

Incorporating BoDiPY Molecular Rotors into Semiconductive Fluorene Polymers

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1 Summary

There is an increasing demand for molecular sensors to measure phenomena at ever smaller scales with minimal invasiveness. Localized rheology can provide insight in many processes at cellular scales, and molecular rotors are ideal molecular sensors for this purpose. Molecular rotors are fluorescent compounds which have a spectral response to rotation around a specific bond in the molecule. Many classes of molecular rotors have been described but in this work molecular rotors based on BoDiPy are considered. BoDiPy-based molecular rotors are easily synthesised and modified after synthesis. A downside however is that time-resolved fluorescence methods are required when the concentration cannot be controlled or determined independently. To resolve this, a secondary fluorophore can be attached covalently to a molecular rotor and be used as an internal standard with the molecular rotor, such constructs being known as radiometric dyes. Polyfluorene has excellent properties to serve as a secondary fluorophore with BoDiPy and therefore copolymers of fluorene and BoDiPy-based molecular rotors were synthesised with both alternating and random sequence. Their fluorescence lifetime response to viscosity was tested in mixtures of toluene and castor oil, to find that the individual properties of polyfluorene and BoDiPY had been retained in the copolymer, and the relation of fluorescence lifetime and radiometric ratio was found to be linear. The radiometric dye was then encapsulated in nanoparticles of poly-[octadecyl acrylate] (pODA), a crystallisable polymer, and the evolution of the fluorescence emission spectrum was recorded over several cycles of melting and crystallising. It was found however that the radiometric ratios had a behaviour opposite to what was expected and this was thought to be caused by the opacity of the nanoparticles in their solid state. Further research should be undertaken to further characterize the BoDiPy-fluorene copolymer system and to apply this in polymer glasses where opacity will not be an issue.

2 Introduction

There is an increasing demand for new methods to measure phenomena at smaller scales. Traditional methods of physical chemistry are often not applicable in cellular phenomena or can be invasive. Many molecular sensors have been developed for this purpose that can quantify properties at a highly localised scale.[11][10] These generally have a spectroscopic read-out, thus allowing for minimal invasiveness. Cellular rheology has the potential to provide insight into many cellular processes. To measure the rheological properties of cellular compartments, molecular sensors have to be developed tailored to the specific process and cellular environment. A particularly useful type of molecular rheological sensor is the molecular rotor.[5]

Molecular rotors are fluorescent compounds which show a spectral response to rotation around a specific bond in the molecule.[4] This response varies between different species of molecular rotors but in general involves a changed conformational energy landscape in the excited state where rotation results in a lower energy and therefore is favoured. This rotation results in a reduction of the band-gap as the excited state energy decreases and the corresponding ground state energy for the given conformation increases, therefore resulting in a red-shifted emission or non-radiative decay. The relation between viscosity and fluorescence lifetime is given by the Förster-Hoffman equation, as described in [7]. Their response to rotation makes molecular rotors useful as sensors for local physical properties influencing this rotation, such as temperature, polarity, confinement and viscosity. Many different classes of molecular rotors have been described, among which are thioflavin[4], 9-(dicyanovinyl)-julolidine and derivative carboxylates[4][7], porphyrin dimers[7] and indocyanines[7]. This work however focuses on BoDiPy based molecular rotors[7]. These different classes of molecular rotors are shown in Figure 1 along with the numbering scheme used for BoDiPy-based molecular rotors within this work[3].

BoDiPy dyes bearing a phenyl on the 8-position (also known as the meso-position[3]) can function as molecular rotors. Their functionality as a molecular rotor involves a non-radiative decay which becomes accessible as the phenyl ring rotates from its ground state conformation at $45\text{-}65^\circ$ relative to the BoDiPy core[2] to 0° relative to the core, while the core itself puckers due to steric repulsion of the nearby hydrogen atoms.[9] This can be measured through simple spectroscopic methods but is more easily determined with time-resolved spectroscopy. BoDiPy-based molecular rotors are easily synthesised and substituents can be installed both pre-synthetically and as post-synthesis modifications, with symmetrically and asymmetrically substituted cores accessible both pre- and post-synthetically [3][6]. The particular reactivity of the BoDiPy core provides excellent capacity for tuning and functionalization, however a few considerations must be taken into account when designing BoDiPy-based molecular rotors. Foremost is that rotation around the core-to-phenyl bond is not hindered sterically. This requires that there are no substituents at positions 1, 7, 2' and 6'. A second consideration is that molecular rotor functionality is very sensitive to manipulation of the electronic structure and therefore introduc-

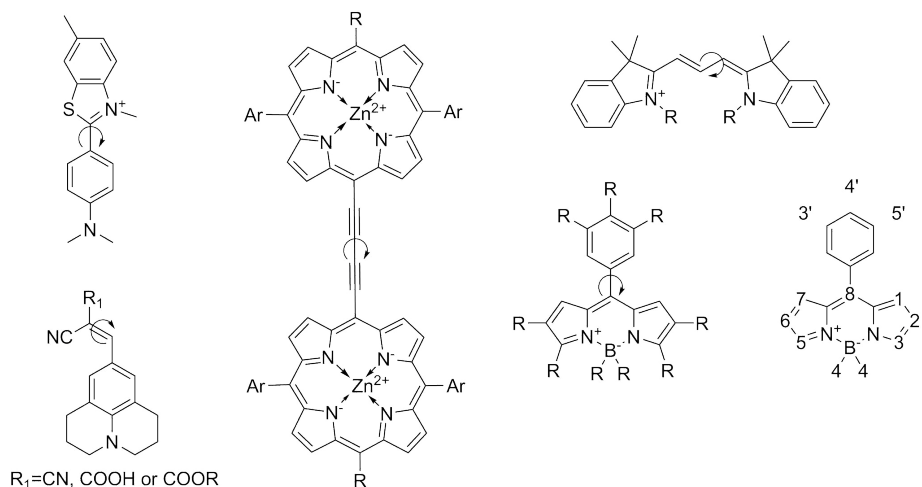


Figure 1: Selected examples of molecular rotors

ing substituents on the core that can extend the pi-conjugation may compromise this functionality. This limits the tuning potential of BoDiPy-based molecular rotors. Overall, BoDiPy-based molecular rotors are a promising class of molecular sensors. A major challenge however remains the need for time-resolved spectroscopy methods to use these in systems where the concentration cannot be known. This can be resolved by incorporating a separate fluorophore with minimal response to viscosity, thus serving as an internal standard, a construct known generally as a rediometric dye[5]. Fluorescence from this internal standard can then be compared to fluorescence from the BoDiPy-based molecular rotor to obtain information about the local viscosity. In this work, polyfluorene is considered as the internal standard.

Polyfluorene is a semiconductive polymer with a characteristic blue fluorescence and a wide range of uses. The fluorescence emission spectrum shows a peak at 415 nm followed by vibronics at 440 and 470 nm. Furthermore, polyfluorene is generally well-behaved from a spectroscopic perspective, having a very short and well-defined fluorescence lifetime that shows minimal response to changing viscosity.

In principle it should be possible to co-polymerise BoDiPy-based molecular rotors with fluorene derivatives to obtain a BoDiPy-doped polyfluorene. Whether such copolymers function as molecular rotors with built-in internal standard remains a question. Sub-questions are; 1) whether the polyfluorene and BoDiPy fluorescence is retained, 2) whether the functionality as molecular rotor is retained, 3) whether such copolymers have a reliable response to local viscosity quantifiable in fluorescence lifetime and fluorescence intensity.

These questions will be answered by synthesizing such molecules with both alternating and random sequences and evaluating their spectroscopic proper-

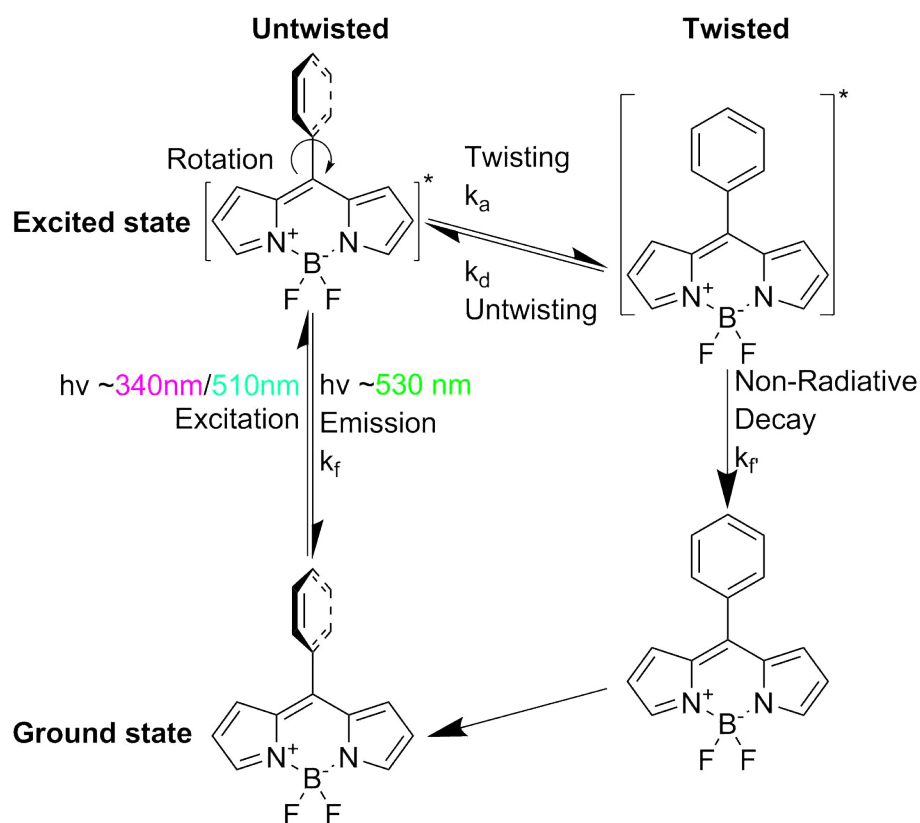


Figure 2: Schematic representation of BoDiPY rotor mechanism

ties through common and time resolved fluorescence spectroscopy in various viscous media. Furthermore, they will be encapsulated in nanoparticles of poly[octadecyl acrylate] (pODA), a crystalizable polymer having a distinct melting point as opposed to a glass transition range. This will be used to examine the extremes of the utility of this molecular rotor system.

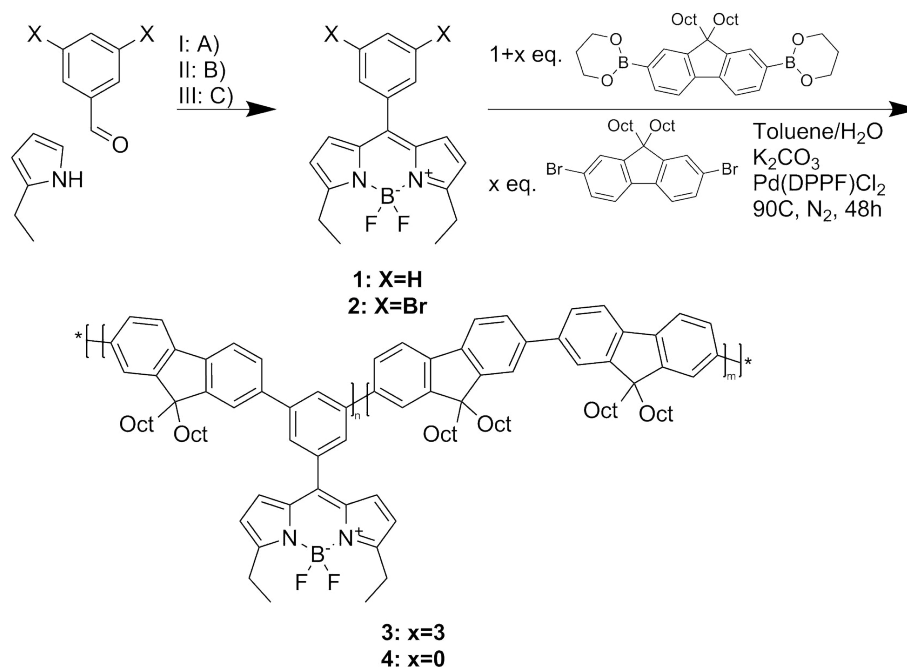


Figure 3: Overview of the synthesis of molecular viscosity sensors. Only **2** goes through the second reaction. Conditions: A) TFA, DCM, N₂, r.t., 12h; B) TCQ, DCM, r.t., 50 min; BF₃*O(Et)₂, DCM, DiPEA, r.t., 45 min

3 Materials and Methods

3.1 Synthesis

The synthesis of **1** and **2** was based on a procedure from Barba-Bon et al.[1].

3.1.1 **1 (3,5-diethyl-4,4-difluoro-8-phenyl-4-bora-3a,4a-diaza-s-indacene)**

To a 250 ml round bottom flask was added 50 ml dry DCM and the flask was closed with a septum. To this flask 1.0 ml (1.06 g, 10 mmol) benzaldehyde was added. The flask was wrapped in aluminium foil and the solution was degassed for 15 minutes. 2.04 ml (1.9 g, 20 mmol) 2-ethyl-1H-pyrrole was added followed by 90 μ L of trifluoroacetic acid (TFA). This was stirred overnight at room temperature under nitrogen atmosphere. To this was added 2.46 g (10 mmol) tetrachloro-1,4-benzoquinone (TCQ) and then stirred for 50 minutes at room temperature. To this 10 ml DiPEA was added followed by stirring for 30 minutes. To this 10 ml BF₃*O(Et)₂ was added and stirred overnight. Solvents were removed under vacuum, then 100 ml methanol was added and heated to reflux to fully dissolve the crude product. This was transferred to a beaker and

washed with a further 40 ml methanol and an approximately equal volume of water was added precipitating the crude product. This was purified by silica column chromatography using DCM as eluent. 800 mg (2.47 mmol, 24.7%) was recovered as a film of green and orange crystalites. ¹H-NMR (400 MHz, CDCl₃, 300 K): (ppm) 7.54-7.48 (m, 5H), 6.75 (d, 2H, J=4.2 Hz), 6.35 (d, 2H, J=4.2 Hz), 3.09 (q, 4H, J=7.6 Hz), 1.36 (t, 6H, J=7.6 Hz) TLC (SiO₂ on Al foil, DCM : heptane (v/v), rf): 4:0, 0.95 ; 3:1, 0.72; 2:2, 0.44, 1:3, 0.17

3.1.2 2 (8-[3,5-dibromophenyl]-3,5-diethyl-4,4-difluoro-4-bora-3a,4a-diaza-s-indacene)

To a 250 ml round bottom flask was added 50 ml dry DCM and 2.64 g (10 mmol) 3,5-dibromobenzaldehyde. The flask was wrapped in aluminium foil and closed with a septum. The solution was degassed for 15 minutes. 2.04 ml (1.9 g, 20 mmol) 2-ethyl-1H-pyrrole was added followed by three drops of trifluoroacetic acid (TFA). This was stirred overnight at room temperature under nitrogen atmosphere. To this was added 2.46 g (10 mmol) tetrachloro-1,4-benzoquinone (TCQ) slurried in 30 ml dry DCM, followed by a further washing with 5 ml dry DCM, and then stirred for 50 minutes at room temperature. To this 10 ml DiPEA was added followed by stirring for 30 minutes. To this 10 ml BF₃*O(Et)₂ was added and stirred for 45 minutes. Solvents were removed under vacuum and the remaining suspension was filtered through a cotton plug and washed with DCM. The resulting filtrate was purified by silica column chromatography using DCM as eluent, recovering 887.4 mg of final product and a further 2.137 g of impure product in a secondary fraction. The secondary fraction was recrystallised from 80 ml methanol, obtaining 2.0796 g, which was dried in a vacuum oven overnight. The total yield is 2.967 g (61.1%) of which 0.8874 g (18.3%) in the first fraction. MS: M+H/z, relative abundance: 481.2, 50; 483.2, 100; 485.2, 50; ¹H-NMR (400 MHz, CDCl₃, 300 K): (ppm) 7.84 (t, 1H, J=1.8 Hz), 7.59 (d, 2H, J=1.8 Hz), 6.71 (d, 2H, J=4.1 Hz), 6.39 (d, 2H, J=4.2 Hz), 3.08 (q, 4H, J=7.6 Hz), 1.35 (t, 6H, J=7.6 Hz) TLC (SiO₂ on Al foil, DCM : heptane (v/v), rf): 4:0, 0.97 ; 3:1, 0.77; 2:2, 0.48, 1:3, 0.21

3.1.3 3 (poly-(9,9-dioctylfluorene-2,7-diyl)-co-(5-[3,5-diethyl-4,4-difluoro-4-bora-3a,4a-diaza-s-indacene-8-yl]-m-phenylene) (7:1))

To a 100 ml round bottom flask was added 2 ml toluene, 15 ml 2M aqueous K₂CO₃, 100 mg (0.2075 mmol) **1**, 341.38 mg (0.6225 mmol) 2,7-dibromo-9,9-dioctyl-fluorene and 463.44 mg (0.8300 mmol) 9,9-dioctylfluorene-2,7-diboronic acid bis(1,3-propanediol) ester. The mixture was deaerated and 25 mg [1,1-Bis(diphenylphosphino)ferrocene]dichloropalladium(II) (Pd(DPPF)Cl₂) was added. The mixture was stirred for 48 hours at 90°C under nitrogen atmosphere. The organic phase was then precipitated into 2 x 40 ml methanol under vigorous stirring and centrifuged at 3000g for 20 minutes. The supernatant was decanted and the precipitate was dried in a vacuum oven. The crude product was purified by successive Soxhlet extractions with ethanol, acetone and finally chloroform

to yield 44.8 mg (7.1%) of dark red flakes. GPC: Mn: 2.0 kDa, Mw: 5.5 kDa, D: 2.7907; ¹H-NMR (400 MHz, CDCl₃, 300 K): (ppm) 7.86, (s, 2H), 7.84 (s, 23H), 7.75-7.61 (m, 62H), 3.09 (q (dq?), 4H, J=6.1 Hz), 2.12 (s br, 53H), 1.26-1.14 (m, 341H), 1.35 (t (dt?), 6H, J=6.1 Hz), 0.82 (m, 172H) TLC (SiO₂ on Al foil, DCM : heptane (v/v), rf): 4:0, 0.99 ; 3:1, 0.98; 2:2, 0.64 (0.77), 1:3, 0.29 (0.73)

3.1.4 4 (poly-(9,9-dioctylfluorene-2,7-diyl)-alt-(5-[3,5-diethyl-4,4-difluoro-4-bora-3a,4a-diaza-s-indacene-8-yl]-m-phenylene) (1:1))

To a 100 ml round bottom flask was added 2 ml toluene, 15 ml 2M aqueous K₂CO₃, 200 mg (0.4150 mmol) **1** and 231.72 mg (0.4150 mmol) 9,9-dioctylfluorene-2,7-diboronic acid bis(1,3-propanediol) ester. The mixture was deaerated and 12.5 mg Pd(DPPF)Cl₂ was added. The mixture was stirred for 48 hours at 90°C under nitrogen atmosphere. The mixture was then dried under vacuum and the remaining solids redissolved in toluene. This solution was then precipitated into 2 x 40 ml methanol under vigorous stirring and then centrifuged at 3000g for 20 minutes. The supernatant was decanted and the precipitate was dried in a vacuum oven. 69.5 mg (23.5%) of red powder was recovered. GPC: Mn: 3.0, Mw: 6.1 kDa, D: 1.9964; ¹H-NMR (400 MHz, CDCl₃, 300 K): (ppm) Insufficient solubility in CDCl₃ or degradation before sample was taken. TLC (SiO₂ on Al foil, DCM : heptane (v/v), rf): 4:0, 0.96 ; 3:1, 0.56; 2:2, 0.00, 1:3, 0.00

3.2 Spectroscopy

Dilute solutions (at most 0.1 g/l) of each compound in toluene were prepared. All spectra were recorded using a 10x10 mm quartz cuvette. Fluorescence spectra were measured on an Agilent Technologies Cary Eclipse Fluorescence Spectrophotometer. Excitation spectra were recorded over 200-580 nm range with 2.5 nm excitation slit and with emission at 600 nm with 10 nm emission slit. Emission spectra were recorded over 380-650 nm range with 2.5 emission slits and excitation at 360 nm with 10 nm excitation slits. All data were normalised with respect to the maximum in the 310-650 nm range.

3.3 Fluorescence Lifetime Response in Castor Oil

Stock solutions of **1**, **3**, **4** and poly-[9,9-dioctylfluorene] (pF8) were prepared using toluene as solvent. The maximum concentration was 5 mg/l but all stock solutions were prepared from saturated solutions of undetermined exact concentration. Stock solutions were stored in containers wrapped in aluminium foil.

Fluorescence decays were measured on an Edinburgh Instruments FS5 using a dilute Ludox solution to determine Instrument Response Function and a 3.5 ml 10x10 mm cuvette with Teflon cap to hold samples. Excitation lasers were used at 404.8 nm (EPL-405) for **3**, **4** and pF8 and 472.6 nm (EPL-475) for **1** with 3 nm monochromator for all but **4** where the monochromator was set to 10 nm on the emission side and a multi-count photon detector was used. Decays of 20 and 50 ns and 4096 channels were recorded with 415 nm and 530 nm emission respectively, all with a peak intensity of 10^4 counts. **3** and pF8 were measured at 415 nm and **1**, **3** and **4** at 530 nm, with the emission spectra of **3** being recorded over a 410-650 nm range with emission correction additionally. Samples were tested in order of increasing polystyrene concentration and returned as much as possible to their original vial after recording the decay. The cuvette was not cleaned between samples.

Decays were analyzed using DecayFit 1.4 running in Matlab R2017b. Up to three exponentials were fitted to each decay. Resulting lifetimes were correlated with other lifetimes determined within the same sample series to obtain a set of three lifetime components for each compound.

3.4 Encapsulation of **3** in pODA Nanoparticles

A stock solution was prepared by dissolving 80 mg poly[octadecyl acrylate] (pODA, T_m 52°C determined by DSC) into a 0.1 g/l stock solution of **3** in toluene. Of this 200 l was added to a wide-necked 50 ml round bottom flask. To this 5 ml of toluene-saturated aqueous sodium dodecyl sulfate (SDS) solution (5mM) was added. The mixture was emulsified by ultrasonication with an ultrasonic needle 5 times for 30 seconds each time. Then 20 ml SDS solution (5 mM) was added. The flask was wrapped in aluminium foil and the solution bubbled with nitrogen at 70°C overnight. Resulting particles (**3**@pODA) were analyzed by static light scattering to be 115 nm radius.

To 2 ml of the resulting solution 15 ml SDS solution (5mM) was added and mixed carefully without foaming. Of this 4 ml was added to a quartz cuvette with stir bar which was closed with a teflon stopper.

Fluorescence emission spectra were recorded with excitation at 380 nm and detection over 400 nm to 600 nm. The sample was cycled 4 times over a temperature range of 10-50-10°C while being stirred, with 5°C steps and 20 minutes to fully equilibrate after each change in temperature before a spectrum was recorded.

4 Results & Discussion

4.1 Synthesis

The synthesis of semiconducting polymers incorporating BoDiPy molecular rotors was successful but in low yield. Nevertheless this low yield is sufficient for characterization and further experiments. The ratio of BoDiPy to fluorene in **3** was intended to be 1:7 however analysis of the ^1H NMR spectrum suggests this ratio is somewhere closer to 1:11 to 1:17. Additionally, some peaks of both fluorene and BoDiPy spectra could not be found at their expected chemical shift. One peak corresponding to a pair of aromatic protons on fluorene expected at 7.39-7.30 ppm appeared to overlap with the peak at 7.75-7.61 ppm (based on the integral. Two peaks corresponding to protons on the 1,7- and 2,6-positions on the BoDiPy core were expected at 6.71 and 6.39 ppm but in this region no peak could be found at all. In a prior NMR spectrum of a sample taken immediately after precipitation but prior to soxhlet extraction, these latter peaks did show up however at 6.96 and 6.43 ppm as singlets with integral of 1H each relative to the ethyl CH_2 . Despite this, the remaining protons of BoDiPy showed peaks at the expected chemical shifts. Especially the ethyl groups attached to the core showed chemical shifts differing by only 0.01 ppm for CH_2 and 0.05 ppm for CH_3 . Close examination showed these peaks were doubled, suggesting BoDiPy occurs in two slightly different chemical environments. Similarly, for fluorene the remaining aromatic protons and alkyl chain protons were found at their expected chemical shift. The loss of signals for BoDiPy 1,7- and 2,6-protons and the shift of signals one pair of fluorene protons to overlap with another pair could suggest the proximity of these protons and some of the aromatic systems in such a way that unexpected shifts are induced. Nevertheless, the incorporation of BoDiPy into polyfluorene appears successful.

TLC analysis showed the presence of a high retention factor fluorescent fraction in **3**. This is likely simple polyfluorene in which no BoDiPy has been incorporated. This conforms somewhat to expectation as the reaction conditions certainly allow for simple polyfluorenes to be formed and this indicates that the methods of separation were not completely successful. The relatively lower retention factor of **4** further confirms this, showing the retention factor is dependent on BoDiPy content.

GPC results show that both **3** and **4** are rather short and it is hard to truly call them polymers. Instead it is perhaps more appropriate to refer to these compounds as synthesised in this work as oligomers.

4.2 Spectroscopy

UV-VIS absorption, excitation and emission spectra of all synthesised compounds are shown in Figure 4 below.

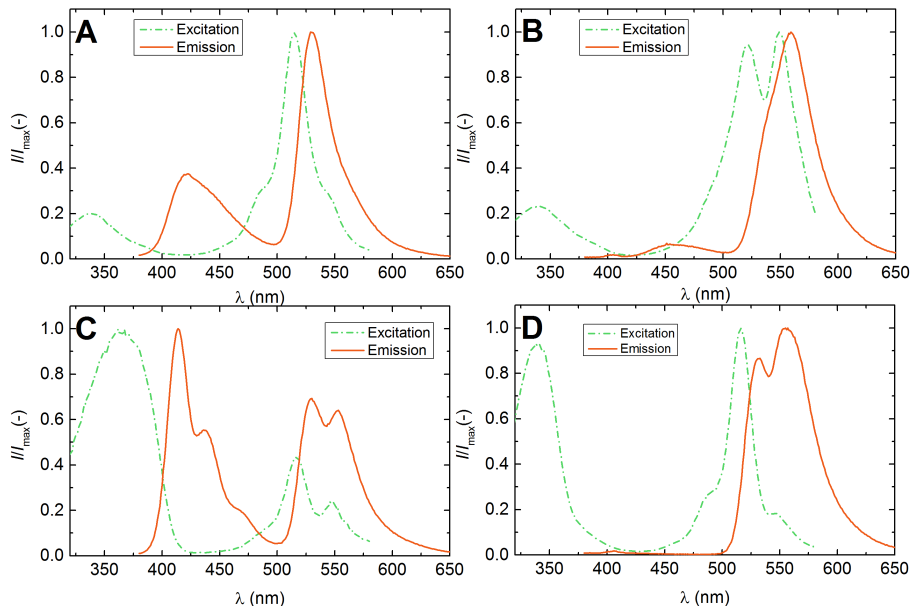


Figure 4: UV-VIS absorption, excitation and emission spectra of **1** (A), **2** (B), **3** (C) and **4** (D) in toluene solution

In the excitation spectra shown in Figure 4 all four compounds show effective excitation in the 340-380 nm range while excitation at 360 nm produces excellent and well separated emission of both fluorophores. In emission **1** (Figure 4A) shows a peak at 530 nm and **2** (Figure 4B) at 560 nm. Both these peaks are reproduced in **3** (Figure 4C) and **4** (Figure 4D) at 530 nm and 555 nm respectively. These peaks in **3** and **4** can therefore be ascribed to the BoDiPy fluorophore incorporated into the oligomer. The presence of two distinct peaks is explained by there being two distinct chemical environments a BoDiPy can occupy in these oligomers, being in the middle of the chain flanked by fluorene on either side and at the end of the chain flanked by fluorene on just one side. Further, **3** shows a peak at 415 nm with further vibronics at 435 and 470 nm. This peak is characteristic of fluorene. Although **4** also contains fluorene in its structure, the same peak is almost absent which may be explained by almost complete transference of excited state energy from fluorene to BoDiPy (FRET), or because the meta-phenylene linkers disrupt the conjugation of polyfluorene, thus diminishing their fluorescence. It is clear however that the fluorescence of polyfluorene is retained if the grade of BoDiPy-doping is low enough. Further, incorporation into polyfluorene has greatly increased the excitation of BoDiPY

by UV, with broader and stronger excitation being seen with lower grades of doping. This further confirms that FRET plays a part in the fluorescence characteristics.

4.3 Fluorescence Lifetime Response in Castor Oil

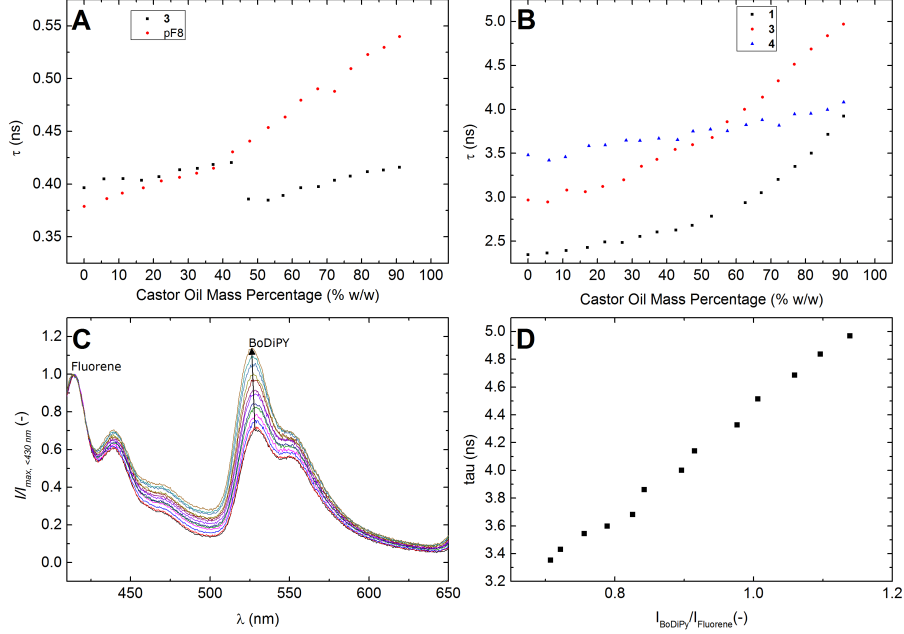


Figure 5: A: Fluorescence lifetime of **3** and poly-bis(octyl)fluorene (pF8) in toluene solution with varying mass percentage of castor oil. B: Fluorescence lifetime of **1**, **3** and **4** in toluene solution with varying mass percentage of castor oil. C: Emission spectra, normalised relative to the maximum emission at the peak labeled A, of **3** in toluene solution with varying mass percentage of castor oil. D: Lifetime of **3** vs. the maximum emission intensity at peak B relative to peak A in figure C.

As shown in Figure 5A, fluorescence lifetimes of polyfluorene in both pF8 and **3** show minimal response to changing viscosity, with less than 0.2 ns increase recorded over the range of viscosities measured here. Curiously **3** shows a sudden drop in lifetime followed by another steady increase. This drop coincides with a break in measurement, the samples before it having been measured one day and the samples after it having been measured the next day, having been kept in a closed box in uncontrolled conditions overnight. This suggests the lifetime of polyfluorenes is perhaps dependent more on extrinsic factors like temperature than on the viscosity of its medium. Nevertheless, incorporating BoDiPy molecular rotors into polyfluorene does not appear to have had a sub-

stantial impact on the fluorescence lifetime response of polyfluorene.

As shown in Figure 5B, fluorescence lifetimes of BoDiPy in **1**, **3** and **4** do show a substantial response to changing viscosity, with over 2 ns increase in lifetime in **3** and a respectable 1.5 ns in **1**, although the response in **4** is minimal reaching only 0.6 ns increase over a similar range of viscosity and also follows a linear response compared to the parabolic response of the others. The latter can possibly be attributed to interference from the secondary peak at 555 nm because of the larger monochromator bandwidth (required because of limited excitation) or could be because the function of the molecular rotor is reduced. Nevertheless, **1** and **3** show typical response to viscosity as described by the Förster-Hoffman equation[7].

In Figure 5C it is shown how the emission spectrum of **3** changes with increasing viscosity. Relative to the maximum intensity of fluorene emission, maximum BoDiPy emission intensity increases substantially and the greatest response is seen at the 530 nm peak. A small blue shift is also seen. In Figure 5D the fluorescence lifetime of BoDiPy in **3** is plotted against the ratio of BoDiPy to fluorene emission intensities. This shows a linear relationship of the fluorescence lifetime and the relative emission intensity of BoDiPy. This linear relationship allows the relative emission to be used as a proxy for fluorescence lifetime which means **3** is a potential single molecule viscosity sensor.

4.4 Encapsulation of **3** in pODA Nanoparticles

Shown below in Figure 6 are the complete spectra recorded in one temperature cycle for **3**@pODA nanoparticles and the evolution of the relative intensity of some interesting peaks in the spectrum over all 4 cycles.

As shown in Figure 6A somewhat counterintuitively, the emission of both fluorene and BoDiPy increases with temperature, showing an almost doubling in the intensity of both from 40°C to 50°C. This may be because the opacity of the micelles reduces as they melt or because fluorescence quenching is reduced. As figures 6B-D show, the first cycle shows a different behaviour compared to subsequent cycles. This is likely because the particles were formed in an amorphous state and need to melt and slowly crystallise over at least one cycle. Furthermore, the relative intensities of different subpeaks change over the temperature cycle. For fluorene the main peak at 415 nm appears above the secondary peak at 415 nm as temperature increases, and the same is the case for the main BoDiPy peak at 530 nm relative to the secondary peak at 555 nm. The relative intensities given in figures 6B-D show how this phenomenon is limited at low temperatures up to 35 degrees but suddenly transitions at 40 degrees in Figures 6B and 6C and at 45 degrees in 6D, representing a melting transition. A hysteresis can also be found on the return of the cycle with relatively constant values at high temperatures followed by a sudden transition at 40 degrees in Figure 6B and at 45°C in Figures 6C 6D, which should represent nucleation events. Although coarse-grained, this shows the complexity of **3** in

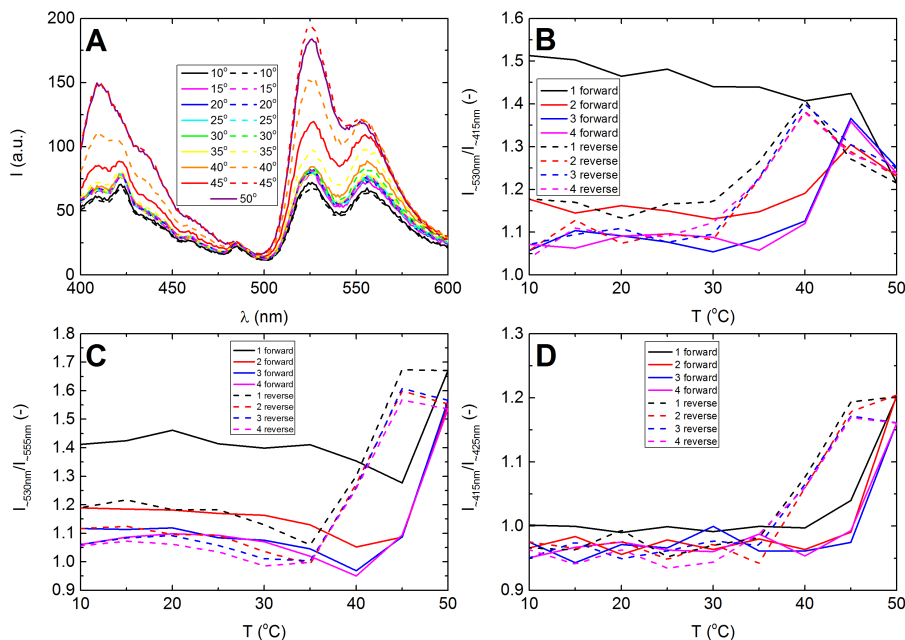


Figure 6: A: Evolution of the emission spectra of **3**@pODA nanoparticles over the course of one temperature cycle, showing spectra recorded on the heating part of the cycle in solid lines and those on the cooling part of the cycle in dashed lines. B: Evolution of the relative emission of BoDiPy compared to fluorene in **3**@pODA over the course of 4 temperature cycles. C: Evolution of the relative emission of the main peak of BoDiPy compared to the secondary peak in **3**@pODA over the course of 4 temperature cycles. D: Evolution of the relative emission of the main peak of fluorene compared to the secondary peak in **3**@pODA over the course of 4 temperature cycles.

the solid state. It is unclear whether **3** still acts as a molecular rotor in such a highly viscous unplasticised polymer matrix, but it is clear that it cannot be interpreted as a simple molecular rotor with attached internal standard. The phase transitions which are detected appear to be fall in line with predicted phase transitions but are clearly caused by phenomena distinct from molecular rotation. Likely a simpler and more versatile molecule could be developed for the same purpose and indeed simple pF8 could perhaps suffice, due to the different behaviour of the various vibronic peaks.

5 Conclusion

BoDiPy-based molecular rotors were successfully incorporated into polyfluorene polymers. These co-polymers however had a low degree of polymerization and could better be described as co-oligomers. The fluorescent properties of both BoDiPy and polyfluorene are retained in such co-oligomers, provided sufficient fluorene is included, otherwise only the emission of BoDiPy was recorded. The absence of emission from fluorene could be because of complete FRET between the fluorophores or because the conjugation of fluorene is disrupted by BoDiPy and that this diminishes their fluorescence. In these co-oligomers BoDiPy shows two distinct emission peaks, one at 530 nm and one at 555 nm, which correspond well with the emission peak of 530 nm for a BoDiPy rotor without substitution on the phenyl group and with 560 nm for a BoDiPy rotor with a 3,5-dibrominated phenyl group. The two peaks are therefore hypothesised to correspond to different positions BoDiPy can occupy with the oligomer chain, with the emission at 530 nm caused by BoDiPy in the middle of the chain flanked on both sides by fluorene and the emission at 555 nm caused by BoDiPy at the chain terminus with one bromine remaining. The effectiveness of BoDiPy as a molecular rotor is retained in such co-oligomers. Fluorescence lifetimes for BoDiPy in co-oligomers were 0.5 to 1.2 ns higher than for free BoDiPy for the corresponding bulk viscosity and the effect occurs over a similar range however the fluorescence lifetime range over which the effect occurs is dependent on the fluorene content of the co-oligomers. Fluorene in these oligomers however shows no signs this effect transferring. The relative intensity of BoDiPy compared to fluorene shows a linear relationship with the lifetime of BoDiPy. This shows these oligomers function as molecular rotors with attached internal standards and can potentially be used as single molecule viscosity sensors. The limits of their utility are however found in solid media where effects other than molecular rotation appear to have greater effect on the spectral properties of these oligomers. Although these effects can be harnessed as sensors for the solid to liquid transition, they are independent of the function as molecular rotor.

6 Outlook

In future work, efforts should be made to synthesise similar sensor molecules with more controlled structures. Ideally, a single BoDiPy should be connected at both 3' and 5' to oligofluorene chains of well-defined length. Oligofluorene chains of different lengths can be used in this synthesis to tune the ratio of fluorescence of BoDiPy compared to fluorene. A method of synthesis based on [8] is shown in Figure 6 below. Methods of separating these from partially reacted material are also required. Given the difference in polarity found by TLC, simple chromatographic methods might work well. The hypothesis given for the presence of two distinct peaks in the BoDiPy emission spectrum of **3** and **4** could also be tested with such molecules, as this hypothesis predicts a single peak at 530 nm for them. Through similar methods BoDiPy-terminated oligofluorene chains could also be synthesised and their emission spectrum should show a single peak at 555 nm. Alternatively, the synthesis of longer polymers (10-100 kDa) should be explored. One synthesis based on the Yamamoto reaction was attempted during this project, however no soluble product of notable length could be obtained. Such long polymers should also show a single peak at 530 nm. Given the molecules described here did not show the expected behaviour in pODA nanoparticles in their phase transition as a result of the opacity of solid pODA, the same behaviour can be reproduced in a transparent system such as a polymer glass. Although the glass transition is a much less sharply defined phase transition than melting and crystallizing, the continuous nature of the transition from liquid to glass and the impact this has on viscosity should make this a candidate for study with radiometric molecular rotors.

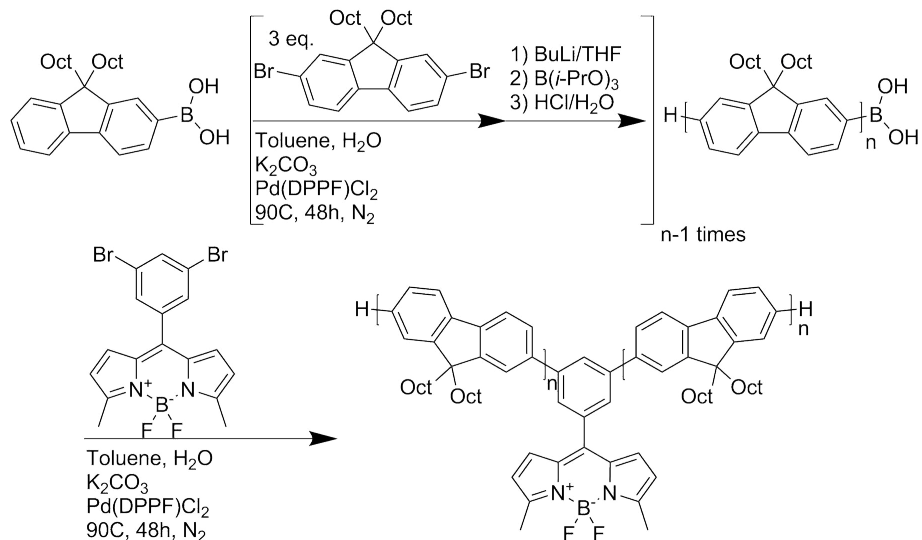


Figure 7: Proposed synthesis of BoDiPy with oligofluorene chains of well-defined length, *n*=4-10.

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References

- [1] Andrea Barba-Bon, Laura Calabuig, Ana M. Costero, Salvador Gil, Ramón Martínez-Máñez, and Félix Sancenón. Off-on bodipy-based chemosensors for selective detection of Al^{3+} and Cr^{3+} versus Fe^{3+} in aqueous media. *RSC Adv.*, 4:8962–8965, 2014.
- [2] Yuting Chen, Liang Wan, Daopeng Zhang, Yongzhong Bian, and Jianzhuang Jiang. Modulation of the spectroscopic property of bodipy derivatives through tuning the molecular configuration. *Photochem. Photobiol. Sci.*, 10:1030–1038, 2011.
- [3] Ulrich Gilles, Ziesel Raymond, and Harriman Anthony. The chemistry of fluorescent bodipy dyes: Versatility unsurpassed. 2008.
- [4] Mark A Haidekker, Matthew Nipper, Adnan Mustafic, Darcy Lichlyter, Marianna Dakanali, and Emmanuel A Theodorakis. Dyes with segmental mobility: molecular rotors. In *Advanced Fluorescence Reporters in Chemistry and Biology I*, pages 267–308. Springer, 2010.
- [5] Mark A. Haidekker and Emmanuel A. Theodorakis. Molecular rotors—fluorescent biosensors for viscosity and flow. *Org. Biomol. Chem.*, 5:1669–1678, 2007.
- [6] Lijuan Jiao, Weidong Pang, Jinyuan Zhou, Yun Wei, Xiaolong Mu, Guifeng Bai, and Erhong Hao. Regioselective stepwise bromination of boron dipyrromethene (bodipy) dyes. *The Journal of Organic Chemistry*, 76(24):9988–9996, 2011. PMID: 22077955.
- [7] Kuimova Marina K. *Molecular Rotors: Imaging Intracellular Viscosity*, chapter 11, pages 243–262. Wiley-Blackwell, 2012.
- [8] Alexander L. Kanibolotsky, Rory Berridge, Peter J. Skabara, Igor F. Perepichka, Donal D. C. Bradley, and Mattijs Koeberg. Synthesis and properties of monodisperse oligofluorene-functionalized truxenes: Highly fluorescent star-shaped architectures. *Journal of the American Chemical Society*, 126(42):13695–13702, 2004. PMID: 15493927.
- [9] Hooi Ling Kee, Christine Kirmaier, Lianhe Yu, Patchanita Thamyongkit, W. Justin Youngblood, Matthew E. Calder, Lavoisier Ramos, Bruce C. Noll, David F. Bocian, W. Robert Scheidt, Robert R. Birge, Jonathan S. Lindsey, and Dewey Holten. Structural control of the photodynamics of borondipyrroin complexes. *The Journal of Physical Chemistry B*, 109(43):20433–20443, 2005. PMID: 16853644.
- [10] Carrie W Rogers and Michael O Wolf. Luminescent molecular sensors based on analyte coordination to transition-metal complexes. *Coordination Chemistry Reviews*, 233-234:341 – 350, 2002.

- [11] Bernard Valeur and Isabelle Leray. Design principles of fluorescent molecular sensors for cation recognition. *Coordination Chemistry Reviews*, 205(1):3 – 40, 2000.