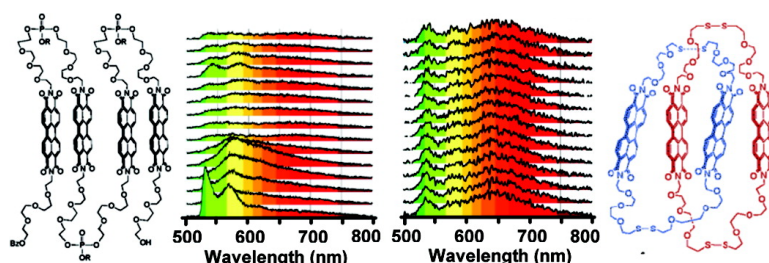Article

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Architecturally Diverse Nanostructured Foldamers Reveal Insightful Photoinduced Single-Molecule Dynamics

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Abstract: Single molecule fluorescence spectroscopy has been used to probe architecturally diverse and unique model oligomers containing exactly two or four perylene tetracarboxylic diimide (PTDI) units: linear foldamers **lin2** and **lin4**, monocyclic complement **cyc2**, and concatenated foldable rings **cat4**. Linear, cyclic, and concatenated foldamers reveal that photoabsorption and excitation induces unfolding and refolding, generating colorful spectral switching from one spectral type to another. Foldamer architectures dictate the unfolding and refolding processes, and hence the spectral dynamics. As a result, linear tetramer exhibits active frame-to-frame spectral switching accompanying dramatic changes in colors, but a concatenated tetramer displays a multicolored composite spectrum with little or no spectral switching. Excited state dynamics causes spectral switching: an electronically decoupled PTDI monomer emits green fluorescence while electronically coupled PTDI π-stacks emit red fluorescence, with longer π-stacks emitting redder fluorescence. A key question we address is the excited-state delocalization length, or the exciton coherence length, in the π-stacks, which has been proven difficult to measure directly. Using foldamers having controlled sequences, structures, and well-defined length and chromophore numbers, we have mapped out the exciton coherence length in π-stacks. Single molecule fluorescence studies on chromophoric foldamers reveal that the maximum domain length is delocalized across just four π-stacked PTDI dyes and no new *pure* color can be found for oligomers beyond the tetramer. Therefore, the range of fluorescent colors in π-stacks is a function of the number of chromophores only up to the tetramer.

1. Introduction

Weak, secondary intermolecular forces drive molecules to self-organize, and nature has been using this strategy to assemble astonishingly complex, yet functional, biological nanostructures.[1] Though we cannot yet match nature's mastery to richly exploit self-organizing processes, knowledge underpinning self-organization processes has emerged and is instrumental in designing molecular architectures.[2] Indeed, our deeper understanding of such driving forces leads us to design molecules that self-organize in solution into simple and often predictable artificial nanostructures. Studying such model systems and their diverse folding-unfolding structural motifs will advance our understanding of folding dynamics which play important roles in complex biopolymers.

Molecular recognition and self-organization[3] highlight the increasing potential for weak forces which have enabled a new generation of functional molecular devices.[4] However, the success of future molecular devices will inevitably rely in part on their ability to maintain resilient shape, usable function, and overall structural integrity while being perturbed externally. Ultimately, both weak and strong forces are important while architectural design responsible for holding the devices together will alter key roles that the forces will play. Herein we contrast weak $\pi-\pi$ interactive dynamics in different architectures using single molecule fluorescence spectroscopy.

Previously, we probed linear foldamers containing three (trimer) and six (hexamer) PTDI units while using the monomer as a reference.[5,6] After laser excitation, the excited states could relax either radiatively (fluorescence) or nonradiatively (triplet shelving, thermal vibrations, etc).[7] In our experiments, we specifically avoided a polymer matrix so as not to restrict large chromophore displacements after the molecules absorb considerable laser excitation energy and undergo structural rearrangements such as unfolding and refolding.[6]

In this report, we present new and complementary results by further investigating six model PTDI-based oligomers and revealing that molecular architecture can, by design, restrict molecular dynamics. Scheme 1 shows four representative oligomers used in this study. The four architecturally diverse foldamers chosen for comparison are the linear PTDI dimer (**lin2**) and its macrocyclic complement (**cyc2**), linear tetramer (**lin4**), and its exotic concatenated complement (**cat4**). We show that results for **cyc2** and **cat4** strongly support our previously

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Scheme 1. Foldamers Used to Probe Single-Molecule Dynamics

LIN2   CYC2   LIN4   CAT4
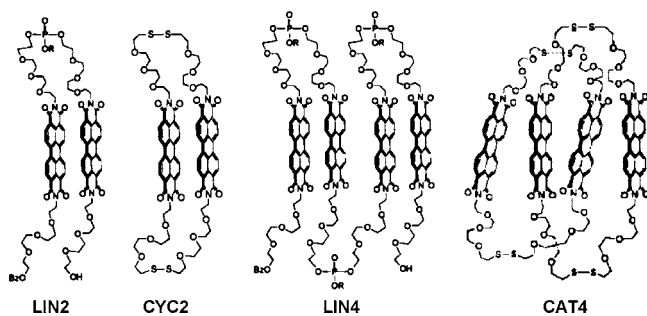
proposed folding model for linear foldamers and provide further insight into photoinduced folding dynamics of linear PTDI foldamers. Using these structurally controlled foldamers, we are able to experimentally measure the exciton coherence length directly in the π-stacks and determine the minimum and maximum number of cofacially stacked chromophores sharing the delocalized, wave-like, excited states.

2. Experimental Procedures

2.1. Materials. Synthesis and characterization have been reported for **lin2** and **lin4**,[5] for **cyc2** and **cat4**,[8] and for linear oligomers through 11-mer.[9] Spectroscopic grade methylene chloride (CH_2Cl_2), methanol (MeOH), and cover glasses (Gold Seal No.1) were purchased from Fisher. Solvents were distilled prior to use for single molecule studies but were used as received for ensemble solution measurements.

2.2. Sample Preparation. Solutions of the four compounds were prepared in 4:1 CH_2Cl_2/MeOH to a concentration of $\sim 10^{-11}$ M and stored in vials cleaned with 2% Micro 90 and Chromerge. Cover glasses were cleaned by sonicating in 2% Micro 90 for 30 min, rinsing thoroughly with high-purity water (18 MΩ), drying in an oven, soaking in Chromerge for 1 h, rinsing thoroughly with 18 MΩ water, and finally heating gently in a methane flame to dryness. After cooling slowly, cover glasses were stored in a dust-free container. Samples were prepared by spin-coating (4000 rpm) 1 drop of diluted foldamer onto a cover glass.

2.3. Instrumental Setup. A custom single molecule confocal setup as described in detail elsewhere[6] was modified to include two APDs for simultaneous collection of two-color dual time traces, in which emission light was split into red and green channels by a dichroic beamsplitter centered at 560 nm. Confocal scanning and time trace acquisitions were collected using custom LabVIEW software, with a 20 ms dwell time for time trace acquisition. Spectra were acquired through WinSpec/32 software using a typical dwell time of 1–2 s. Typical laser power used for diffraction limited analysis was $\sim 8 \times 10^2$ W/cm^2 at the cover glass surface.

2.4. Ensemble Spectra. Steady-state fluorescence spectra were recorded with a SPEX Fluorolog-3-21 spectrofluorometer with excitation at 489 nm. UV–vis spectra were recorded with a Varian Cary 100 spectrophotometer. Time-resolved fluorescence decays were collected over the full spectral window on a Hamamatsu 3680 High-Speed Streak Camera equipped with a mode locked Ti: sapphire laser. Fitting of mono- and biexponentials was performed in Igor Pro 6.0 software. All dilutions for ensemble measurements were performed using 4:1 CH_2Cl_2/MeOH solvent.

3. Results and Discussion

3.1. Ensemble Spectra. It is instructive to first consider solution phase ensemble spectra. In dilute CH_2Cl_2/MeOH (4:1)

solvent, the vibronic 0–0 and 0–1 bands in the absorption spectra reveal that all four compounds have diagnostic intensity reversal (Figure 1) in the A^{0-0}/A^{0-1} ratio when compared to a non-self-assembled PTDI monomer (superimposed). All PTDI foldamers (dimer through 11-mer) exhibit this intensity reversal, which indicates that weak π–π molecular orbital overlap and solvophobic interactions drive cofacial stacking of perylene units.[5] Linear and macrocyclic dimers have similar 0–0/0–1 vibronic peak ratios (**lin2**: $A^{0-0}/A^{0-1} = 0.84$ and **cyc2**: $A^{0-0}/A^{0-1} = 0.73$), indicating that the folding equilibrium and configurations for both dimers are quite similar in the *ground state*, with approximately 90% of the linear dimers and essentially all of the cyclic dimers completely coupled.[10] Likewise, the ratios for **lin4** (0.66) and **cat4** (0.63) reveal that tetramers are also stacked almost identically and that all four chromophores are preferably folded together and not as two separately stacked dimers.[8] For all foldamers, the equilibrium strongly favors the folded state and the absorbance ratios persist even as solutions are diluted to nanomolar concentrations used to prepare samples for single molecule studies.

Although absorbance spectra indicate the linear and cyclic foldamers are similarly folded in the *ground state*, fluorescent spectra reveal the model oligomers behave significantly different in the *excited state*. With the spectra in Figure 1 normalized to the 0'−0 monomer-like peak, it is apparent that cyclic species have a relatively higher π-stack red emission than linear species, with tetrameric species showing a much more prominent red emission than dimeric species. Interestingly, all spectra also retain a significant monomer-like green emission component. However, the substantial magnitude of green fluorescence does not translate to a large monomer-like *ground-state* population, as optical absorption and NMR studies reveal that cyclic and linear compounds are almost completely folded before photo-excitation.[5,8] Considering that the ground state exhibits minimal monomer-like characteristics, the substantial monomer-like emission can only hint that the foldamer excited states have active and relatively fast dynamics shuffling folded, partially folded, and unfolded motifs.

Absorbance spectra confirm the molecules are mostly folded in the ground state, but fluorescence data report significant unfolding in the excited state. This discrepancy is consistent with a photoinduced unfolding mechanism: following absorption of a photon, the excitation may delocalize across two or more chromophores in a stack to form a traveling exciton state, which sometimes produces a broad red emission. Alternatively, the excitation may cause the folded oligomer to partially unfold, generating one or more uncoupled chromophores that may emit a green monomer-like emission. It is unclear if the photon which causes the oligomer to unfold directly results in emission, or if the molecule absorbs another photon during the ultrafast transient unfolding and emits green monomer-like fluorescence. In either case, linear foldamers have high degrees of freedom, can actively undergo dynamic conformation exchange, and emit relatively strong green fluorescence. Conversely, the cyclic complements cannot completely unfold in the same manner as the linear foldamers because structurally imposed constraints limit conformation dynamics (Scheme 2). Thus, the cyclic dimer and concatenated tetramer have less freedom, remain mostly folded as π-stack nanorods, and emit predominantly red fluorescence.

(8) Wang, W.; Wang, L. Q.; Palmer, J.; Exarhos, G.; Li, A. D. Q. *J. Am. Chem. Soc.* **2006**, *128*, 11150–59.

(9) Li, A. D. Q.; Wang, W.; Wang, L. Q. *Chem.—Eur. J.* **2003**, *9*, 4594–4601.

(10) Calculations for ground and excited state folded/unfolded populations by steady-state or time-resolved techniques are detailed in the Supporting Information.
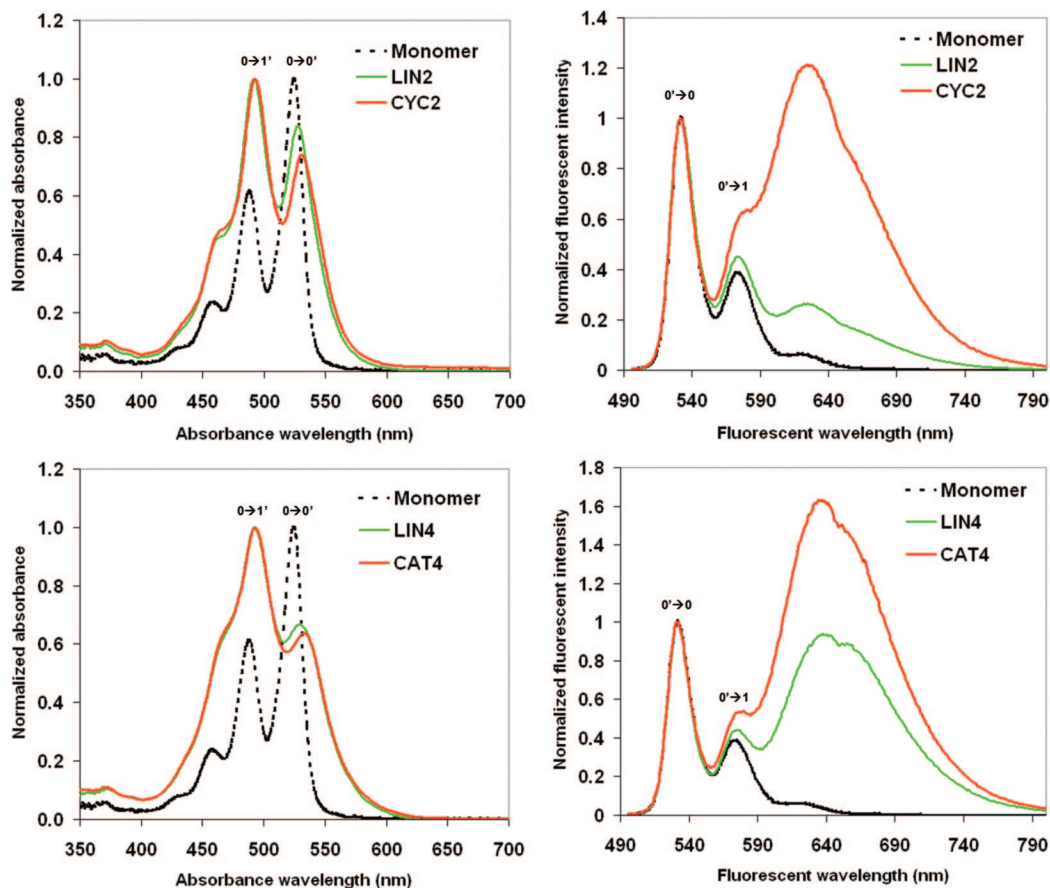
Figure 1. Normalized absorption (left) and emission (right) spectra of the dimer (top) and tetramer (bottom) oligomers at nanomolar concentrations in 4:1 CH₂Cl₂/MeOH. The free monomer (dashed line) is shown for comparison. The cyclic compounds (red) have almost identical absorbance spectra as the linear compounds (green) showing that cyclic and linear compounds are folded similarly in the ground state. The linear compounds display higher relative monomer-like emission which is indicative of photoinduced unfolding.

Scheme 2. Model Illustrates a Few Unfolding and Refolding Pathways Induced by Photoexcitation
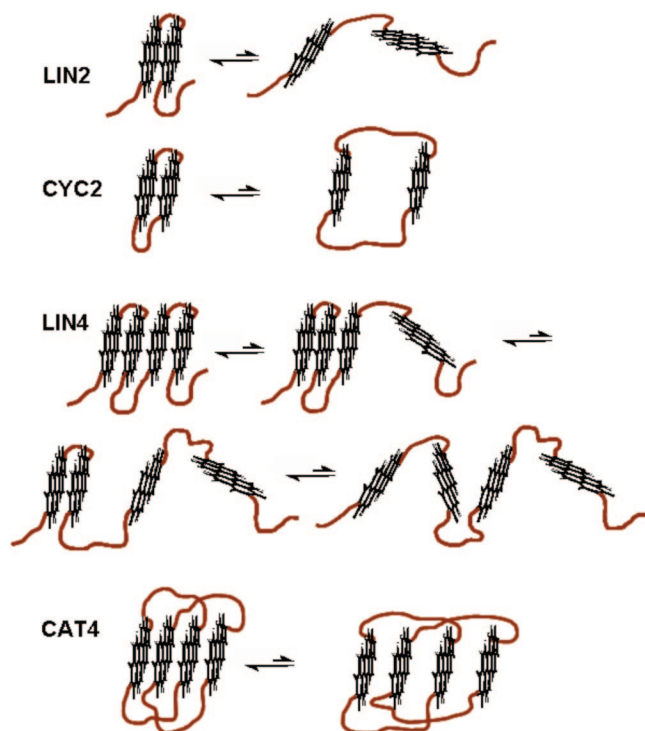
The relative population of transiently unfolded green-emitting chromophores in the excited state was estimated from the ensemble spectra by comparing the relative green and red emission components.[10] Accounting for differences in quantum yields and lifetimes,[11] we estimate that one transiently unfolded chromophore is potentially 10 times brighter than a π-stack emission. Due to such differences and uncertainty in the brightness, an alternative method is to compare the monomer-like emission intensity for the linear and cyclic dimers to the free monomer emission. This method does not depend on red emission and provides the relative population of chromophores emitting monomer-like fluorescence in the excited state. We calculate that ~20% of **lin2** fluorophores exhibit monomer-like emission while only 3% of **cyc2** chromophores display such an emission.[10] Thus, both dimers reveal an increase in unfolded spectroscopic signatures between the *ground* and *excited* states, with the linear dimer exhibiting much greater dynamics due to its less constrained architecture. Similarly, **lin4** and **cat4** exhibit 5% and 1% monomer emission, respectively. These architecture-dependent differences in the folding equilibrium between ground and excited states are consistent with transient unfolding during photoexcitation, and the results are summarized in Table 1.

To complement the steady-state measurement results and further probe the folding and unfolding dynamics of the excited state, we collected time-resolved fluorescence decays to obtain

(11) Ahrens, M.; Sinks, L.; Rybtchinski, B.; Liu, W.; Jones, B.; Giamo, J.; Gusev, A.; Goshe, A.; Tiede, D.; Wasielewski, M. *J. Am. Chem. Soc.* **2004**, *126*, 8284–94.

Table 1. % Chromophores Unfolded

	lin2	cyc2	lin4	cat4
ground state	10%	<1%	<1%	<1%
excited state[a]	19.8%	3.4%	5.1%	1.7%
excited state[b]	23.8%	3.7%	5.8%	3.5%
excited state[c]	43.0%	–	6.1%	–

[a] Determined by comparing green oligomer fluorescent intensity to monomer fluorescent intensity. [b] Determined by comparing ratio of green and scaled red oligomer steady-state emission. [c] Determined by time-resolved fluorescence. The calculations are detailed in the Supporting Information.

lifetimes of the monomer-like and red emissive species and to estimate %-monomer-like emission across the linear dimer through hexamer series. As shown in the Supporting Information, the PTDI monomer consistently fits well to a single exponential, yielding a lifetime of 4.00 ± 0.01 ns. In contrast, oligomer decays obtained over the full spectral window consistently fit well to a biexponential to yield two lifetimes: a faster ∼4 ns component attributed to transiently unfolded states and a slower ∼10 ns component corresponding to the red emission from folded π-stacks. By analyzing the pre-exponential factors and accounting for differences in quantum yields, we calculate up to 45% of **lin2** fluorophores exhibit monomer-like emission while 7−5% of the linear trimer through hexamer display such an emission.[10] Values for trimer through hexamer agree well with the values obtained from steady-state spectra, while **lin2** exhibits approximately a 2-fold increase in the unfolded population when compared to steady-state measurements. Regardless of method, there is an unambiguous increase in the unfolded population between the ground and excited states as summarized in Table 1; the difference is the most dramatic in the least restricted architectures.

Importantly, both solution-phase and single-molecule emission spectra provide the extent to which the red-emitting exciton state is delocalized. The π-stack emission involves delocalized excitons that relax radiatively while emitting red fluorescence to the Franck−Condon ground state, which is in a repulsive high vibrational level of the relaxed ground state and eventually loses its energy nonradiatively, reorganizing to the lowest vibronic level (ΔE_g).[12] When a locally excited (LE) state evolves into an exciton, there is another energy loss (ΔE_e) to delocalization. The two-energy losses ($\Delta E_e + \Delta E_e$) account for the exciton red shift from the 0−0 vibronic band of the monomer. Thus, the number of chromophores involved in the delocalized state governs the exciton emission wavelength.

These oligomers exhibit two π-stack emission peaks, which are broad, closely overlapping, and separated by only 15 nm as discussed in detail elsewhere.[13] The linear and cyclic dimers have identical spectral shapes peaked at 624 nm in solution (DCM/MeOH 4:1), indicating that the excitons have identical π-stack structures regardless of linear or cyclic motifs. Similarly, the linear tetramer and concatenated tetramer have identical spectral shapes with a 638-nm maximum; the four chromophores also form identical exitonic structures regardless of linear or concatenated architectures. Compared to ensemble emission for all foldamers (dimer through 11-mer), the π-stack emission shows no further red shift beyond the tetramer. This is in contrast to the $A^{0−0}/A^{0−1}$ absorbance ratio, which continues to decrease

(12) Saigusa, H.; Lim, E. *Acc. Chem. Res.* **1996**, *4*, 171–8.
(13) One possible explanation of the overlapping excimer-like peaks has been discussed in ref 16. We intend to provide a different explanation in a future article.
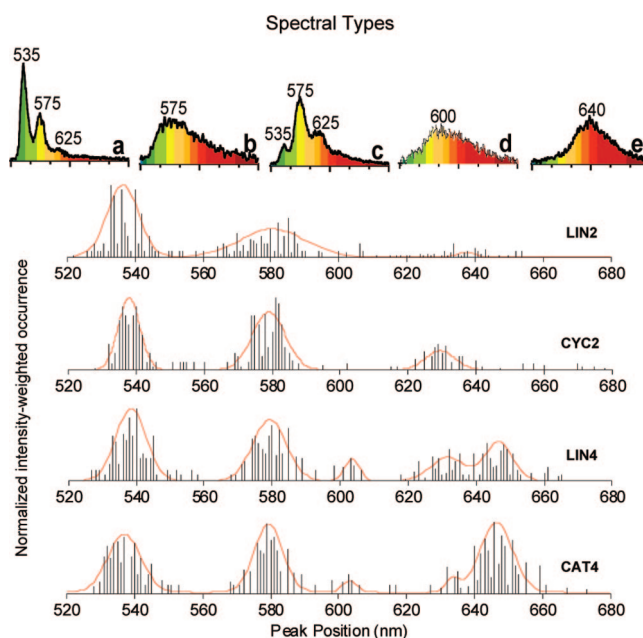
Figure 2. Top: Most common single molecule spectral types observed. Bottom: Average relative intensities of spectral peaks observed in the first frame of spectral trajectories. A 1-nm bin size was used. Gaussian fits were scaled up for clarity but retain their relative intensity ratios.

through the 11-mer, demonstrating that stack length continues to increase in higher oligomers.[5] Thus, solution-phase ensemble measurements clearly reveal that the maximum delocalization length is essentially achieved with four PTDI units, although the wave-like excited state may tail slightly into adjacent chromophores. Additionally, the minimum delocalization length is achieved with just two chromophores; the excitons delocalize from two to four chromophores, and longer π-stacks generate no further red-shifted fluorescence.

3.2. Single Molecule Spectra. While contrasting solution ensemble measurements, we observed that solventless PTDI single molecule measurements exhibit dramatically different emission spectra. When a continuous wave laser excites linear foldamers **lin2** and **lin4** and cyclic compounds **cyc2** and **cat4**, their fluorescence spectral trajectories collectively display various characteristic shapes and vibrant colors. Five common spectral types (Figure 2), including a pure monomer-like green emission (type "a") and pure π-stack red emission (type "e"), have been identified as representative of all spectra observed, although many spectra are slightly shifted from those shown, or are composites of multiple spectral types. These five spectral types are representative and initially identified for the linear trimer and hexamer.[6]

The relative peak intensities and observed spectral positions reflect the degree and strength of electronic coupling and therefore the conformational state in which the PTDI molecule exists on the glass surface.[6,8] Integrating all emission wavelengths, we typically count $5 \times 10^{4−6}$ emission cycles per second using the experimental conditions described here. The fact that we see spectra that are not completely representative of the ensemble spectra suggests that the energy landscape and local energy minima (traps) play important roles in the folding/unfolding.

3.2.1. First Frame Spectra. Spin coating is a physically tortuous and violent process to prepare single molecule samples, in which analyte molecules randomly physisorb as they are

rapidly cast across the substrate. This can impose formidable structural perturbations to a folded nanostructure held together by weak π-stacking. The relative peak intensities and spectral positions in the first snapshot provide the degree of π-stacking retained after the spin coating process. The initial 2 s integrated emission spectrum also minimizes other undesired effects such as photobleaching and substrate heating.

Figure 2 shows the intensity-weighted spectral peak occurrence observed in the *first frame* of all spectral trajectories. The relative intensities indicate molecular populations having that type of electronic coupling; thus a suitable method to quantify both peak occurrence and relative intensity is to weight occurrence by intensity. Peaks in a given spectrum are first identified; the highest intensity peak is normalized and given an occurrence of 1. All remaining peaks are given an occurrence of I_j/I_{max}, where I_{max} = maxiumum peak intensity and I_j = intensity of the jth peak.

All four molecules routinely showed green and yellow fluorescence bands, although their relative intensities and exact positions varied. The Gaussian fit for **lin2** displays a mostly type "a" monomer-like emission. Two-occurrence maxima match the $0 \rightarrow 0$ (green, 535 nm) and $0 \rightarrow 1$ (yellow, 575 nm) vibronic transitions observed for a monomer-like emission (type "a"). The $0 \rightarrow 2$ (red, 620 nm) vibronic transition has a lower intensity and often could not be discriminated in the occurrence spectra. Hence, this band is notably absent from Figure 2. A weak red band centered near 628 nm emerged for **cyc2** and appears again for **lin4** and **cat4**. Compounds **lin4** and **cat4** have an additional red band centered at 647 nm, except **cat4** exhibits a higher relative intensity. The Gaussian fits to these intensity-scaled bands represent relative intensity ratios and can be compared directly from one oligomer to the next. The cyclic dimer and concatenated tetramer reveal a significant π-stack emission (type "e") because architectural constraints favor an exciton-delocalization likelihood among PTDI units while their linear counterparts have low abundance in red fluorescence. It is expected but noteworthy that the single-molecule averaged Gaussian peaks roughly reflect the ensemble fluorescent spectra seen in Figure 1.

Spin coating brings out the architectural differences of the four molecules in Scheme 2. The centrifugation forces may render a completely folded (pure red fluorescence) π-stack into many possible conformations, including completely unfolded (pure green fluorescence) structures and many possible intermediary folded states. In solution, however, low kinetic barriers promote folded structures. The first frame single molecule spectra capture the random nature that spin coating and solvent evaporation create and subsequently render molecules immobilized on the substrate. A simplistic view of the linear dimer has two states: a folded conformation (delocalized exciton and red-emission) or one representing many possible unfolded and uncoupled conformations. Since the monomer-like emission is potentially 10 times brighter than that of the folded state, it easily dominates over the characteristic π-stack emission. In contrast, **cyc2** cannot "unfold" in the same manner as **lin2**, and therefore a significant amount remains folded and emits characteristic red fluorescence centered at 628 nm.

The tetrameric molecules exhibit similar results. However, because there are four chromophores instead of two, more intermediary folded states have electronically coupled chromophores, increasing red-fluorescence occurrence, and probability. The prominent 647-nm red bands observed for **lin4** and **cat4** are attributed to exciton states. As with **cyc2**, however,

cat4 does not completely unfold due to constraints imposed by the chemical structure. As a consequence, $\pi-\pi$ molecular orbital overlap across the entire structure is better preserved during spin coating, and excimer-like emission for **cat4** takes place with higher occurrence than that for **lin4**, which is not structurally bound to the folded state.

It is interesting that we observe type "c" spectra so prevalently in single molecule spectra. Type "c" appears to be a reversal of the normal Franck–Condon progression and the "mirror" emission that reverses the folded absorbance spectra. In solution, the $0-1$ vibrational peak emission intensity is always about 50% of the $0-0$ peak which is similar to the ratio we have measured for a free monomer. Thus, if there is a type "c" emission component in solution, it must be relatively small and masked by the monomer-like emission. It is possible that in the single molecule experiments the relaxed excited state has a better probability to emit before excimer formation due to the absence of solvent which might facilitate the π-stack and hence exciton formation.

3.2.2. Spectral Dynamics. Histograms of initial spectra as presented above reveal the distribution of folded states immediately following spin coating. Spectral trajectories provide more insightful information, and photoinduced folding and unfolding occur when single molecules are excited. The previously studied linear trimer and hexamer taught us that spectral trajectories frequently switch from one spectral type to another, sometimes displaying dramatic frame-to-frame color changes.[6] For instance, spectral switching would freeze, a particular spectral shape (e.g., red fluorescence) would persist for several frames, and then the spectral trajectory would suddenly resume switching. Spectral shapes relate to folded or unfolded nanostructures, and spectral switching indicates active nanostructural dynamics. A corollary is that linear foldamers experience a stochastic folding and unfolding process driven by absorption of laser energy. As laser excitation power was increased, spectral activity increased, and so did the number of actively switching molecules. Thus, experimental data support this photoinduced model.

The linear trimer and hexamer exhibit spectral trajectories that are actively switching. The linear tetramer further verifies these observations, showing frequent switching from one spectral type to another while displaying all spectral types. More importantly, the cyclic compounds reveal only minimal switching trajectories, supporting a photoinduced folding and unfolding model. Figure 3 shows portions of **lin4** and **cat4** spectral trajectories, where **lin4** exhibits switching between different spectral types and **cat4** displays a marked and unambiguous decrease in spectral switching and steadily emits one multicolored composite spectral type. Although a small percentage of **cat4** molecules did show minor spectral switching, Figure 3 represents statistical emission of **cat4**. The cyclic compounds mostly display composite spectra, having simultaneous monomer-like and π-stack emission characteristics, which implies that coupling/uncoupling dynamics occurs faster than the experimental 2 s integration time.

Spectral trajectories for **lin2** and **cyc2** compare somewhat differently than the tetramers (see Figure S1 in the Supporting Information). While being excited, **lin2** preferentially and frequently adopts the unfolded configurations, unlike **lin4**, and thus shows mostly a monomer-like emission and only occasionally visits the folded state that fluoresces red. In contrast to **cat4**, **cyc2** spends more time traveling between folded and unfolded states and displays a smaller relative red peak. After correcting
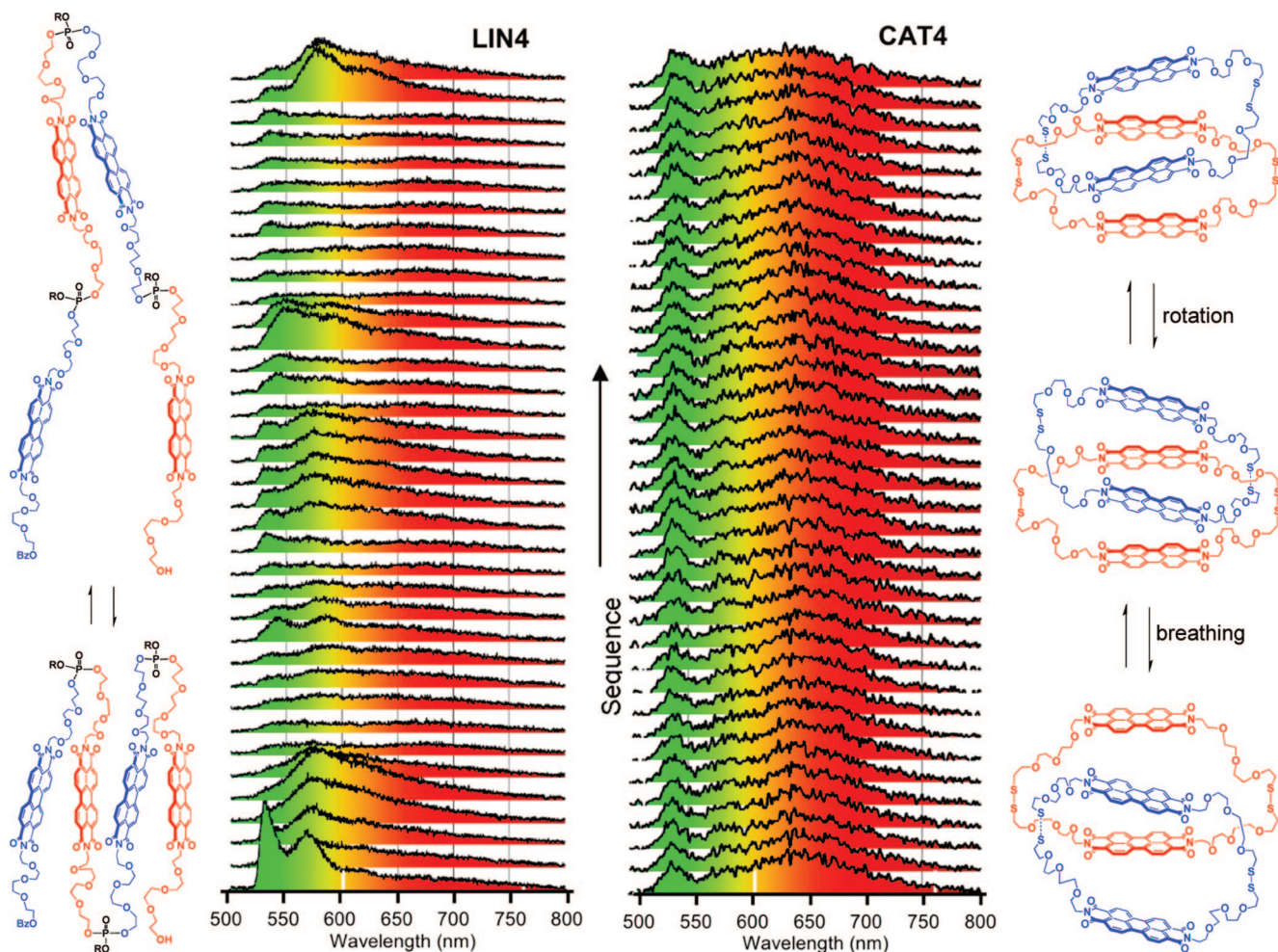
Figure 3. Portions of spectral trajectories for a single **lin4** molecule (left) and a single **cat4** molecule (right). Emission from **lin4** often shows dynamic spectral switching attributed to photoinduced folding and unfolding while **cat4** tends to show "steady-state" emission due to its concatenated structure. 37 frames at 2 s integration/frame = ~74 s total elapsed time.

the quantum yield and lifetime differences, we find that **cyc2** spends more than $^3/_4$ of the time in the folded state and less than $^1/_4$ of the time in unfolded states. The concatenated tetramer, however, spends more than 90% of the time in folded states. The difference is that **cyc2** is not intercalated like **cat4** and thus allows chromophores to displace significantly and spend more time in the uncoupled excited state.

To statistically analyze these observations we defined the parameter "spectral activity," SA = switch/t, where "switch" is the number of times a spectrum changed from one type to a distinctly different type over the duration time, t (min), while acquiring spectral trajectories. Figure 4 plots spectral activity histograms for all four compounds. The fractions in Figure 4 summarize the number of trajectories with SA > 2 over the total number trajectories investigated. Each model oligomer has 40−50% of the molecules showing no spectral switching, which indicates the molecule−substrate interactions limit conformations and generate deep kinetic traps following spin coating. Linear dimer, **lin2**, behaves like a monomer and therefore has a low probability transitioning to a coupled conformation and thus shows limited switching. Similarly, **cyc2** and **cat4** display mostly multicolored conformations and rarely exhibit resolved switching between the green and red emission. In contrast, half of **lin4** molecules surveyed exhibited dramatic spectral switching with a prominent feature occurring at ~6 switch/min (0.1 Hz), which is consistent with the previously studied linear trimer
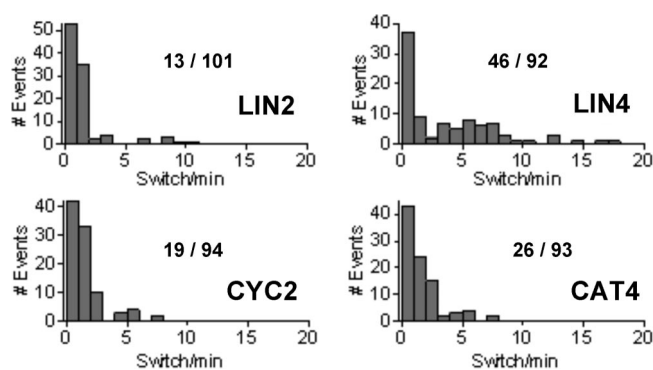

Figure 4. Distribution of SA (switch/min, bin size = 1) values for the four molecules. A shift in the distribution from **cat4** to **lin4** is clearly evident and emphasizes the greater photoinduced dynamics in the linear versus the structurally restrained cyclic compounds. The number of trajectories with SA > 2 over the number investigated is inset for each oligomer.

and hexamer. Figure 4 reveals that **lin4** tends toward a switching rate of 0.1 Hz, whereas the structurally restrained and concatenated **cat4** prefers to cluster near the origin, having little or low switching activities.

The compelling spectral trajectory data reveal that structurally constrained cyclic compounds have dramatically different dynamics when compared to the series of linear foldamers and strongly support that photoinduced displacements (folding/
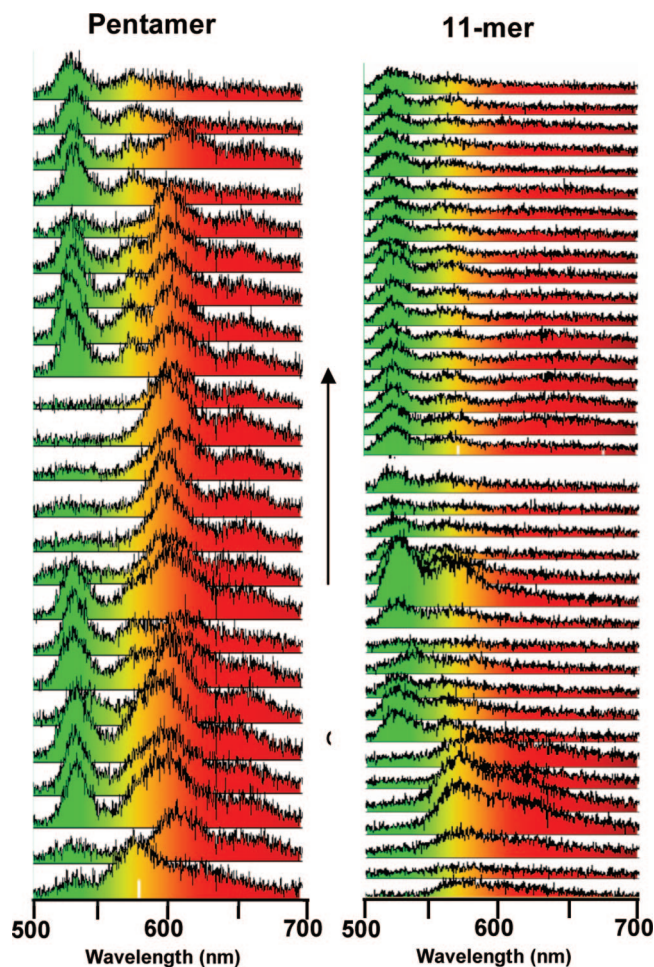
Figure 5. Spectral trajectories for a single pentamer (left) and two single 11-mers (right). The 11-mers display no greater spectral range than the 5-mer during either active switching (bottom right) or quasi-steady-state (upper right) trajectories. Each frame represents 2 s.

unfolding) create dynamic spectral switching, both disrupting and reforming PTDI π-stacks. Chromophore separation or translation of only 1−2 Å can sufficiently quench excited-state delocalization or annihilate excitons, which explains why the cyclic compounds also display a substantial monomer-like emission that is even observed in very long perylene stacks.[14,15] Following photoinduced unfolding, the cyclic compounds are much more likely to return to the folded state more quickly than we can detect and therefore spectral trajectories show little activity (Figure 3). In contrast, the linear compounds may visit many available unfolded states after photoexcitation, some having large entropy traps, and consequently taking a longer time to return to the folded state.

3.2.3. π-Stack Emission. Linear foldamers have active spectral dynamics: the larger the foldamers (more chromophore units), the more colorful the spectral range, up to a point. For example, Figure 5 displays typical spectral trajectories for the linear pentamer and 11-mer, which exhibits no greater spectral range than the tetramer. Figure 6 shows the spectral range within which the molecule averaged peaks λ_{max} (Gaussian peaks in

Figure 2) appear as a function of degree of foldamer polymerization, from monomer to hexamer and 11-mer. The emission-peak range ($\Delta\lambda_{max}$) is shown numerically. Single molecule measurements reveal that longer foldamers result in larger $\Delta\lambda_{max}$ for the dimer through the tetramer, after which no further red shift or $\Delta\lambda_{max}$ increase is found. The longest wavelengths λ_{max} from all trajectory frames locate closely to the average maximum peaks obtained from the first frame and do not show an increasing trend for oligomers larger than the tetramer. Single molecule spectra display emission containing significant infrared tailing because a π-stack emission involves high vibrational levels of the ground state; however, no *peaks* are ever observed with wavelengths greater than ~675 nm.

The maximum exciton coherence length apparently dictates the extent π-stack emission will red-shift.[13] Extended π-stacked perylene arrays tens of nanometers long including self-organized nanofibers[15] and covalently bound photonic wires[14] have shown a red-shifted π-stack emission. However, their fluorescence is not any redder than that of tightly folded **lin4**. In these systems, trap states were speculated to determine the average exciton domain length and similarly individual photobleached chromophores would fragment the extended arrays and limit exciton domains. The same π-stack emission has been described when perylene monomers self-assemble into π-stacks in solution.[16] These also do not show emission wavelength maximums longer than **lin4**. The model is similar to ours in that photoexcitation generates a relaxed state from which emission takes place. Yet in these systems, it is impossible to determine the minimum number of monomers required to achieve such an emission or the maximum number of monomers possible, forming an exciton that subsequently emits remarkably red-shift fluorescence.

The exciton diffusion length and coherent domain length along *conjugated polymers* attracts intensive experimental and theoretical studies and debate and are currently believed to range from 20 nm to 1.2 μm and 4.5 to 11 nm, respectively.[11,17] The domain length across π-stacked systems has not been directly measured, although Barton and co-workers recently estimated the molecular wire domain in DNA to be 4−5 stacked base pairs.[18] The foldamer structural series presented here offer unique opportunities to determine the maximum exciton coherent domain length in π-stacked perylene chromophores. Unlike the self-assembled or polymer systems mentioned above, each foldamer has the precise known number of monomers.

Here, single molecule experiments have determined that the peak maximum red shift $\Delta\lambda_{max} = 112$ nm is achieved with just four chromophores and that no longer wavelength emissions have been found when the foldamer π-stacks have been increased from tetramer to 11-mer. Ensemble solution phase measurements confirm this apparent peak maximum red shift, $\Delta\lambda_{max}$.

3.3. Single Molecule Time Trace Analysis. To probe fluctuations at faster time scales than the 2 s integrated spectra, we collected time traces using two APDs where the emission was split into a green (<560 nm) and red (>560 nm) channel at a 20 ms bin time. Because the red channel receives yellow, orange, and red photons, it is difficult to discriminate various nongreen types switching (c, d, and e) in the time traces. However, pure green type "a" emission and spectral types with a very small

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(15) Yan, P.; Chowdhury, A.; Holman, M.; Adams, D. *J. Phys. Chem. B* **2005**, *109*, 724−730.

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(17) Kakitani, T.; Kimura, A. *J. Phys. Chem. A* **2002**, *106*, 2173−9.

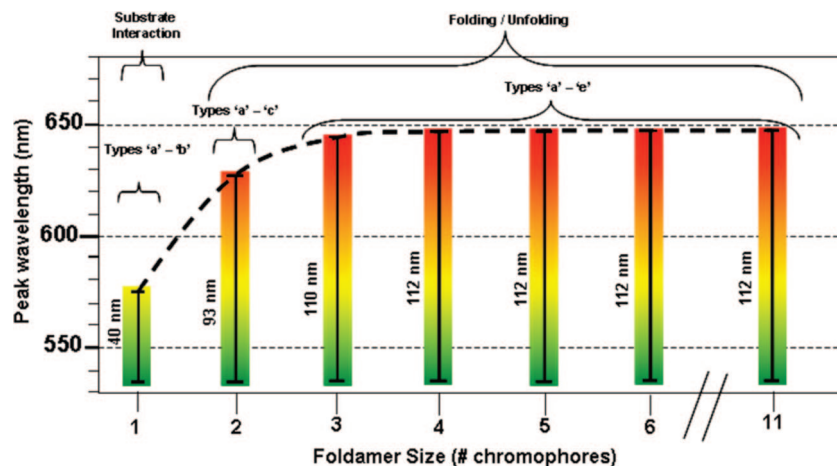(18) O'neill, M.; Barton, J. K. *J. Am. Chem. Soc.* **2004**, *126*, 11471−11483.

Figure 6. Maximum range within which first-frame molecule-averaged λ_{max} were observed as a function of foldamer length. The $\Delta\lambda_{max}$ is presented numerically for each oligomer. A wider range of colors are seen as foldamer size increases up to the tetramer, after which no further increase in $\Delta\lambda_{max}$ is found. A dashed line helps visualize the λ_{max} trend.

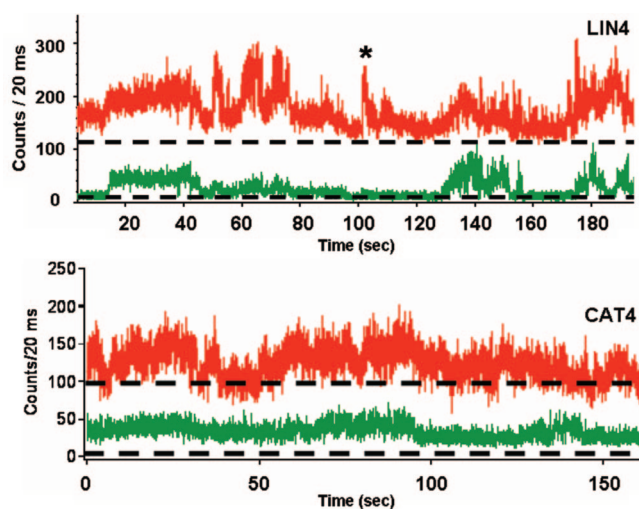**Figure 7.** Dual time trace for the Lin4 (top) and Cat4 (bottom). The red trace is photons > 560nm while the green trace represents photons < 560nm. Data was collected in 20 ms bins. The red trace is off-set from the baseline by 100 counts for better clarity.

Figure 8. Idealized schematic representation of proposed folding model. In response to laser excitation, foldamers can assume one of many possible folded states. The completely unfolded state (uf) will exhibit type "a" emission, whereas partially (pf) and completely folded (f) states with two or more coupled chromophores may emit from yellow to red.

green component (types d and e) are readily observable in the time traces. Two examples, one each for **lin4** and **cat4** are shown in Figure 7. **Cat4** tends to exhibit a steady-state like emission with very little switching. Thus, the green and red channels are generally synchronized and rarely show regions where only one channel is receiving photons while the other is not. Conversely, **lin4** often demonstrates correlated switching from green to red, although the switching rarely creates completely unfolded (~85% green channel) or completely folded (100% red channel) nanostructures. Many of these switching events are slow enough to resolve even with the CCD spectra. However, there are some switching events observable in the time traces that are not apparent in the slower spectra. For example, a red-channel peak (marked with a *) at ~100 s in the **lin4** dual time trace, Figure 7, displays red emission in the absence of a green emission with a time-dependent spectral detail that is unresolved during a 2 s spectral trajectory.

3.4. Photoinduced Dynamics Model. Figure 8 represents our current photoinduced unfolding—refolding model using the linear trimer as an example. Similar schemes for longer foldamers would include additional partially folded states. The three conformations shown in Figure 8 represent folded states
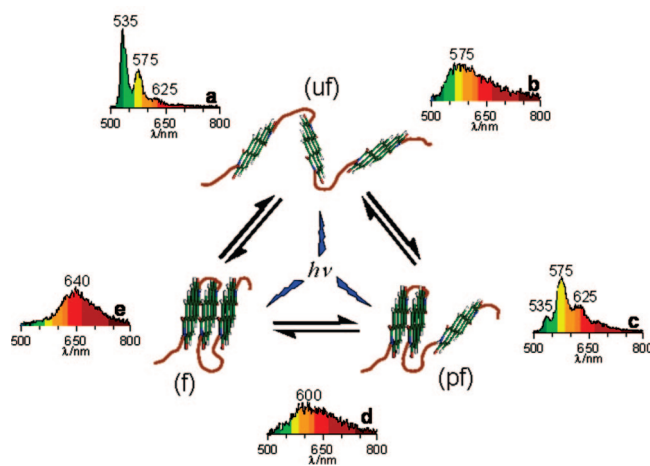
in which three, two, and zero chromophores are electronically coupled via π-stacking. States containing both coupled and uncoupled PTDI units can behave as two independent quantum emitters: uncoupled chromophores yielding green photons; π-stacked segments yielding yellow to red photons. Photon absorption, in which a single 488-nm photon delivers 58.5 kcal/mol of energy and subsequently disrupts $\pi-\pi$ interactions (determined to be 2−7 kcal/mol in organic solvent),[5] is believed to actuate switching between different conformers. It is likely that loss of excess vibrational energy as heat to the environment via vibrational energy transfer occurs much less efficiently than in solution because there is no homogeneous solvent shell intimately surrounding the molecule. Excessive heat build-up in the foldamer as well as the substrate may contribute to overpowering kinetic barriers to actuate folding and unfolding. Single molecule polarization anisotropy measurements suggest that dye molecule rotation embedded in polymer matrices may be both photoinduced and thermally induced.[19] The absence of a polymer matrix significantly lowers the rotation and translation

(19) Bartko, A. P.; Xu, K.; Dickson, R. M. *Phys. Rev. Lett.* **2002**, *89*, 026101/1−4.

barriers when the foldamers are excited, and the same thermal and optical energy that can physically drive apart adjacent chromophores can in turn reunite them.

Figure 8 describes a pictorial model, emphasizing that large conformational displacements change the associated spectral types, but similar spectral effects are expected on an ultrafast time scale, for example, photoinduced displacements such as rotations, stacked chromophore slipping, interplanar dihedral angle changes, or small separations on the order of 1 Å. However, such displacements are expected to quickly return to the enthalpy-favored folded state. Unfortunately, our single-molecule setup is too slow to resolve such ultrafast dynamic processes.

Two or more independent quantum states in the same foldamer simultaneously emit colorful photons and typically generate multicolored spectra. However, the quasi-steady-state single molecule emission as displayed by **cat4** (Figure 3) exhibits a green-yellow-red composite emission, suggesting that multicolored fluorescence is also occurring from a process faster than the time resolution used for spectral acquisition. Interestingly, spectra for **cat4** often match well to dilute solution spectra; despite the excimer-like states beam prominent red photons, a green monomer-like band still exists. The evidence presented here suggests that **cat4**, which cannot achieve long-range unfolded configurations because it is structurally restrained, experiences mostly subtle and/or ultrafast translational displacements and quickly returns to the folded configuration while being excited. Thus, both green and red emissions are detected simultaneously as the single molecule undergoes ultrafast breathing processes involving both coupled and uncoupled configurations within the detection time.

Finally, it is constructive to contrast the solution-phase ensemble measurement with single molecule results. Both thermodynamic and kinetic effects govern the folding and unfolding dynamics. In solution, the thermodynamically favorable equilibrium state for foldamers is the completely folded π-stacks, having presumably low kinetic folding barriers. Absorbance and NMR data report that the molecules are mostly folded in the ground state, but fluorescent spectra show they can become unfolded during photoexcitation. However, spin cast molecules on silica are complicated because the random nature of spin casting renders all possible unfolded nanostructures and substrate interactions as not well-defined. Such substrate interactions frequently result in large kinetic barriers to achieving thermodynamic equilibrium. These slowed photodynamics significantly change the folding conditions from the solution phase. Yet, it is these constipated folding equilibria that allow us to capture the emission from partially folded configurations and provide more insightful photoinduced folding dynamics.

4. Conclusion

Single molecule fluorescence spectroscopy, complementing ensemble fluorescence spectroscopy, has been used to probe unique well-defined π-stacked foldamers containing exactly two or four PTDI units: linear foldamers **lin2** and **lin4**, and their cyclic complements, **cyc2** and concatenated **cat4**. Ensemble measurements report the molecules are mostly folded in the ground state but significant portions unfold following excitation.

Single molecule fluorescence captures the emission from several of the folded and unfolded configurations, which are distinctly different than the ensemble spectra.

Fluorescence spectral trajectories were collected for all four compounds and analyzed statistically to reveal common spectral types and switching frequency. Spectral activity showed an unambiguous difference between **lin4** and **cat4**. PTDI units in **cat4** undergo small photoinduced displacements, and multicolored spectra suggest that subtle but fast individual PTDI conformational changes may intermittently disrupt the delocalized excitons. Spectral trajectories for **lin4** confirm the previously studied linear trimer and hexamer in that they frequently switched between spectral types and displayed all spectral types, including pure green, multicolored composites, and pure red. The fact that linear and cyclic compounds have dramatically different spectral trajectories strongly supports that photoinduced phenomena actively fold and unfold foldamers on glass substrates and hence switch on-and-off many spectral types.

Chromophoric interplay within the foldamers generates fluorescence spectra that span a colorful range: the greater the number of chromophores, the richer the color until a point. The π-stack emission continuously shifts toward red from dimer to tetramer, but without further increase for larger oligomers, suggesting the maximum domain length is delocalized across mainly *four π-stacked PTDI dyes*.

The optical behavior of molecular aggregates is both fundamentally and technologically interesting, and the results presented here have broader ramifications. Photoinduced unfolding itself has many possible applications. For example, the distinctly different folded conformations having different emission properties can be used for biosensors and nanotensil strength detectors. It has been demonstrated that perylene self-assembles above its critical concentration[2] and that self-assembly directs specific reactions and enhances the reaction rate, thereby serving as a catalyst.[8] Combining directed self-assembly and controllable photoinduced disassembly could create nanoscale shuttles or other devices in which photoinduced actuation is to occur within defined nanoscale architectures. The cyclic versus linear structures discussed here provide insightful information regarding how the assembly distances and rates might be controlled. Furthermore, direct measurement of the exciton domain length is important for building molecular wires and self-assembled molecular circuitry.

Supporting Information Available: Spectral time trajectories and calculations. This material is available free of charge via the Internet at http://pubs.acs.org.

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