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Folding and Unfolding of Chromophoric Foldamers Show Unusual Colorful Single Molecule Spectral Dynamics

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Nature uses exquisitely folded biopolymers such as proteins to generate an astonishing array of novel biological functions. Despite its importance, our current understanding of folding in polymers is still rather limited since they lack optical chromophores within their natural sequences, making them difficult to probe.[1] Synthetic foldable polymers (foldamers) made of fluorophores, however, offer promising insight into intrinsic folding dynamics and folding behavior. Furthermore, probing synthetic foldamers at the single molecule (SM) level should allow observation of complex folding dynamics normally obscured in ensemble measurements. Toward this end, we have examined a series of well-defined foldamers[2] with a specific sequence of alternating rigid perylene chromophores and flexible ethylene glycol chains that exist as free π-stacked folded nanostructures in dilute 4:1 CH_2Cl_2/MeOH solution.[3] Using the 488-nm line of an argon ion laser and the perylene monomer (**1**) as the control, we interrogated single foldable trimers (**3**) and hexamers (**6**) (Scheme 1) nonspecifically adsorbed on cover glasses and monitored both single foldamer emission spectra and intensity temporal profiles at room temperature.

Single chromophore spectral fluctuation due to both conformational (intrinsic) and environmental (extrinsic) changes has been observed.[4] In addition, multichromophoric perylene systems often show broad red-shifted single molecule emission, a feature that is generally attributed to intramolecular coupling of adjacent chromophores within the single molecule or nanostructure.[5−8] As **1** displays minor spectral fluctuations, we necessarily use the results of **1** as a reference against which linear foldable multichromophoric **3** and **6** are compared.

The unique feature exhibited by **3** and **6** is the degree of dynamics in their spectral trajectories. Figure 1a−e shows the five most common spectral types observed, though some spectra were either slightly shifted (<10 nm) or intermediary in shape between those shown, or a combination of one or more types. All spectra of **1** (103 molecules, 1000 spectra) are represented by "type a" (64%) or a variant of "type b" (36%) where the peak max is 540−580 nm. The situation for **3** and **6** is much more complex. Switching between different spectral types (Figure 1f,g) occurred for 38% (40/104) and 62% (81/130) of all trimer and hexamer molecules, respectively. In addition, spectral switching from frame to frame for a single trimer or hexamer could be quite dramatic in terms of both spectral shape and intensity. Differences in peak maxima of up to 110 nm for **3** and 140 nm for **6** were observed.

While interaction with the local environment can explain minor spectral fluctuations as seen in **1**, they cannot account entirely for the observed unusual color shifts of **3** and **6**. The flexibility of perylene foldamers is in contrast to perylene-based photonic wires[6] and nanofibers,[7] in which the relative degree of motional freedom of individual perylenes is limited by chemical structure. Therefore, we attribute the rich dynamics to a photoinduced stochastic folding/unfolding phenomenon in which varying degrees of electronic coupling between adjacent chromophores occurs.

Scheme 1

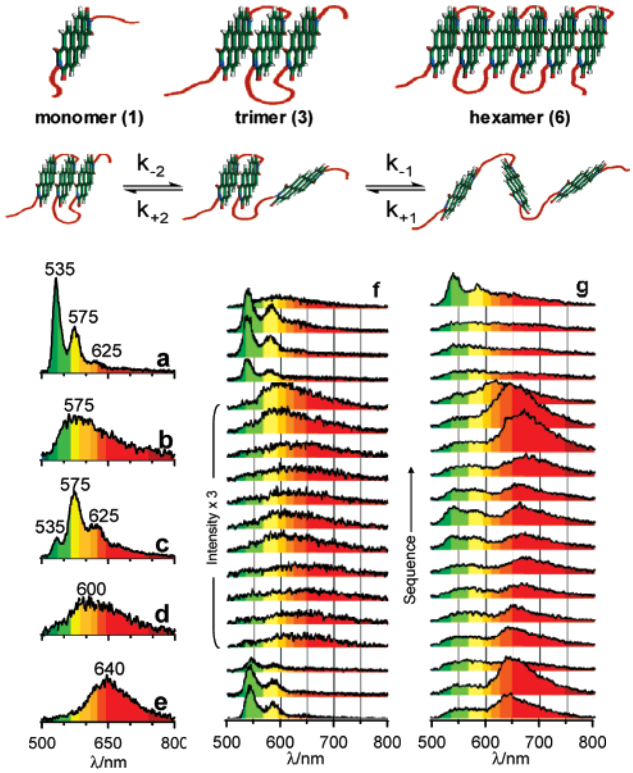

Figure 1. Most common single molecule spectral shapes observed for monomer (a,b), trimer (a−e), and hexamer (a−e). Portions of spectral trajectories for a single trimer (f) and hexamer (g) showing dynamic emission color switches, indicating folding, unfolding, and refolding. Twenty frames at 2 s integration/spectrum = ~40 s total elapsed time.

The range of spectra observed is an indicator of the range of $\pi-\pi$ interaction between adjacent chromophores. Emission following normal Franck−Condon progression (535, 575, 625 nm, Figure 1a) is attributed to the unfolded structure, while vibronically resolved yellow (Figure 1c) emission originates from two or more folded perylene units. This diagnostic change in intensity ratios between the first two vibronic transitions is seen in optical absorption and emission of covalently bound perylene cyclophanes[9] and folded dimers[2] and is attributed to $\pi-\pi$ interaction. Broad structureless fluorescence centered at 600−640 nm is attributed to delocalized states across extensively folded and coupled π-stacks.[6,7,10]

By analyzing each molecule's spectral trajectory for dominant fluorescent color and frequency of spectral switching, we define two parameters, $K_{fold} = \tau_{fold}/\tau_{unfold}$, where τ_{fold} and τ_{unfold} are times spent in the folded and unfolded states, respectively, for a given spectral trajectory, and "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), of the spectral acquisition. Figure 2 shows histograms of measured K_{fold}

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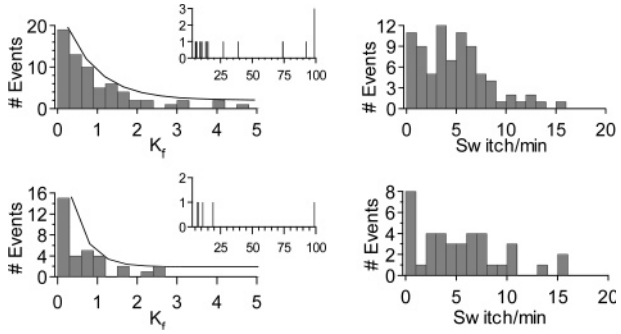

Figure 2. Distribution of K_{fold} (bin size = 0.3) and SA (switch/min, bin size = 1) for the 81 hexamer (top) and 40 trimer (bottom) molecules showing spectral switching. Solid lines are offset exponential fits. Insets: continuation of K_{fold} showing large, but rare, values.

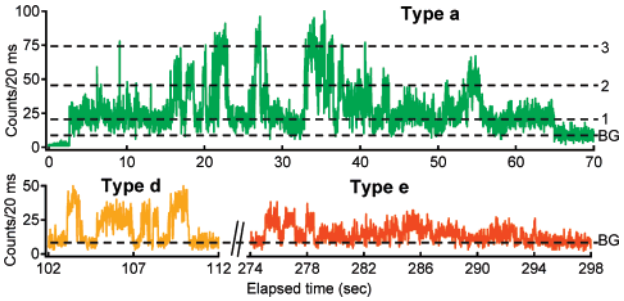

Figure 3. Trimer time trace collected simultaneously with spectra showing three characteristic intensity regions and their corresponding spectral types. "Type a" emission showed multilevel (1,2,3) intensity jumps above background (BG). Hexamer showed similar behavior.[3]

and SA values. While K_{fold} and SA are limited in resolution by the 2 s integration time, they provide insight into both equilibrium and kinetics, respectively, of folding. The expected exponential decay in the K_{fold} distribution indicates no preference for a particular equilibrium under continuous excitation, supporting that folding and unfolding are photoinduced. A single photon ($h\nu$) excitation (58.6 kcal/mol) is sufficient to disrupt $\pi-\pi$ interaction energy, determined to be 2−7 kcal/mol in $C_2H_2Cl_4$.[2,10a,c] Spectral activity shows a prominent feature at 6 switch/min (0.1 Hz) and scales with excitation power,[3] further supporting a photoinduced model. This slow folding/unfolding process is probably due to local energy minima traps for intermediary folded states and is indicative of directed motion involving highly correlated non-Markovian dynamics (Scheme 1 and Figure 1f,g).[11]

Fluorescence time traces for both **3** and **6** at 20 ms bin size show distinct emission behavior corresponding to different unfolded/folded states. Figure 3 shows sections of a trimer time trace obtained simultaneously with spectra. Green (type a) emission shows three intensity levels, corresponding to an unfolded state where the three chromophores emit individually. Interestingly, cooperative jumps between two levels are observed, indicating that two perylene units are likely involved in a single photophysical process involving charge/energy transfer.[5] Multilevel cooperative intensity jumps are also observed for trimer and hexamer.[3] The folded states showing yellow fluorescence typically exhibit one intensity level at or slightly above the single green level, while folded states with red emission typically show lower variable intensity than the single green level. These results support the folding and unfolding model. In dilute solutions, where foldamers undergo a folding/unfolding dynamic,[2] fluorescence decays exhibit multiexponential behavior, with typical short 4.1-ns and long 10-ns lifetimes, corresponding to green (unfolded) and red (folded) emission, respectively. In the unfolded structures, quantum interactions between chromophores are not established, and thus each perylene diimide functions as an individual quantum emitter. In the folded structures, perylene stacks exhibit strong quantum coupling through $\pi-\pi$ molecular orbital overlaps, which could either yield a Franck−Condon factor reversal or develop into a delocalized exciton traveling across the π-stacks. As a result, the folded structures function as a single collective quantum emitter and yield only one characteristic in the time trajectories with one spectral shape. This is further supported by the appearance of photoblinking between on and off states in the yellow and red emission regions.

Our results have revealed unusual folding/unfolding dynamics. Additional kinetic analysis[3] suggests the existence of multiple pathways occurring at variable rates between folded and unfolded states. The fact that we have observed distinct behavior from **1**, **3**, and **6** indirectly proves that we are monitoring single molecules, not aggregates. Thus, single molecule spectroscopy is a powerful tool and chromophoric foldamers are informative systems to provide a wealth of information on fundamental mechanisms of folding.

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Supporting Information Available: Experimental details, ensemble spectra, spectral trajectories, time traces, power dependence data, and kinetic analysis (PDF, AVI). This material is available free of charge via the Internet at http://pubs.acs.org.

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