Electronic Supplementary Material (ESI) for Physical Chemistry Chemical Physics.
This journal is © the Owner Societies 2016

Electronic Supplementary Information

Structure–Property Relationships in Two-Dimensionally Extended Benzoporphyrin Molecules Probed Using Single-Molecule Fluorescence Spectroscopy

Sujin Ham, Sang Hyeon Lee, Heejae Chung, and Dongho Kim*

Spectroscopy Laboratory for Functional π-Electronic Systems and Department of Chemistry, Yonsei University, Seoul 03722, Korea

Corresponding Authors

dongho@yonsei.ac.kr (D. Kim)

Table of Contents

1. Experimental details

Single-Molecule Spectroscopy

Samples for single-molecule measurements were prepared by spin-coating a chloroform solution of **BP*N***s (~10^{-10} M) containing poly(methyl methacrylate) (PMMA) (20 mg/mL; Sigma-Aldrich, Mw = 97,000) on rigorously cleaned glass cover slips at 2,000 rpm for 60 s, yielding thin polymer films (~100 nm thickness, as determined by atomic force microscopy). In the films, the average molecular density was maintained as low as five single molecules in a 5 × 5 μm^2 area.

Detection of single-molecule fluorescence was performed using an inverted microscope equipped with a sample scanning stage. Picosecond pulsed excitation light at 470 (**BP2** and **BP3L**) or 485 nm (**BP3I**, **BP4**, and **BP5**) with an irradiation power of 0.4 μW and repetition rate of 10 MHz (LDH-F-C-470 or LDH-P-C-485, Picoquant) was rendered circularly polarized using a Berek compensator, directed into the microscope by passing it through a laser line filter (z470/10× or z485/10× , Chroma Technology) and collimating lens, and then focused on the sample via an oil immersion objective (Plan Fluor, 1.3 NA, 100×, Nikon). Fluorescent signals were collected using the same objective, passed through a dichroic mirror (470dcxru or z488rdc, Chroma Technology), spectrally filtered using a notch filter (HNPF-470.0-1.0 or HNPF-485.0-1.0, Kaiser Optical Systems) and a band pass filter (LP02-488RE and FF01-496/LP-25, Semrock), and then split using a non-polarizing 50:50 beam splitter. Half of the fluorescence was dispersed via a spectrograph (SpectraPro 2150i, Princeton Instruments) and projected onto an EMCCD camera (PL PROEM:512B EMCCD, Princeton Instruments) in order to record the fluorescence emission spectrum. The other half was split further using a polarizing beam splitter (05FC16PB.5, Newport) and detected by two avalanche photodiode (APD) modules (SPCM-AQR-16-FC, EG&G) in order to record the fluorescence intensity, lifetime, and linear dichroism. The fluorescent signals detected by the two APDs were registered by the PC card operated in first-in-first-out (FIFO) mode; for each detected photon, the system recorded the arrival time after the beginning of acquisition (with 50 ns resolution), the time lag with respect to the excitation pulse (with 6 ps resolution), and the detection channel (APD1 or APD2). The data were processed using the BIFL data analyzer (Scientific Software Technologies Center) in order to reproduce the fluorescence intensity and linear dichroism trajectories with a user-defined binning time. By using photons belonging to a user-defined region in the intensity trajectories, we were able to reproduce the fluorescence decays.

Defocused fluorescence imaging measurements of single **BP*N*** molecules were performed using a wide-

field fluorescence microscopy system consisting of an inverted optical microscope (IX71, Olympus) equipped with an oil immersion objective (1.4 NA, 100×, Plan Fluorite, Olympus) and a highly sensitive, cooled, 512 × 512 pixel EMCCD camera (Andor, iXon Ultra). For excitation, 488 nm light from a CW laser (Coherent, Cube) with an irradiation power at the sample of approximately 250 W/cm^2 was used. The circular polarized laser beam was sent to the microscope after passing through a laser line filter (LL01-445-25, Semrock), collimating lens, and dichroic mirror (Di02-R488-25x36, Chroma Technology) and then focused on the back-focal plane of the objective in order to achieve wide-field illumination (Köhler illumination mode). The defocused fluorescence image was obtained by shifting the sample plane by 0.9 μm toward the objective from the focus position. The defocused image was magnified 3.4 times using a relay lens and then spectrally filtered using a notch filter (HNPF-488.0-1.0, Kaiser optical systems) and band pass filters (FF-01-496/LP-25, Semrock). the image integration time was 1 s. The defocused fluorescence images were analyzed using a pattern matching routine written in MatLab software to roughly determine the transition dipole moment orientation through calculation of the two-dimensional correlation coefficients (r) of the defocused images obtained experimentally (A) and theoretically (B) using the equation[1]:

$$r = \frac{\sum_m \sum_n (A_{mn} - \overline{A})(B_{mn} - \overline{B})}{\sqrt{(\sum_m \sum_n (A_{mn} - \overline{A})^2)(\sum_m \sum_n (B_{mn} - \overline{B})^2)}} \quad (1)$$

where $\overline{A}$ and $\overline{B}$ are the means of A and B, respectively.

Photobleaching processes can be induced by several dynamical processes such as physical motions of the molecule such as translational and rotational diffusion,[2–5] spectral diffusion induced by conformational changes,[6–8] triplet dynamics,[9] and formation of radical ion and photooxidation.[10–14] The possibility of molecular rotation can be eliminated because the molecules are likely to be immobilized tightly in PMMA matrix, hindering the molecules from rotating in the polymer matrix. Also, the molecules are not allowed to change its conformation in the polymer matrix because the molecules are tightly enclosed by PMMA polymer matrix. Although it is possible to transit from higher intensity level to lower intensity level, that might cause only large intensity fluctuations in the fluorescence intensity trajectories of **BP*N***s.[15] The possibility of photobleaching being induced by triplet dynamics can be ruled out, because oxygen quenches triplet state when a sample is exposed to air.[16,17] Consequently, we think that the formation of "defects" such as radical cations and/or oxidized molecules behaves as fluorescence quenchers. The porphyrin molecules

are known to generate radical cations and can be oxidized due to oxygen penetrated through the PMMA matrix,[18,19] and consequently act as fluorescence quenchers.

Ensemble Solution Spectroscopy.

Steady-state absorption spectra were recorded using a UV/Vis spectrometer (Cary5000, Varian). Steady-state emission spectra were recorded using a fluorometer (F-2500, Hitachi) with excitation of the low-energy Soret band.

2. Supporting figures and table (Figure S1–S5, Table S1)

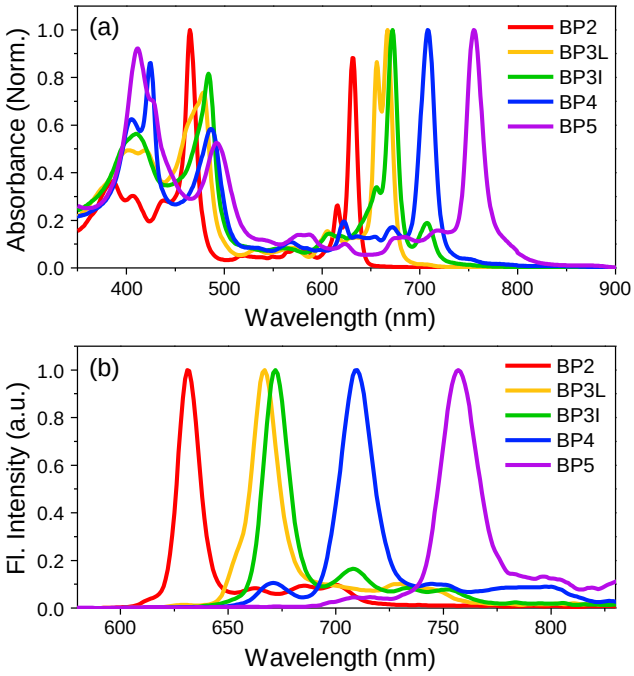

Figure S1. Steady-state (a) absorption and (b) fluorescence spectra of the **BP*N*s** in THF containing a small quantity of pyridine.[20,21]

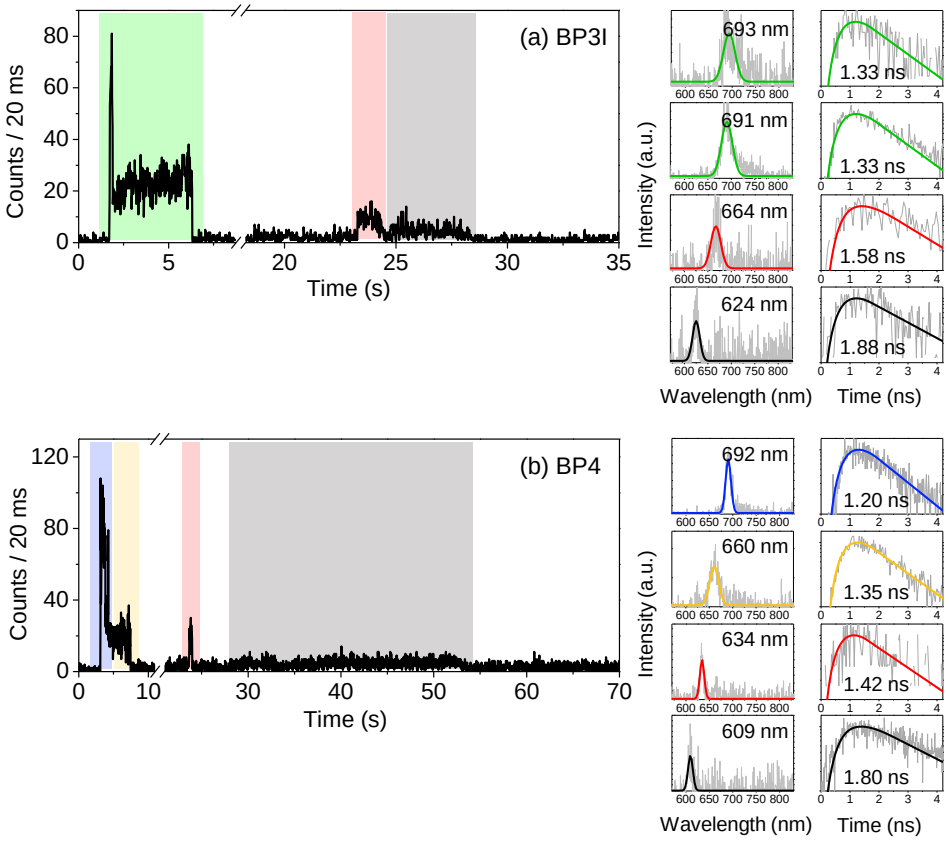

Figure S2. Representative multi-step photobleaching behaviors in the fluorescence intensity trajectories (FITs) and the corresponding fluorescence decay profiles and spectra for (a) **BP3I** and (b) **BP4**. Each spectrum is an average of a multiple single-molecule spectra obtained at the same intensity level for 1 s. The FITs are indicated by different colors that correspond to the fluorescence decay profiles and spectra.

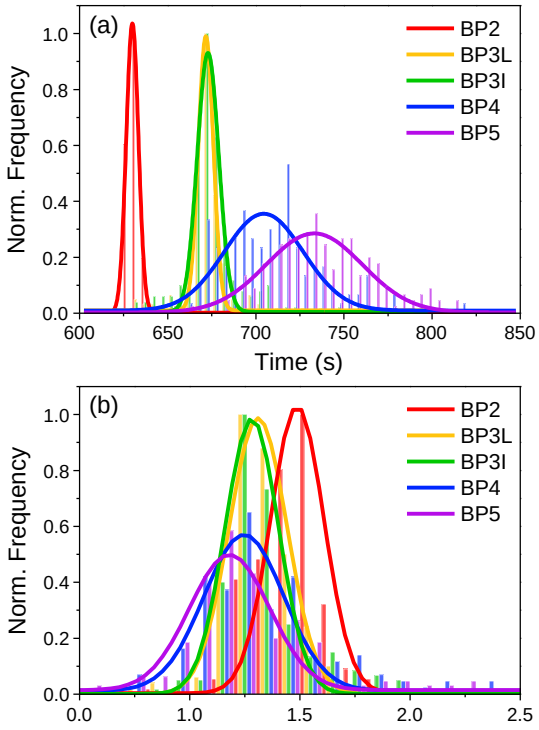

Figure S3. Distributions of the fluorescence (a) spectral peak positions and (b) lifetimes of the first emissive levels of the fluorescence intensity trajectories for the **BP*N*s**.

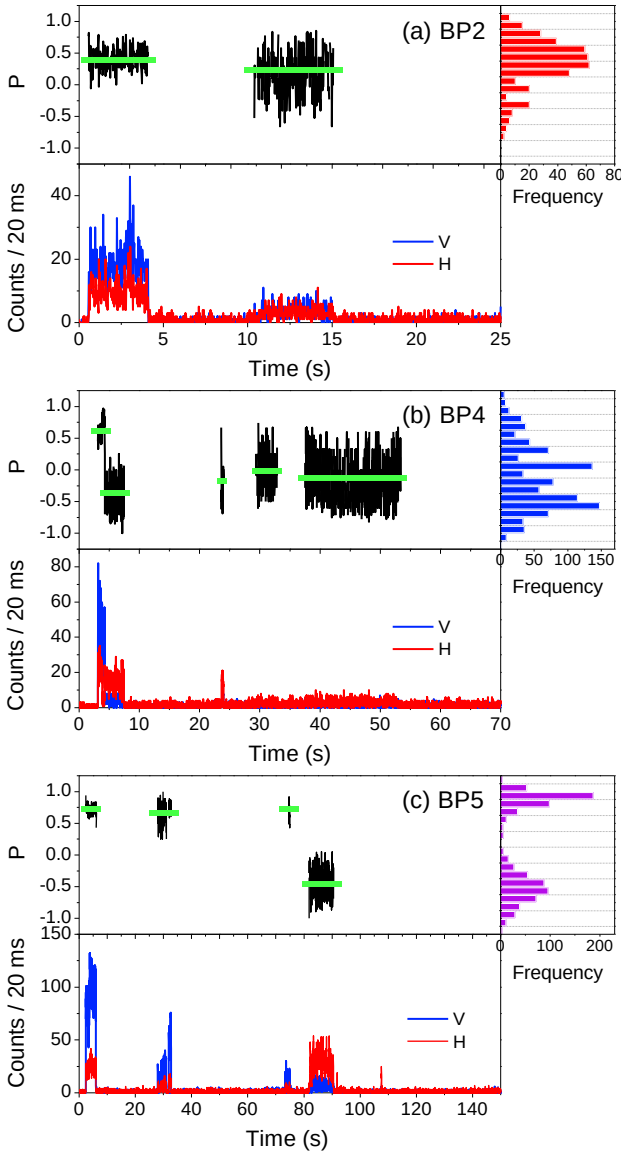

Figure S4. Representative examples of polarization trajectories, *p*-value histograms, and fluorescence intensity trajectories with horizontally (H) and vertically (V) polarized components of (a) **BP2**, (b) **BP4**, and (c) **BP5**.

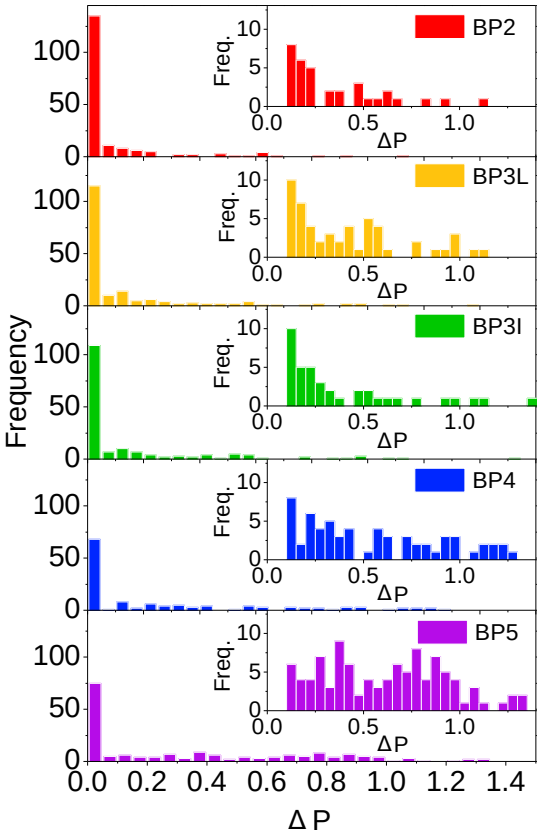

Figure S5. Histograms of the differences in the minimum and maximum *p* values in the fluorescence intensity trajectories for the **BP*N*s**.

Table S1. Fluorescence lifetimes and spectral peak positions of the first emissive levels in the fluorescence intensity trajectories of the **BP*N*s**.

	τ (ns)	**FWHM (ns)**	**λ (nm)**	**FWHM (nm)**
BP2	1.5 ± 0.16	0.28	630 ± 3.8	8.2
BP3L	1.3 ± 0.21	0.31	671 ± 13.3	9.9
BP3I	1.3 ± 0.26	0.29	673 ± 15.3	14.3
BP4	1.2 ± 0.39	0.42	705 ± 27.1	52.8
BP5	1.2 ± 0.40	0.43	733 ± 48.6	64.2

3. References for supporting information

(1) S. Habuchi, T. Oba and M. Vacha, *Phys. Chem. Chem. Phys.*, 2011, **13**, 7001.

(2) Y. Hou and D. A. Higgins, *J. Phys. Chem. B*, 2002, **106**, 10306.

(3) J. Hofkens, W. Verheijen, R. Shukla, W. Dehaen and F. C. De Schryver, *Macromolecules*, 1998, **31**, 4493.

(4) H. Uji-I, S. M. Melnikov, A. Deres, G. Bergamini, F. De Schryver, A. Herrmann, K. Müllen, J. Enderlein and J. Hofkens, *Polymer*, 2006, **47**, 2511.

(5) D. Wöll, H. Uji-i, T. Schnitzler, J. Hotta, P. Dedecker, A. Herrmann, F. C. De Schryver, K. Müllen and J. Hofkens, *Angew. Chem. Int. Ed.*, 2008, **47**, 783.

(6) K. D. Weston and S. K. Buratto, *J. Phys. Chem. A*, 1998, **102**, 3635.

(7) K. D. Weston, P. J. Carson, H. Metiu and S. K. Buratto, *J. Chem. Phys.*, 1998, **109**, 7474.

(8) H. P. Lu and X. S. Xie, *Nature*, 1997, **385**, 143.

(9) F. Köhn, J. Hofkens, R. Gronheid, M. Van der Auweraer and F. C. De Schryver, *J. Phys. Chem. A*, 2002, **106**, 4808.

(10) D. A. Vanden Bout, W. T. Yip, D. Hu, D. K. Fu, T. M. Swager and P. F. Barbara, *Science*, 1997, **277**, 1074.

(11) F. Vargas, O. Hollricher, O. Marti, G. De Schaetzen and G. Tarrach, *J. Chem. Phys.*, 2002, **117**, 866.

(12) R. Zondervan, F. Kulzer, S. B. Orlinskii and M. Orrit, *J. Phys. Chem. A*, 2003, **107**, 6770.

(13) E. K. L. Yeow, S. M. Melnikov, T. D. M. Bell, F. C. De Schryver and J. Hofkens, *J. Phys. Chem. A*, 2006, **110**, 1726.

(14) J. Schuster, F. Cichos and C. Von Borczyskowski, *Appl. Phys. Lett.*, 2005, **87**, 051915.

(15) H. Yoo, J. Yang, Y. Nakamura, N. Aratani, A. Osuka and D. Kim, *J. Am. Chem. Soc.*, 2009, **131**, 1488.

(16) M. Forster, D. Thomsson, P. R. Hania and I. G. Scheblykin, Phys. Chem. Chem. Phys., 2007, 9, 761.

(17) F. Schindler, J. M. Lupton, J. Feldmann and U. Scherf, *Adv. Mater.*, 2004, **16**, 653.

(18) E. Mei, S. Vinogradov and R. M. Hochstrasser, *J. Am. Chem. Soc.*, 2003, **125**, 13198

(19) R. W. Wagner, J. S. Lindsey, J. Seth, V. Palaniappan and D. F. Bocian, *J. Am. Chem. Soc.*, 1996, **118**, 3996.

(20) P. Kim, S. Ham, J. Oh, H. Uoyama, H. Watanabe, K. Tagawa, H. Uno and D. Kim, *Phys. Chem. Chem. Phys.*, 2013, **15**, 10612.

(21) H. Uoyama, K. S. Kim, K. Kuroki, J. Y. Shin, T. Nagata, T. Okujima, H. Yamada, N. Ono, D. Kim and H. Uno. *Chem. Eur. J.*, 2010, **16**, 4063.