supporting) showed the brightness peaked at *ca* 4:1 (donor:acceptor). This observation reflects a compromise between effects that increase the brightness (*eg* the cross section of the donor runs) and those that decrease it (*eg* acceptor-acceptor quenching, structural perturbations, and average separation of the fluorene units to the next BODIPY in the chain). No significant change in either the absorbance or emission wavelengths was observed for the acceptor when increasing its concentration in the polymer. These changes were monitored via Energy Transfer Efficiency (ETE %) and Energy Transfer (ET %) as indicated in Table 2. We define ET as the percentage of total fluorence of the polymers excited at the donor attributable to the acceptors. ETE % is a measure of the quantum yield of the cassette when irradiated at the donor. It reflects the extent of energy transfer including the negative effects of non-radiative loss in the transfer process.

$$\text{ETE }\% = \frac{\text{quantum yield of the acceptor fragment in the cassette excited at the donor}}{\text{quantum yield of the acceptor fragment in the cassette excited at the acceptor}} \times 100$$

Overall the observations described above motivated us to make a series of polymers that contained *ca* 0.19 equivalents of the acceptor monomers; this was achieved using 0.54 equivalents of the BODIPY acceptor. A 48 h polymerization run was used; shorter times gave less brilliant polymers (see supporting). Table 2 gives the essential parameters of polymers **1a** – **e** formed via the conditions mentioned above.

Emissions from the polymers occur mainly from the acceptors with ET % ranging from 86–98 % (Figure 1). The emission maxima of polymers **1a–d** are at wavelengths slightly lower to that of the free acceptors **2a–d**. For example, polymer **1d** has an emission maximum at 685 nm while acceptor **2d** has a maximum at 677 nm. Polymer **1e** shows a considerable increase (43 nm) in its emission wavelength compared to acceptor **2e**. This could be because the phenyl groups on azaBODIPYs in **1e** can adopt conformations that are more planar with the heterocyclic core than the *ortho*-substituted *meso*-aryl groups of **1a** – **d**. In other words, polymerization with fluorene extends the conjugation of the azaBODIPY.

Energy transfer efficiencies (ETE) in the series decreased as the $\lambda_{max/emiss}$ values for the acceptors increased. Throughout the series, and most markedly for the azaBODIPY systems **1e** and **2e**, fluorescence quantum yields of the acceptor fragments *decreased* in the polymers; in fact, the quantum yield of the azaBODIPY-polymer **1e** is only 0.21% and even with less acceptor, the same type of quenching was observed, see supporting.

McNeill *et al.* have reported efficient generation of organic nanoparticles from polyfluorene and similar systems.[24,25] We investigated whether comparable nanoparticles could be constructed from doped polymer **1**. In the event, slight modifications were found to be more suitable for these polymers; slower addition rate of a more dilute THF solution of the polymer to water gave particles of 48.4 ± 13.3 nm by dynamic light scattering in the case of **1b**. TEM investigations of particles made from **1a–d** led to aggregation before the analysis though smaller particles averaging 20 to 60 nm could be seen.

Uptake of the organic nanoparticles from **1** was studied on normal rat liver cells (Clone 9). After 40 h incubation at 37 °C in Ham's + 5% fetal bovine serum (FBS) culture medium, the nanoparticles were observed as bright dots inside the cells (Figure 2); they did not specifically target organelles. Polymers incubated under same conditions did not enter the cells.

The work reported here features cassettes with donor and acceptor fluorophores joined via linkers that would allow conjugation if the molecules became planar.[26–33] However, the twist between these fragments "insulates" the donor and acceptor parts so that the overall emission characteristics of the composites mirror those of the free acceptor. This makes it possible to design of materials with predictable fluorescence emission wavelengths. This property coupled with the fact that there are several donors per acceptor part, offers potential for fluorescent probe design, OLEDs and lasing materials.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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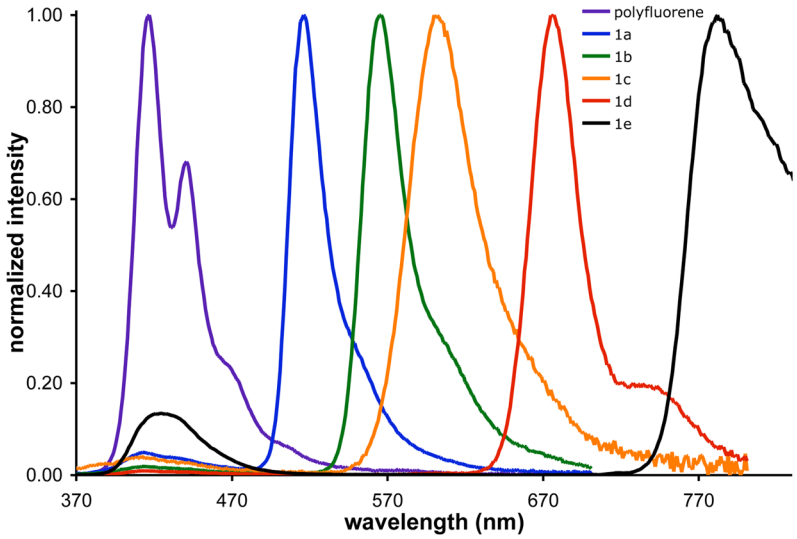


Figure 1.
Normalized fluorescence of **1** in CH_2Cl_2 at 10^{-6} M excited at 358 nm.

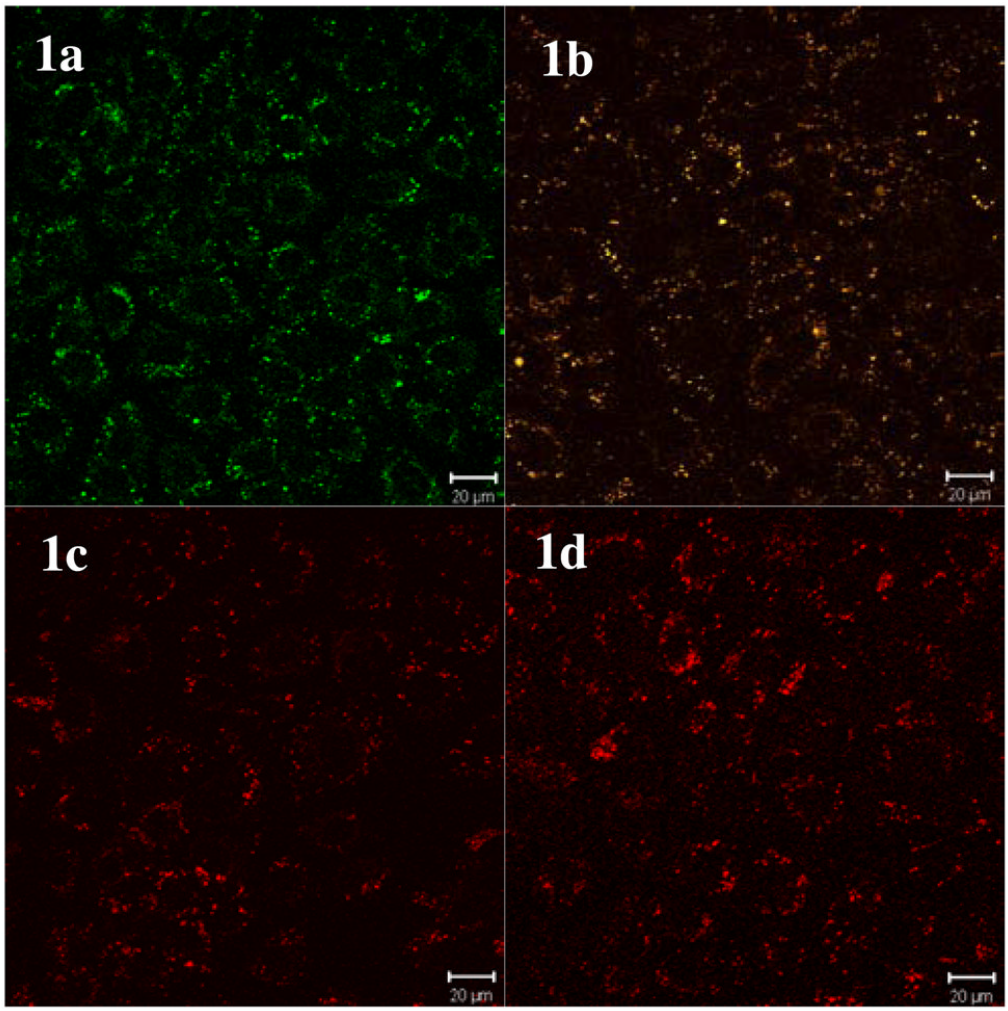
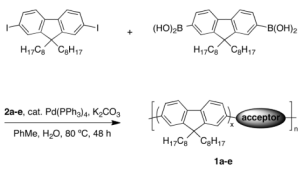


Figure 2.
Confocal imaging of Clone 9 rat liver cells with polymeric nanoparticles **1a–d**.



Scheme 1.

Syntheses of the polymers **1a–e** yielding M_w ranging from 7 to 15 kDa (supporting).

Table 1

Spectroscopic properties of fluorene and acceptors in CH_2Cl_2

	$\lambda_{abs\ max}$(nm)	$\varepsilon \times 10^{-4}$ ($cm^{-1}\bullet M^{-1}$)	$\lambda_{em\ max}$(nm)	φ	fwhm[a] (nm)	Stokes Shift (cm^{-1})
fluorene	266	14.7	308	0.28[b]	26	5126
2a	510	8.0	521	0.36[c]	26	414
2b	556	12.0	573	1.0[d]	32	534
2c	556	5.6	609	0.48[d]	46	1565
2d	667	15.0	685	0.46[e]	37	394
2e	678	6.0	710	0.05[e]	47	665

[a] Fluorescence full width at half maximum peak height

[b] Naphthalene in cyclohexane as standard (φ = 0.92)[19]

[c] Fluorescein in 0.1M NaOH as standard (φ = 0.92)[20]

[d] Rhodamine B in EtOH as standard (φ = 0.65)[21]

[e] Zinc phthalocyanine in pyridine as standard (φ = 0.30)[22]

Table 2

Photophysical properties of polyfluorenes incorporated with acceptors in CH_2Cl_2

polymer	mol % acceptor[a]	$\lambda_{abs\ fluorene}$ (nm)	$\lambda_{abs\ bodipy}$ (nm)	λ_{em} (nm)[b]	ETE %[c]	ET %[d]	$\varphi_{acceptor}$	$\varphi_{polymer}$[h]	molar absorptivity × 10^{-5} (cm^{-1}•M^{-1})
1a	19	343	505	517	70.8±0.5	90.5±0.6	0.147±0.002[e]	0.104±0.002	5.1±0.9
1b	27	343	549	566	60±2	97.2±0.3	0.24±0.01[f]	0.14±0.05	5.74±0.07
1c	22	344	547	602	48±8	95.9±0.5	0.13±0.02[f]	0.06±0.02	5.18±0.03
1d	25	347	657	677	37±3	98±1	0.25±0.07[g]	0.09±0.03	4.3±0.3
1e	40	336	703	753	49.4±0.3	86±2	0.002[g]	0.001	4.58±0.07

[a] Estimated by NMR.

[b] Emission maximum when excited at 358 nm (fluorenes)

[c] Energy transfer efficiency (ETE) was calculated by dividing the quantum yield of acceptor when excited at fluorene donor by the quantum yield of acceptor when excited at acceptor.

[d] Percent of the total fluorescence being emitted by the acceptor.

[e] Fluorescein in 0.1M NaOH as standard (φ = 0.92).[20]

[f] Rhodamine B in EtOH as standard (φ = 0.65)[21]

[g] Zinc phthalocyanine in pyridine as standard (φ = 0.30)[22]

[h] Quantum yield of polymer calculated as the product of ETE and the quantum yield of the acceptor.