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Fluorescence Dynamics of Directly Meso−Meso Linked Porphyrin Rings Probed by Single Molecule Spectroscopy

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Abstract: Porphyrin rings **CZ4**, **CZ6**, and **CZ8** that respectively comprise four, six, and eight porphyrins, immobilized in a thin PMMA film, have been investigated using single molecule fluorescence spectroscopy with a focus on the influences of the overall structural rigidity as the ring size of porphyrin array increases. Neighboring porphyrin moieties were linked directly to enhance through-bond electronic interactions and, as a consequence, efficient excitation energy migration processes like the natural LH2 complex. Unlike the ensemble study, the single molecule study using confocal microscopy could eliminate the averaging effect, and consequently provide detailed information on individual molecular behaviors. Indeed, in solution, as a dihedral angle between neighboring porphyrins decreases in the order of **CZ6** > **CZ8** > **CZ4**, red-shifted Q-absorption bands and faster excitation energy hopping rates were observed. However, at the single molecule level, we found that they show longer survival times, less frequent on−off behaviors, narrower fluorescence lifetime distributions, and high relative single molecular brightness in the order of **CZ8** > **CZ6** > **CZ4**, by recording fluorescence intensity trajectories. Especially, **CZ4** reveals high photostability with its rigid structure, and about 3 porphyrin units among the 4 chromophores-constituted molecule behave as a collective coherent domain. Thus, our results single out **CZ4** as a potential and promising candidate for application in artificial light harvesting solid-state devices.

Introduction

There have been tremendous efforts on making artificial light harvesting (LH) arrays using porphyrin pigments as mimicry of natural systems such as B800 and B850 LH complexes.[1−6] As prerequisite requirements for the fabrication of artificial LH arrays, numerous research activities have been made toward the elucidation of excitation energy transfer (EET) processes occurring in multichromophoric systems.[7−10] Especially, we have witnessed a variety of cyclic arrangements of porphyrins of which the energy migration efficiency has been characterized by ensemble and single molecule spectroscopic methods.[11−13] In an ongoing effort in making efficient LH porphyrin arrays, we have prepared directly meso−meso linked porphyrin rings **CZ*N*s** ($N = 4$, 6, and 8) that comprise four, six, and eight zinc(II) porphyrins to possess effective electronic interactions due to direct linkages between neighboring porphyrin units attested by their broad B-bands (Chart 1 and see the Supporting Information). The previous ensemble spectroscopic study on these porphyrin rings reported that the smaller dihedral angle in the order of **CZ6** > **CZ8** > **CZ4** leads to their EET processes becoming faster, that is, 342 ± 59, 236 ± 31, and 119 ± 2 fs, respectively, indicating that the fast EET processes are mediated by through-bond electronic coupling mainly determined by the dihedral angle between neighboring porphyrins.[14]

Single molecule fluorescence spectroscopy (SMFS) has continued to emerge as a powerful tool to study the individual

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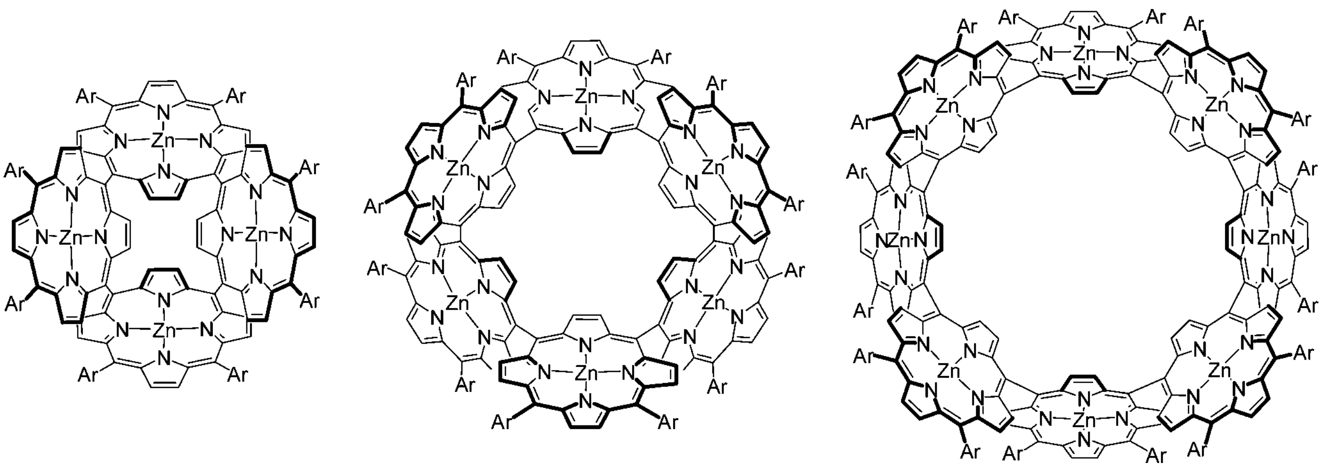
Chart 1. Molecular Structures of Directly Meso−Meso Linked Zn(II) Porphyrin Rings **CZ4**, **CZ6**, and **CZ8**[a]



[a] Ar = 3,5-di-*tert*-butylphenyl.

behaviors of fluorescent single molecules. Through this technique, it has become possible to measure the distributions of spectroscopic behaviors rather than ensemble-averaged values from bulk measurements. The SMFS can also provide direct information on the conformational heterogeneities[15,16] as well as EET efficiencies[10,17] of individual molecules. Despite the similarities in structures and electronic properties of porphyrin arrays to the natural LH complexes, the application of SMFS has been focused on highly fluorescent dye molecules such as perylene diimides[18−22] mainly due to low fluorescence quantum yields of porphyrin moieties. Nevertheless, it is crucial to investigate single molecular photophysical behaviors of porphyrin-based molecular system not only for direct observation of salient properties but also for future applications as LH apparatus in the solid state.

In this work, to unfold molecular photostability and structural rigidity, which are heavily required for molecular photonic devices, of individual **CZ*N***s with an increase of the size of porphyrin rings, survival times and on−off behaviors in fluorescence intensity trajectories (FITs), relative single molecular brightness, and fluorescence lifetimes have been measured with particular attention on the correlation between the number of porphyrin units and the efficiency as LH devices. Also, we have observed the exciton delocalization in the cyclic porphyrin ring **CZ4** by analyzing FITs and their corresponding fluorescence lifetimes simultaneously. Our efforts to apply SMFS to low fluorescence quantum yield molecular systems such as porphyrin arrays are aimed at a detailed understanding of how closely they are related to the natural LH complexes. Such information is necessary for gaining insight into the EET processes of individual multichromophoric LH complexes for future applications as molecular photonic devices.

Experimental Section

Sample Preparation. A detailed description of the synthesis of **CZ4**, **CZ6**, and **CZ8** is reported in a previous paper.[14] Samples for single molecule spectroscopic measurements were prepared by spin-coating (2000 rpm) of the solution of porphyrin ring ($\sim 10^{-10}$ M) in THF (Aldrich, anhydrous, ≥ 99.9%, spectrophotometric grade) containing 10 mg/mL poly (methylmethacrylate) (PMMA) onto thoroughly cleaned glass coverslips. All glass wares used for the sample preparation were cleaned by sonication in acetone, NaOH solution (10%), and Milli-Q water.

Ensemble Spectroscopy. Steady-state absorption spectra were taken via a UV−vis−NIR spectrometer (model Cary 5000, Varian), and steady-state fluorescence spectra were obtained via a fluorescence spectrometer (model F-2500, Hitachi) at an excitation wavelength of 420 nm. All spectra were measured in THF (Aldrich, anhydrous, ≥99.9%, spectrophotometric grade). Relative fluorescence quantum yields were determined with reference to the value ($\Phi_f = 0.033$) of zinc(II) tetraphenylporphyrin in benzene. A picosecond time-correlated single photon counting (TCSPC) system was used for time-resolved fluorescence decay measurements. The system consisted of a self-mode-locked and cavity-dumped femtosecond Ti:sapphire laser pumped by a continuous wave (cw) Nd:YAG laser (Coherent, Verdi). The full width at half-maximum of the instrument response function obtained by a dilute solution of coffee cream was typically 50 ps in our TCSPC system.

Single Molecule Spectroscopy. A confocal microscope system based on the inverted-type microscope, coupled to a picosecond pulsed 470 nm diode laser (10 MHz repetition rate, pulse width <90 ps, PicoQuant), was employed for the detection of fluorescence of single molecules. The collimated laser beam was sent to wave plate to ensure circularly polarized light and delivered to the input port of the confocal microscope after passing a beam expander to make enough beam size at the back focal plane. The laser beam was then reflected by a dichroic mirror and focused onto the sample through an oil-immersion objective lens (100×, N.A. = 1.3, Nikon). Fluorescence was collected by the same objective lens, passed through a dichroic mirror, cleaned with a notch filter and long-pass filters, and focused through a 100 mm focal length lens into an active area of an avalanche photodiode (SPCM-AQR-16-FC, EG&G, Perkin-Elmer Optoelectronics) (APD). The sample positions were controlled using a piezoelectric tube scanner (XE-120, Park

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system). The signal from APD was delivered into a TCSPC card (SPC-830, Becker & Hickl GmbH) operated in FIFO (first-in−first-out) mode, which can construct the fluorescence intensity trajectory with a specific bin time and fluorescence decay profiles with an experimental instrument response of 500 ps. We convoluted the monoexponential fitting function with the response function to arrive at the early time rise displayed by the fits. The maximum likelihood estimation (MLE) was used to fit the fluorescence decays. Maus et al. demonstrated that as the MLE method is based on the more adequate multinomial statistics, it can provide a precise fluorescence lifetime determination for a few photons in single molecule fluorescence. In that paper, the LS (least-squares) and MLE methods were experimentally compared, so that it was verified that the MLE method estimates the lifetimes 5% larger than the LS method by the distribution of the fitted lifetimes against the number of counts per decay.[23,24] The detailed description of the setup and the data acquisition process has been published previously.[25]

We defined fluorescence lifetimes of the solid bulk film as the lifetime of fluorescence decay made up of the data of all of the pixels of a scanned area. The sample area of 20 × 20 μm^2 (128 × 128 pixels) was scanned at an average power of 0.3 μW, and the concentration of the sample solution was about $\sim 10^{-7}-10^{-8}$ M.

Results and Discussion

Fluorescence Intensity Trajectories. The representative FITs of 119 single molecules of **CZ4**, 90 single molecules of **CZ6**, and 93 single molecules of **CZ8** are given in Figure 1. The FIT of **CZ4** exhibits four-step photobleaching behavior in accordance with the number of constituent porphyrin units; however, those of **CZ6** and **CZ8** show less intensity levels than the number of chromophores and more complex behaviors with repetitive on−off behaviors as well as large intensity fluctuations. The FIT of **CZ6** reveals four intensity steps with a few tens of millisecond times in the first intensity level. After 10 s, the molecule goes to the long off-state and re-emits at 18 s for awhile at the second intensity level before the irreversible photobleaching occurs. Similarly, the FIT of **CZ8** displayed in Figure 1 shows repetitive on−off behaviors. This feature implies that **CZ6** and **CZ8** have complex emission dynamics that tend to inhibit continuous radiation.

To explain this behavior in a concrete manner, we have collected the FIT data for statistical analysis of on−off behaviors, which are one of the characteristic features in photodynamics. As the porphyrin ring becomes larger, they go to the off-state more frequently. In the FITs, 39% of **CZ4**, 50% of **CZ6**, and 52% of **CZ8** molecules exhibit on−off behaviors while they emit fluorescence. Off-states can be induced by several dynamical processes such as (1) triplet blinking,[26] (2) physical motions of the molecule such as translational and rotational diffusion,[27−30] (3) spectral diffusion due to changes in the absorption cross section and emission yield by conformational changes,[15,16,31] and (4) formation of radical ion and photooxidation.[32−36] Because we have applied the binning time of 30 ms to acquire sufficient signal-to-noise ratio in the FITs, we can observe only a few seconds off-state. Accordingly, the occurrence of on−off behaviors caused by the triplet state populations is excluded because the triplet-state lifetimes of porphyrins are shorter than the binning time.[37] Also, the possibility of molecular rotation can be ruled out because the porphyrin rings are likely to be immobilized tightly in PMMA matrix, hindering the molecules from rotating in the polymer matrix. Yet unlike bulky rotational and translational motions, intramolecular conformation changes are still possible. As the changes in molecular structures induce the changes in emission spectrum and emission yield, it is possible to transit from higher intensity level (on) to lower intensity level (dim). Therefore, large intensity fluctuations in the FITs of **CZ6** and **CZ8** can be explained by their conformational changes in dihedral angles

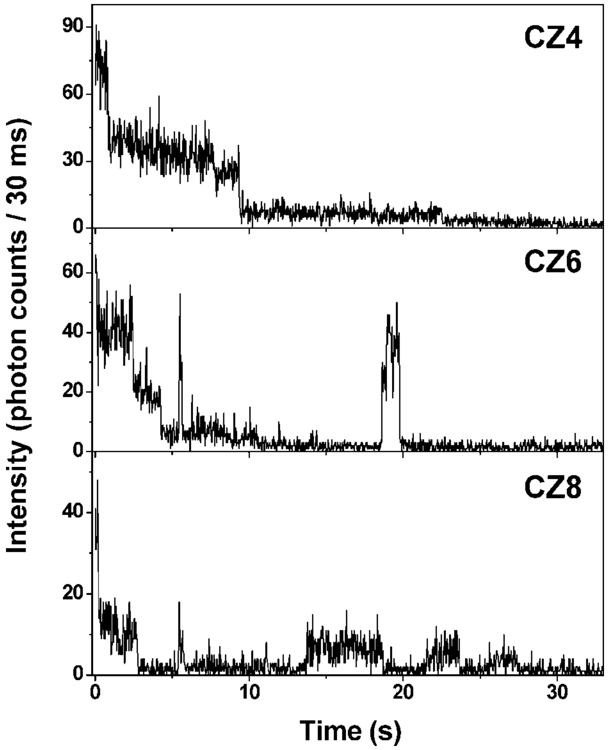

Figure 1. Fluorescence intensity trajectories of **CZ4**, **CZ6**, and **CZ8** embedded in PMMA matrix at an average power of 0.9 μW ($\lambda_{\mathrm{exc}} = 470$ nm).

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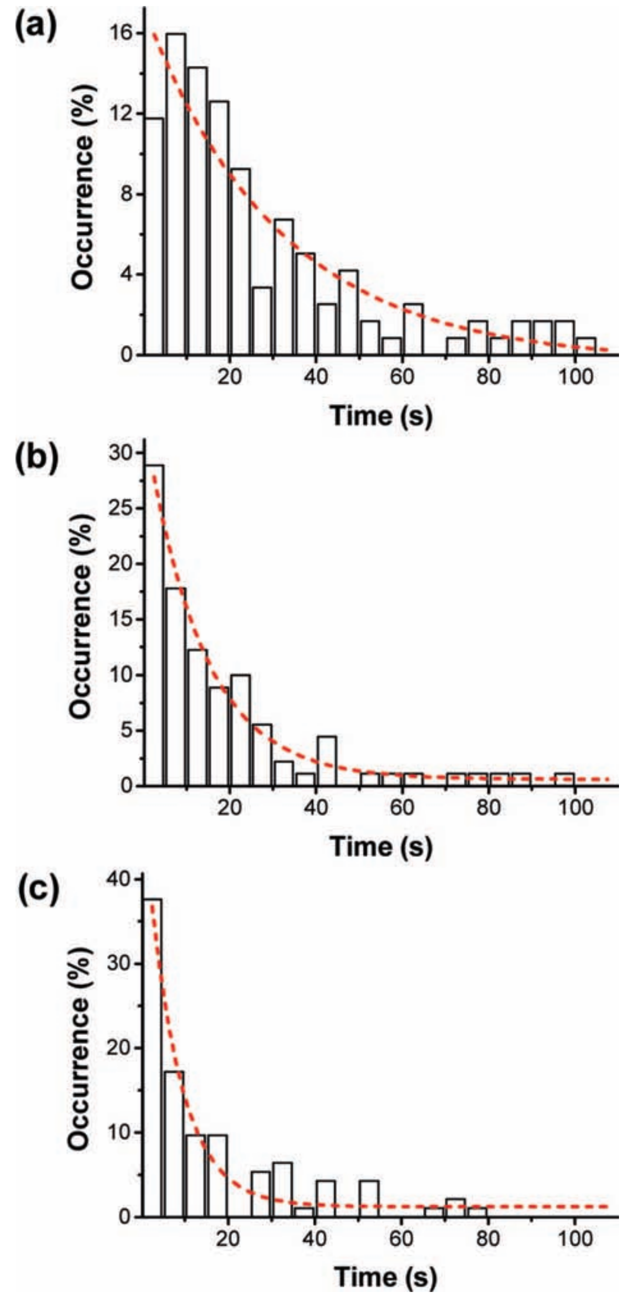

Figure 2. Survival time distributions for (a) 119 **CZ4** molecules, (b) 90 **CZ6** molecules, and (c) 93 **CZ8** molecules. The dashed curves are single exponential fits of each distribution, yielding average survival times of 31.4 s (a), 13.3 s (b), and 7.4 s (c). The survival time distributions of **CZ6** and **CZ8** are shifted to shorter time than that of **CZ4**.

Table 1. Fitted Fluorescence Lifetimes of **CZ4**, **CZ6**, and **CZ8** in Each Environment (Solution/Bulk Film/Single Molecule)

	in solution (ns)[a]	in film (ns)[b]	single molecule (ns)[c]
CZ4	1.84 ± 0.02	1.78 ± 0.01	1.75 ± 0.17
CZ6	1.76 ± 0.04	1.74 ± 0.01	1.68 ± 0.22
CZ8	1.80 ± 0.02	1.70 ± 0.01	1.67 ± 0.26

[a] $\lambda_{exc} = 420$ nm in THF. The fluorescence lifetimes in solution were obtained by averaging the fitted single fluorescence lifetimes at several emission wavelengths (625, 650, 700 nm). [b] $\lambda_{exc} = 470$ nm. Bulk films were prepared by spin-coating the sample solution ($\sim10^{-7}-10^{-8}$ M) containing PMMA/THF (10 mg/mL) solution on cleaned coverslips. [c] Averaged single molecule fluorescence lifetimes were estimated at the first emissive level of each fluorescence intensity trajectory. The error range indicates the standard deviation of fluorescence lifetime distribution. The standard errors ($\sigma/N^{1/2}$) for lifetime distributions of **CZ*N*s** are 0.016, 0.024, and 0.027, respectively. In a large number of lifetime data sets, each average value might be converged into the standard error range within 99% confidence interval.

between neighboring porphyrin moieties in a directly linked cyclic ring structure.

A more persuasive interpretation for the fluorescence intermittency can be considered as that these long off-times may arise from the formation of "defects" such as radical cations and/or oxidized molecules. The porphyrin molecules are known to generate radical cations temporarily and can be oxidized due to oxygen penetrated through the PMMA matrix,[38] and consequently act as fluorescence quenchers. The smallest molecule **CZ4** experiences the dark state less frequently than do **CZ6** and **CZ8**, because the high photostability of **CZ4** may prevent efficient radical formation.

FITs also provide information on the survival time, which corresponds to the duration time from when the molecule starts to emit photons to the background intensity level by irreversible photobleaching. Generally, the survival time is related to the number of chromophores in a multichromophoric system;[39,40] the more chromophores they have, the longer survival times can be expected. However, the average survival times of **CZ4**, **CZ6**, and **CZ8** are 31.4, 13.3, and 7.4 s, respectively, by fitting the histograms with a single exponential function (Figure 2). The decreasing survival times in going from **CZ4** to **CZ8** are contrary to the expected behaviors, but are coincident with the above-mentioned tendency, providing evidence that **CZ4** is the most photostable molecule among them.

Our findings that as the porphyrin rings become larger they bleach faster can be related to their conformations; direct linkages in porphyrin cyclic rings can make it difficult to accommodate porphyrins to form exact cyclic structure as the number of porphyrin units increases, leading to structural congestions. This feature infers that, due to the existence of many chromophores in a cyclic multichromophoric molecule with steric hindrances, inevitable conformational heterogeneities like kinked structures. They can induce exciton traps (such as radical cations) that quench the fluorescence within a molecule and lead it to the dark state, and as a consequence the survival time becomes shorter. Thus, in **CZ6** and **CZ8**, their average survival times become shorter by structural heterogeneities.

Fluorescence Lifetimes. We have also measured the fluorescence decays in THF and PMMA matrix. All of the fluorescence decay profiles show single exponential decays, and the fitted fluorescence lifetimes are presented in Table 1. The fluorescence lifetimes of **CZ*N*s** are similar due to the strong electronic coupling in porphyrin rings. Shorter fluorescence lifetimes at the single molecule level as compared to those in ensemble measurements are attributable to a difference in the conformational dynamics[39] and a formation of fluorescence quenching sites induced by higher excitation rate associated with single molecule measurements.[41]

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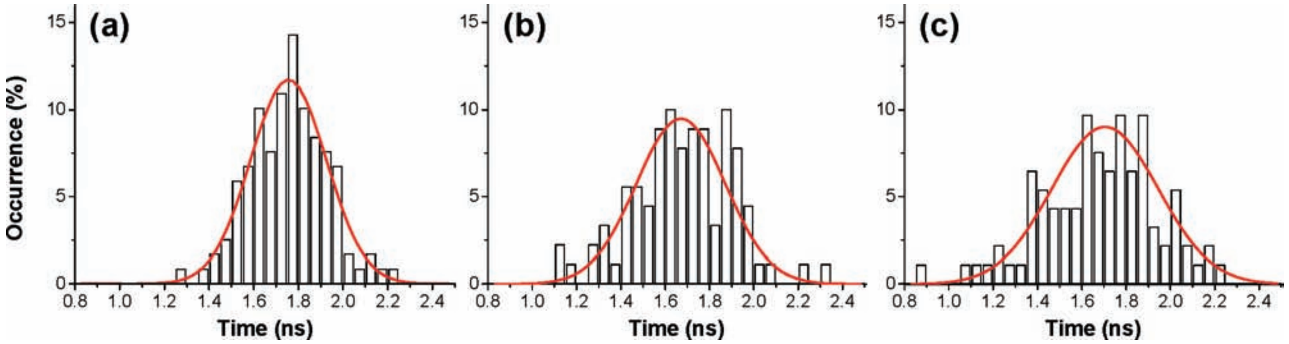

Figure 3. Fluorescence lifetime distributions of **CZ4** (a), **CZ6** (b), and **CZ8** (c). The fluorescence lifetimes of single molecules in the distribution were obtained by fitting all of the photons in the first emissive level, and their average fluorescence lifetimes are 1.75, 1.68, and 1.67 ns, individually. The lines correspond to fitted Gaussian distribution with the 0.40, 0.55, and 0.67 ns fwhm values. The distribution of **CZ4** (a) is narrower than those of others.

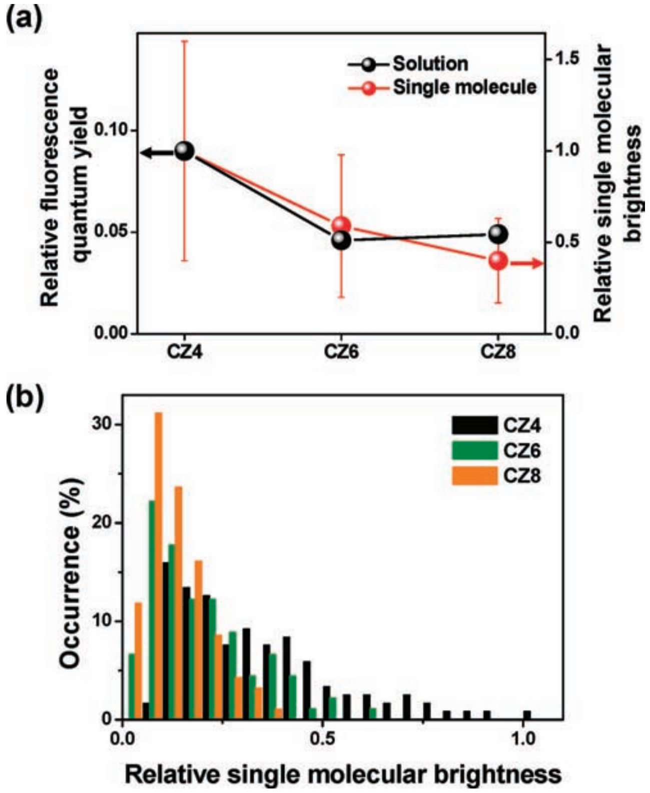

Figure 4. Plot of average relative brightness of single molecules (red) and relative fluorescence quantum yield in THF (black) (a) and the distributions of relative single molecular brightness (b). The relative single molecular brightness is decreased in the order of **CZ4** > **CZ6** > **CZ8**. Error bars represent the standard deviation of mean relative single molecular brightness.

At the single molecule level, both **CZ6** and **CZ8** exhibit broader distributions of fluorescence lifetimes (Figure 3). Although we cannot distinguish a difference in the lifetime distributions between **CZ6** and **CZ8** clearly, the difference between the distribution width of **CZ4** and those of the other two rings is noticeable. The broader distribution means that there are more conformational heterogeneities;[39] on the basis of these results, we can demonstrate that in **CZ4** there are less conformational heterogeneities producing nonradiative decay channels.

Relative Single Molecular Brightness. The relative single molecular brightness, which is evaluated as the maximum photon counts in the first emissive level in FITs divided by the molecular absorptivity,[12] can be regarded as a direct criterion of fluorescence efficiency of single molecules. While the molecular extinction coefficients increase remarkably in the order of **CZ4**, **CZ6**, and **CZ8** (2.9×10^5, 4.9×10^5, and 7.3×10^5 M^{-1} cm^{-1} at 470 nm), the average photon count rate at the first emissive level is nearly around 60 counts per 20 ms for all three porphyrin rings. Unlike the tendency of the fluorescence quantum yields in solution phases, the relative single molecular brightness of **CZ8** is slightly decreased from that of **CZ6** (Figure 4a). This is evidence that the molecular size can be a decisive factor for determining the fluorescence efficiency in the solid state; that is, the larger molecule is prone to be affected by surroundings and has more difficulty maintaining the structural rigidity.

The fluorescence quantum yield of **CZ4** ($\Phi_f = 0.071$) is larger than those of the others (0.031 for **CZ6**, and 0.040 for **CZ8**) despite that **CZ4** contains only four porphyrin units,[14] and as shown in the brightness distribution in Figure 4b, **CZ4** is the most effective fluorescence emitting unit. Many nonradiative relaxation pathways through which the fluorescence emission is quenched rapidly in larger **CZ6** and **CZ8** molecules may lead to lower quantum yields.

Radiative Coherence Length. In 119 single molecules of **CZ4** studied here, there are 9% for one-step, 33% for two-step, 28% for three-step, and 30% for four-step photobleaching behaviors in the FITs of **CZ4**. Except the FITs showing four stepwise intensity levels, the others show collective photobleaching behaviors. Also, there might be diverse photobleaching mechanisms in molecules that reveal four-step intensity traces. Among them, 24 (20%) **CZ4** molecules show stepwise photobleaching behaviors from tetrameric to monomeric intensity level with a gradual increase in their fluorescence lifetimes at each step (Figure 5).[42,43] This behavior well coincides with our previous study on the change in the fluorescence lifetimes of directly meso−meso linked linear zinc(II) porphyrin arrays.[44] The fluorescence lifetimes of linear porphyrin arrays become shorter as the number of porphyrin units increases. This feature was

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(43) The theoretical correlation between the standard deviation of lifetimes and the total photon counts can be defined by the following equation from ref 42: $\mathrm{var}_N(\tau,T,k) = (1/N)\tau^2(k^2/r^2)[1-\exp(-r)] \times ((\exp(r/k)[1-\exp(-r)]/[\exp(r/k)-1]^2)-(k^2/\exp(r)-1))^{-1}$, where N is the total photon counts, τ is the fluorescence lifetime, T is the total width of the measurement window, k is the number of channels, and r is the number of lifetimes in the measurement window. In Figure 5, we have calculated the error for each fluorescence lifetime at stepwise intensity levels by this equation. An error analysis indicates that, within a degree of uncertainty of ~±3%, these lifetime differences are statistically significant.

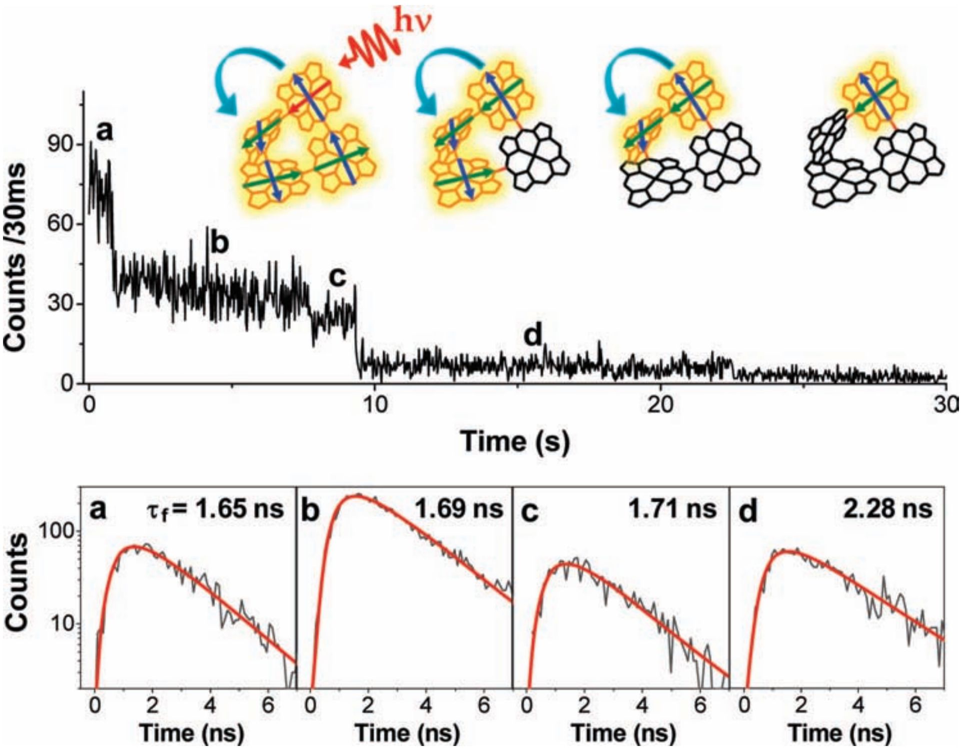

Figure 5. The representative FIT of **CZ4** showing stepwise photobleaching behavior with the fluorescence lifetimes at each intensity levels. The increase fluorescence lifetime with photobleaching is evidence for exciton delocalization of **CZ4**. The fluorescence decay profiles were obtained from each intensity step in the FIT. By BIFL (burst-integrated fluorescence lifetime) data analyzer, we could extract a bunch of photons at each intensity level to fit their lifetimes. Because of a small number of emitted photon counts from porphyrin molecules, we bound up the photons from 16 channels of 4096 channels into one part and fitted by MLE method, which provides a reasonable lifetime fit in low fluorescence intensity. The fluorescence lifetimes and errors are (a) 1.65 ± 0.06, (b) 1.69 ± 0.03, (c) 1.71 ± 0.07, and (d) 2.28 ± 0.06 ns. In the case of a small number of counts or low signal to background ratio, the fitting accuracy is affected by the background signal.

explained by exciton delocalization, which results in the enhancement of radiative decay rates of a complex of chromophores and thus a decrease in the fluorescence lifetimes. Accordingly, the gradual increase in the fluorescence lifetimes while the FIT shows stepwise photobleaching behavior observed in **CZ4** can be ascribed to a decrease in the exciton delocalization length as the constituent porphyrin unit is sequentially photobleached.

We can evaluate the degree of exciton delocalization in individual **CZ4** molecules by using the concept of radiative coherence length (N_{coh}). When one chromophoric unit interacts strongly with another in a multichromophoric system, all units go into one specific exciton state of which the transition possesses much larger dipole strength than that of the monomer, and the radiation of this system occurs in phase (superradiance).[45] In such case, the radiative decay rate (k_{rad}) noticeably increases with a linear correlation with the number of constituent units, because the dipole strength is directly related to the radiative rate. Therefore, the radiative coherence length is defined as the ratio of radiative decay rate of multichromophore divided by that of monomer ($k_{\mathrm{array}} = Nk_{\mathrm{monomer}}$)[4,19] in which the radiative decay rate can be calculated according to the relationship between the fluorescence quantum yield and the fluorescence lifetime ($k_{\mathrm{rad}} = \Phi_f/\tau_f$). This calculation requires an assumption that the size of the cyclic array should be large enough that there is no collision between coherent domains from both directions.[46]

To examine the exciton delocalization of **CZ4** molecules at the intact state, we have calculated the radiative coherence length by employing the fluorescence lifetimes at the tetrameric intensity level and monomeric intensity level in the FIT, respectively. The radiative coherence lengths for 24 **CZ4** single molecules are presented in Table S1 (see the Supporting Information). Note that the relative fluorescence quantum yields measured at the ensemble level are used to estimate the radiative decay rates due to the incapability of measuring the fluorescence quantum yields at the single molecule level; hence this calculation contains the assumption that the single molecules can emit photons as efficiently as they do in solution state. The average radiative coherence length of **CZ4** is 2.74, which is shorter than that of ~4 of directly meso–meso linked linear porphyrin tetramer arrays.[4] The decrease in radiative coherence length of **CZ4** as compared to that of linear porphyrin tetramer array can be explained by a structural difference. The cyclic structure of **CZ4** probably cannot cause exciton to be fully delocalized over the four porphyrin units because the transition dipoles of porphyrin units are arranged nearly orthogonal to one another; therefore, this arrangement of **CZ4** reduces the excitonic interactions among the four porphyrin units, and thus the extent of exciton delocalization.

In **CZ6** and **CZ8**, the radiative coherence length could not be examined because their FITs do not show clear stepwise

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(45) Monshouwer, R.; Abrahamsson, M.; van Mourik, F.; van Grondelle, R. *J. Phys. Chem. B* **1997**, *101*, 7241–7248.

(46) Kakitani, T.; Kimura, A. *J. Phys. Chem. A* **2002**, *106*, 2173–2179.

photobleaching behaviors with a tendency of increasing fluorescence lifetimes at the same time.

Conclusion

To explore the EET efficiencies of porphyrin cyclic rings at the single molecule level, the fluorescence behaviors depending upon the number of porphyrin units in **CZ*N*s** have been investigated using confocal fluorescence microscopy. Because **CZ4** exhibits the largest electronic communication by much reduced dihedral angle between neighboring porphyrins,[14] and high photostability by rigid structure, we believe that it can be a good candidate as LH apparatus. In contrast to time-resolved ensemble study, because **CZ8** might experience the largest strain in the solid state, it exhibited the most complicated FIT, and the lowest relative single molecular brightness. Although **CZ6** and **CZ8** contain more porphyrin units than does **CZ4**, they turn out to be not so efficient as emitting units. Thus, not only the number of chromophores but the structural arrangement of constituent LH units is of utmost importance in the application of single molecule devices based on porphyrin cyclic arrays in the solid state.

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Supporting Information Available: Steady-state absorption and emission spectra, optimized structures, and time-resolved fluorescence decays of **CZ*N*s**; fluorescence lifetimes and radiative coherence lengths of **CZ4** with discussion. This material is available free of charge via the Internet at http://pubs.acs.org.

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