Supporting Materials

Highly Emissive Benzoxazole Nanoparticle of Excited State Intermolecular Proton Transfer Process and its Application in the Selective Bio-Labeling

Hyong-Jun Kim,[1] Jiseok Lee,[3] Tae-Hoon Kim,[1,5] Taek Seung Lee,[5] and Jinsang Kim[1,2,3,4,]*

[1]Department of Materials Science and Engineering, [2]Biomedical Engineering, [3]Chemical Engineering, [4]Macomolecular Science and Engineering, University of Michigan, Ann Arbor, MI 48109

[5]Department of Advanced Organic Materials and Textile System Engineering, Chungnam National University, Daejon, 305-764 Korea

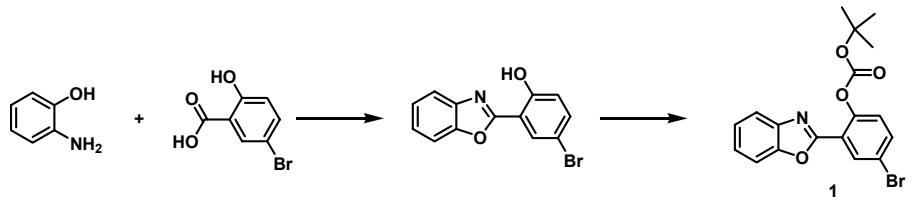
Supplemental Data

Synthesis of DBO

2-(benzoxazol-2-yl)-4-bromophenol. 2-Aminophenol (2.51 g, 23.03 mmol) and 5-bromosalicylic acid (5.5 g (23.03 mmol) were added in 30 ml of polyphosphoric acid and the mixture was heated to 130 ℃ and stirred for 4h. After cooling to room temperature, the reaction mixture was precipitated in ice-water, filtered and washed with DI water. The product was recrystallized from acetic acid and dried in vacuo (yield 5.94 g, 88.9%). ^{1}H NMR(300MHz, $CDCl_3$) : δ 11.44 (s, 1H), 8.16-7.04 (m, 7H).

***tert*-butyl-2-(benzoxazol-2-yl)-4-bromophenyl carbonate (1).** 2-(benzoxazol-2-yl)-4-bromophenol (0.8 g, 2.76 mmol) was dissolved in dry, distilled tetrahydrofuran (THF) with di-tert-butyldicarbonate and (4-dimethylamine) pyridine (5 mol-%). When the reaction appeared complete by thin-layer chromatography (TLC), the solution was concentrated and precipitated in DI water,

washed with ethanol. The product was dried in vacuo (yield 0.77g, 74.5 %). ^{1}H NMR(300MHz, $CDCl_3$) : δ 8.44 (s, 1H), 7.77 (d, 1H), 7.65 (d, 1H), 7.58 (d, 1H), 7.38 (m, 2H), 7.2 (d, 1H), 1.58 (s, 9H).

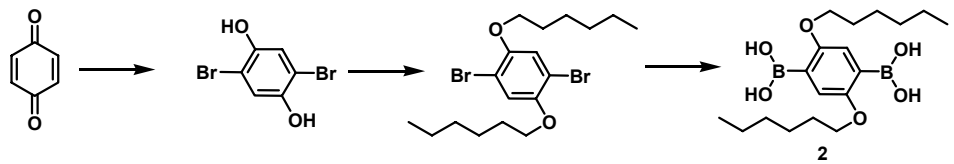


Scheme S.1. Synthesis of *tert*-butyl-2-(benzoxazol-2-yl)-4-bromophenyl carbonate (1).

2,5-dibromo-1,4-dihydroxybenzene. Hydroquinone (50 g, 0.454 mol) was added in acetic acid (240 ml) and stirred vigorously. Bromine (46.6 ml, 0.91 mol) in acetic acid (200 ml) was dropwised to above suspension at 10 ~ 15 ℃. The mixture solution was stirred at room temperature for 12 h and filtered off the pale crystal. The filtrate was reduced to the minimum under vacuum and the white crude product was recrystallized in methanol, filtered and dried in vacuo (yield 29.86 g, 24.55%). ^{1}H NMR(300MHz, $CDCl_3$) : δ 6.98 (s 2H).

2,5-dibromo-1,4-dihexyloxybenzene. A suspension of KOH powder (10.47 g, 186.6 mmol) in dried DMSO (360 ml) was degassed under vigorous stirring for 1h. 2,5-dibromo-1,4-dihydroxybenzene (5 g, 18.66mol) and 1-bromohexane (5.8 ml, 41.06 mmol) were added and stirred for 12 h. The solution was concentrated and precipitated in water, filtered, washed with methanol. The product was recrystallized in ethanol, dried in vacuo (4.518g, 55.5 %). ^{1}H NMR(300MHz, $CDCl_3$) : δ 7.08 (s, 2H), 3.95 (t, 4H), 1.8 (m, 4H), 1.49 (m, 4H). 1.37 (m, 8H), 0.9 (t, 6H).

2,5-(1,4-dihexyloxyphenyl)diboronic acid (2). 2,5-dibromo-1,4-dihexyloxybenzene (2 g, 4.58 mmol) was dissolved in ethyl ether (20 ml) and Butyl-Litium (2.5 M in hexane, 5.05 ml) was added and stirred at room temperature for 12 h under argon gas. Triisopropylborate (2.3 ml, 10.05 mmol) was added at -40℃ and stirred over night from -40℃ to room temperature. The reaction was quenched by the addition of HCl 2M solution (50 ml) and the resulting precipitation was collected, washed with water, ethyl ether, and dried under vacuo (yield 0.53 g, 31.61 %). ^{1}H NMR(300MHz, $CDCl_3$) : δ 7.16 (s, 2H), 3.95 (t, 4H), 1.68 (m, 4H), 1.8 (m, 4H), 1.25 (m, 8H), 0.85 (t, 6H).

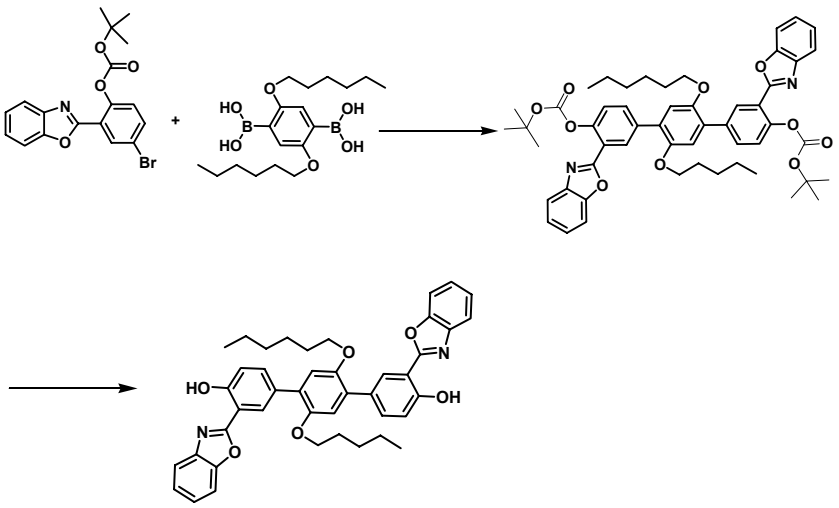


Scheme S.2 Synthesis of 2,5-(1,4-dihexyloxyphenyl)diboronic acid (2).

t-Boc benzoxazole oligomer. The oligomerization was carried out between **1** and **2**. **1** (0.3 g, 0.8 mmol), **2** (0.148 g, 0.4 mmol), palladium catalyst (5 mol %) were placed in a two-necked round-bottom flask charged with 7 ml of THF under argon. 1 M Na_2CO_3 solution was added and stirred for

48 h at 80 ℃. After cooling, the reaction mixture was poured into methanol. The precipitates were isolated by filtration and washed with DIwater and methanol and dried under vacuo. ^{1}H NMR(300MHz, $CDCl_3$) : δ 8.56 (s, 2H), 7.78 (m, 4H), 7.58 (m, 2H), 7.36 (m, 6H), 7.06 (s, 2H), 3.98 (t, 4H), 1.7 – 01.2 (m, 16H), 0.78(t, 6H).

Benzoxazole oligomer. The t-Boc benzoxazole oligomer was dissolved in chloroform (3 ml) ands trifluoreacetic acid (2 ml) was added. After stirring for 12 h, solvents was removed by evaporation and dried under vacuum oven (yield 0.217 g). ^{1}H NMR(300MHz, $CDCl_3$) : δ 11.55 (s, 2H), 8.35 (s, 2H), 7.79-7.68 (m, 4H), 7.62 (m, 2H), 7.41 (t. 4H), 7.2 (d, 2H), 7.06 (s, 2H), 3.99 (t, 4H), 1.78 − 1.23 (m, 16H), 0.78 (t, 6H).



Scheme S.3. Synthesis of DBO

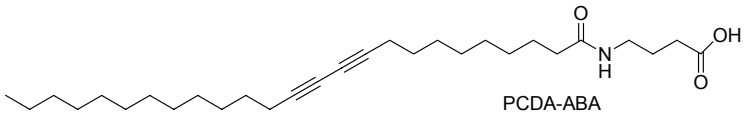
Synthesis of PDA molecules

The diacetylene monomers investigated in this study were prepared by coupling *N*-hydroxysuccininic esters of PCDA. A typical procedure for the preparation of PCDA-ABA and PCDA-biotin is as follows.

PCDA-NHS: To a solution containing 1.00 g (2.67 mmol) of 10,12-pentacosadiynoic acid in 10 mL of methylene chloride was added 0.38 g (3.47 mmol) of N-hydroxysuccinimde and 0.38 g (4.01 mmol) of N-(3-Dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride at room temperature. The resulting solution was stirred at room temperature for 2 h. The solent was removed in vacuo, and the reside purified by extraction with ethyl acetate to give1.08 g (86.2 %) of the desired diacetylene monomer PCDA-NHS as a white solid.: 1H NMR (300 MHz, CDCl3): *ä*)0.85 (t, 3H), 1.20-1.62 (m, 36H), 2.21 (t, 4H), 2.60 (t, 2H), 2.85 (s, 4H), 7.18 (brs, 1H).

PCDA-ABA: To a solution containing 0.56 g (0.50 mmol) of 4-aminobutylic acid in 5 mL of tetrahydrofuran was added 0.25 g (2.55 mmol) of tri-ethylamine, and 1 mL of di-water was added to

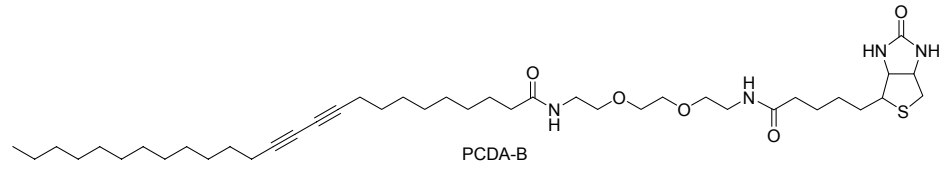
dissolve completely at room temperature. PCDA-NHS 0.20 g (0.42 mmol) in 5 mL of tetrahydrofuran was added dropwise to a mixture solution. The resulting solution was allowed to stir for overnight at room temperature. The solvent was removed in vacuo, and the residue was purified by extraction with methylene chloride. The organic layer was dehydrated with $MgSO_4$ and recrystallized in methylene chloride to give 0.15 g (77.6 %) of the desired diacetylene monomer PCDA-ABA as a pale blue solid.: 1H NMR (300 MHz, CDCl3): *ä*)0.85 (t, 3H), 1.20-1.62 (m, 36H), 1.86 (q, 2H), 2.21-2.38 (m, 8H), 3.35 (t, 2H), 7.28 (brs, 1H).



PCDA-ABA

PCDA-EDEA: To a solution containing 1.055 mL (7.21 mmol) of 2,2′-(Ethylenedioxy)bis(ethylamine) in 60 mL of methylene chloride was added dropwise 1.00 g (2.12 mmol) of PCDA-NHS for 3h at room temperature. The resulting mixture was allowed to stir for 2 h at room temperature. The resulting mixture was concentrated in vacuo, and the residue was purified by column chromatography (9:1 chloroform : methanol) to give 0.51 g (46.7 %) as a white solid.: 1H NMR (300 MHz, CDCl3): *ä*)0.89 (t, 3H), 1.27-1.76 (m, 35H), 2.23 (m, 6H), 2.91 (t, 2H), 3.42-3.63 (m, 12H), 6.21 (t, 1H), 7.27 (brs, 1H).

PCDA-biotin: To a solution containing 0.23 g (0.94 mmol) of biotin in 5 mL of DMF was added dropwise 0.40 g (0.79 mmol) of PCDA-EDEA in 5 mL methylene chloride. The mixture solution was allowed to stir for overnight at room temperature. The solvent was removed in vacuo, and the residue was purified by column chromatography (9:1 chloroform : methanol) to give 0.18 g (33.9%) of the desired diacetylene monomer PCDA-EDEA-biotin as a white solid.: 1H NMR (300 MHz, CDCl3): *ä*)0.89 (t, 3H), 1.27-1.76 (m, 41H), 2.23 (m, 8H), 2.74 (d, 1H), 2.94 (m, 1H), 3.17 (m, 1H), 3.42-3.65 (m, 13H), 4.32 (q, 1H), 4.54 (q, 1H), 4.92 (s, 1H), 5.87 (s, 1H), 6.27 (t, 1H), 6.31(t, 1H), 7.27 (brs, 1H).



PCDA-B

Characterization

UV/Vis absorption spectra were obtained at Cary50 (Varian) and the photoluminescence spectra and the absolute quantum efficiency were collected with QM4 (PTI, Inc) equipped with an integrating sphere and a Nitrogen dye laser. Fluorescence images were taken with Olympus BX51 fluorescence microscope. The size of the vesicles was measured with the scanning electron microscopy.

Excitation spectrum of DBO nanoparticles

10 μM DBO in THF solution shows its large Stokes' shift at 518nm by maximum excitation of 340 nm. Nanoaggregates in THF: H_2O 1:9 mixture show red shift of the excitation and absorption by 40 nm. While the aggregate dispersed in well defined PCDA vesicle structure shows two distinct absorption and excitation at 320 nm and 370 nm. The latter is from the red shift of the syn-enol species and the former is from the anti-enol species.

To check the photo stability, the DBO-PCDA was exposed with 254nm UV for 30min. 6W UV lamp was held 1 cm above the sample. There is 15% decrease in the photoluminescence, but still hold its emissive nature.

And the lifetime measurement was done to check the fluorescence decay lifetime of the DBO.

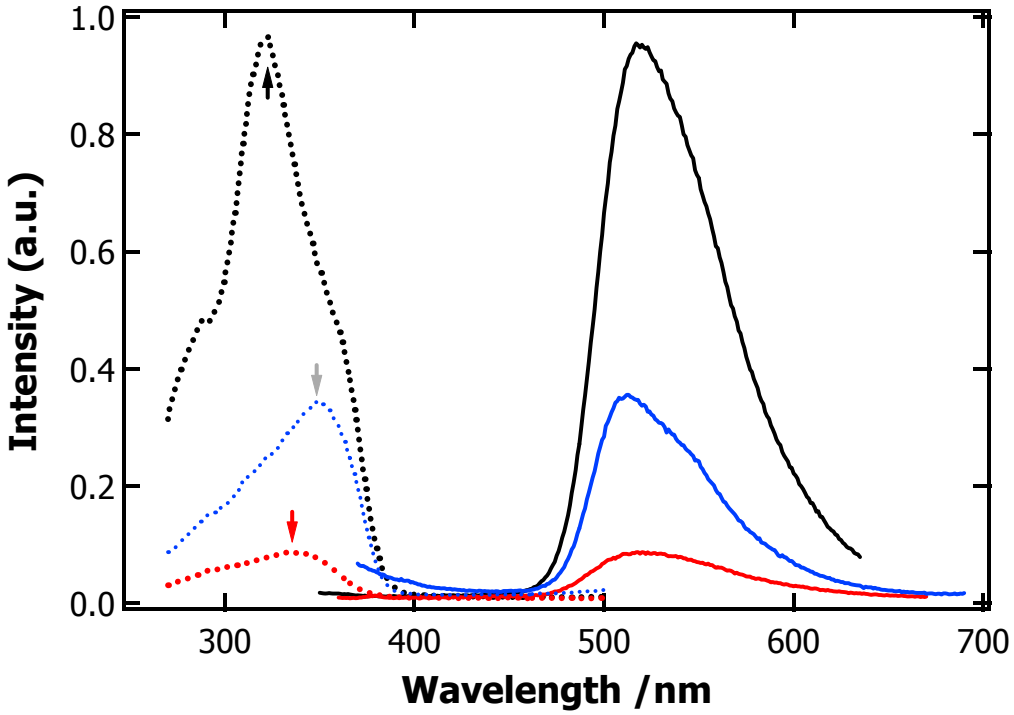


Figure S1. Excitation (dotted lines) and emission (solid lines) spectra of DBO in THF solution (red), dispersion in deionized water (blue), and dispersion in diacetylene liposome (black). The concentrations of DBO were 10 μM. There is red shift for the nanoparticles in THF/H_2O mixture. The anti-enol species (320 nm) formation is seen for the DBO-PCDA vesicle.

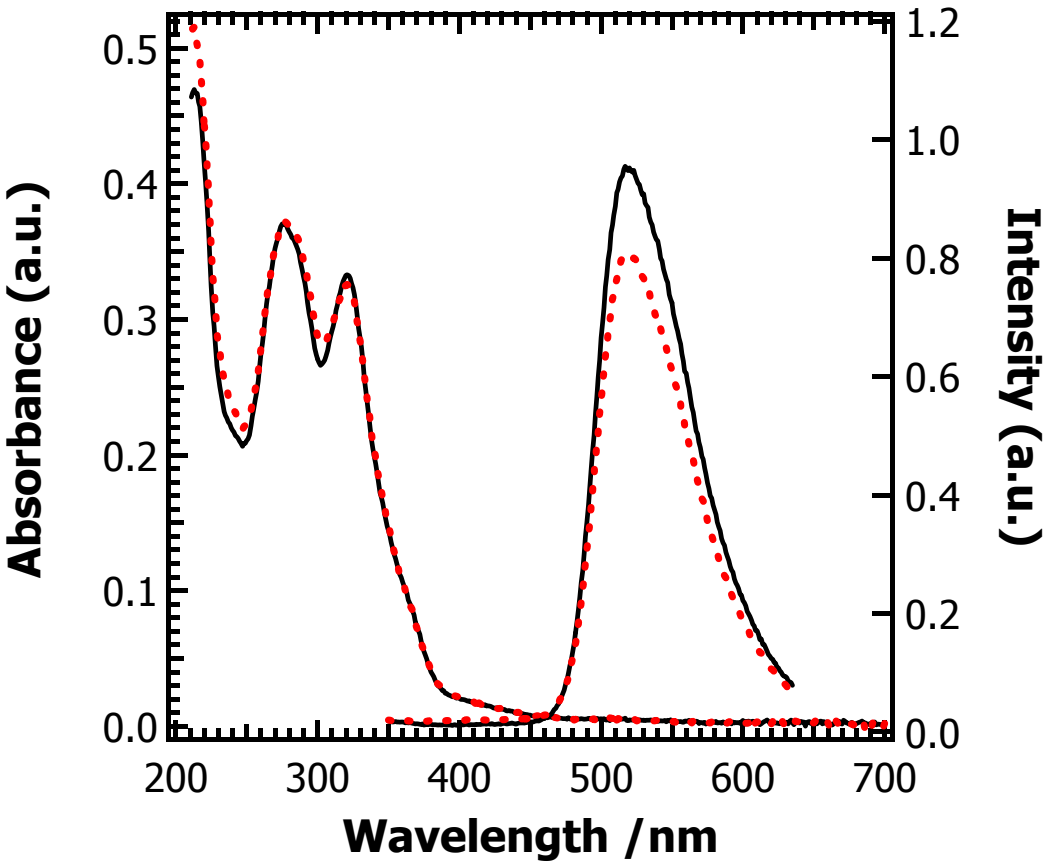


Figure S2. UV irradiation experiment was done with DBO nanoparticles in THF/H_2O mixture. 6W 254nm UV was illuminated 1cm above the suspension. The black line is before UV irradiation and the red lines were obtained after 30min of exposure.

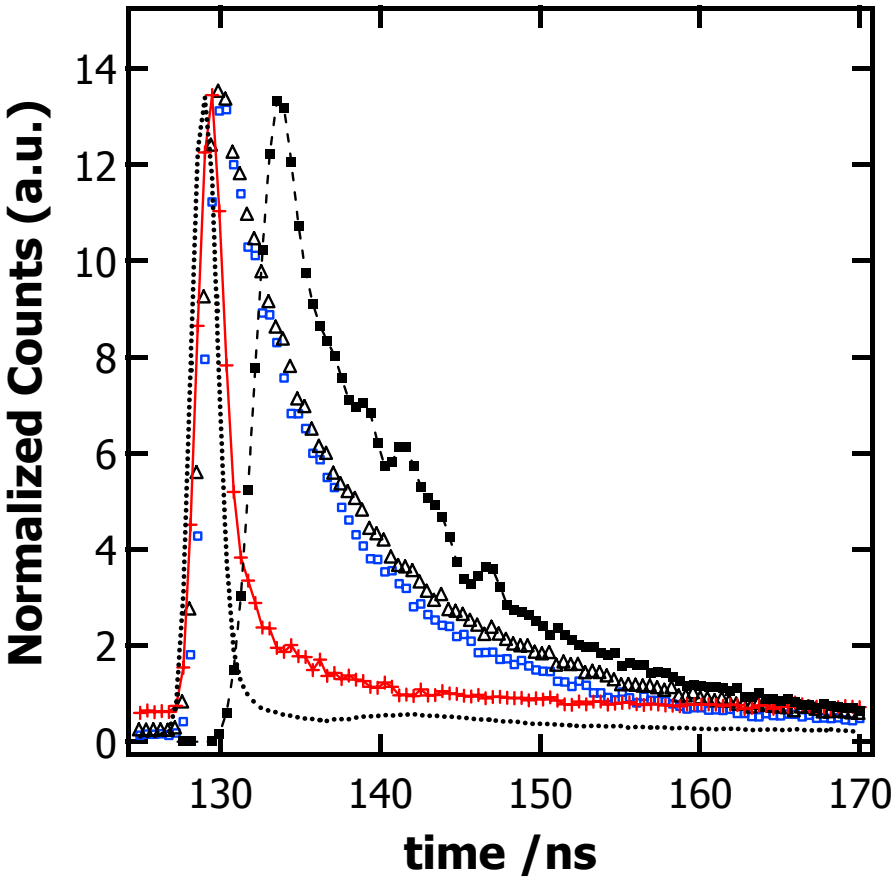


Figure S3. Fluorescence lifetime of DBO in THF solution (red), dispersion in THF:H_2O 1:9 v/v mixture (blue), and dispersion in diacetylene liposome (black) observed at 380nm excitation wavelength. Lifetime measurement was also done at 310nm excitation for the DBO-PCDA nanoparticles (closed black). Instrument response function is plotted in dotted line.