# To Fold or to Assemble?

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Folding (intramolecular association) and self-assembly (intermolecular association) play vital roles in molecular science.[1−4] Indeed, folded proteins and their assemblies can accomplish remarkable functions including catalysis,[5] DNA repair,[6] and information translation.[7] When driven by the same force, will an oligomer fold or assemble first? In this report, we describe a class of oligomers, which consist of rigid chromophores linked by flexible ethylene glycols. Diagnostic changes occur in ${}^1$H NMR, UV−vis, and fluorescent spectra when the chromophores come into close contact.

Scheme 1 summarizes the general strategy for synthesizing oligomers capable of folding and self-organization. First, we monobenzoylated bis-*N,N*-(2-(2-(2-(2-hydroxyethoxy)ethoxy)ethoxy)-ethyl)perylenetetracarboxylic diimide (HPTD) to form a chain anchor (**1a**) that permits extension at only one hydroxyl group. Second, we synthesized the building block of HPTD with one hydroxyl group protected with dimethoxyl trityl (DMTr) and the other hydroxyl group activated with phosphoramidite (**1b**). Thus, we used the building block (**1b**) to attack the monobenzoylated anchor (**1a**) to synthesize a dimer. The DMTr group was then removed by hydrolysis to generate the new hydroxy group necessary for chain extension (**2**).[8] Using this method of coupling followed by detritylation, we have prepared specific oligomers including dimer (**2**), trimer (**3**), tetramer (**4**), pentamer (**5**), and hexamer (**6**).

In dilute solutions (<1 mM), compound **1** exists as mostly "free" monomer. The oligomers **2−6**, however, exist as "free" folded structures as evidenced by the upfield shift of the perylene aromatic proton resonances (Ha and Hb). The chemical shift separation ($\Delta\delta$) between Ha (four outer protons) and Hb (four inner protons) is a reliable indicator of folded structures versus nonfolded structures. The larger $\Delta\delta$ values observed for the folded structures arise because the inner proton (Hb) experiences a larger ring-current than the outer proton (Ha) when the two aromatic rings are $\pi$-stacked. For free monomer (**1**), $\Delta\delta$ is 0.061 ppm, whereas, the $\Delta\delta$ values for dimer, trimer, tetramer, pentamer, and hexamer are 0.26 (Figure 1a), 0.33, 0.38, 0.38, and 0.38 ppm, respectively, confirming that they all exist as folded structures.

Strong exciton−phonon coupling was observed in the folded nanostructures as the absorption maximum blue shifts by 0.17 eV from 0→0 transition to 0→1 transition (Figure 1b).[9] The relative intensities of the vibronic bands are governed by the Franck−Condon factors. The intensity reversal indicates the optimum overlap has shifted from $\langle\chi'_{v'=0}|\chi_{v=0}\rangle$ in free monomers to $\langle\chi'_{v'=1}|\chi_{v=0}\rangle$ in the folded structures, where $\chi$ and $\chi'$ are ground- and excited-state vibronic wave functions. The strong exciton−phonon coupling indicates that the HPTD molecules adopt mostly eclipsed structures with interplanar distance of ∼3.38 Å; this value is below van der Waals contact, suggesting molecular $\pi$-orbital overlaps.[9d]

Excitation of eclipsed stacks of perylenetetracarboxylic diimide could generate both molecular (Frenkel) and charge transfer (CT)

**Scheme 1** ${}^a$



$R = -CH_2CH_2CN$
$Bz = -COC_6H_5$

**dimer (2)**       **hexamer (6)**
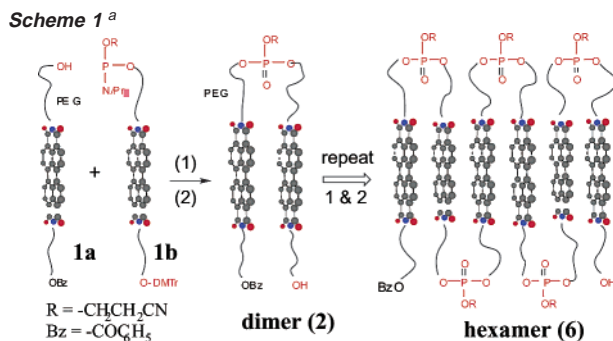
${}^a$ Conditions: (1) $CH_2Cl_2$/N-Ph-IMT, rt; Then $I_2$ ($CH_2Cl_2$/Py/$H_2O$; 1:3:1), rt. (2) $CHCl_2CO_2H$/ $CH_2Cl_2$, rt. Yield for **1** and **2**: ∼74%.
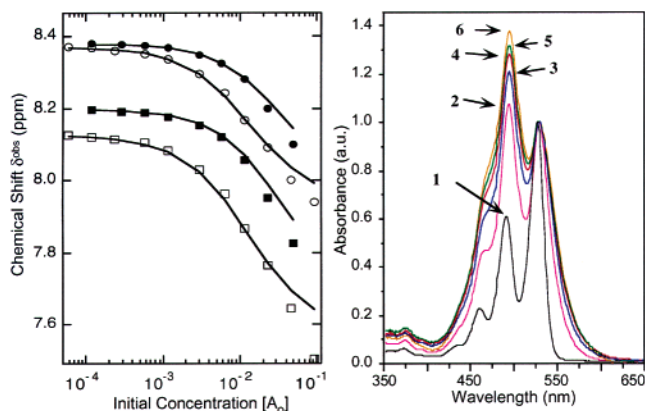


**Figure 1.** (a) Observed (Ha: circles; Hb: squares) and theoretical (solid lines) chemical shifts for dimer **2** in TCE (filled) and CHCl₃ (open). (b) Absorption spectra of free monomer **1** and folded oligomers **2-6** at 6.6 $\mu$M monomeric units in CHCl₃ with the 0 → 0 transition normalized.

states coupled to a molecular vibration. If there were Frenkel and CT excitons, the mixing of Frenkel−CT excitations must be small because unlike solid thin films well-resolved vibronic bands are observed in the folded oligomers. Thereby, the broadening of the absorption peaks may be due to exciton coupling and mixing within the folded nanostructures.[9]

In most solvents, oligomers **2−6** exist as folded structures. In 1,1,2,2-tetrachloroethane (TCE), however, we found a minor amount of unfolded species of the dimer (**2**). Since folded and unfolded species are exchanging rapidly, the equilibrium constant $K_{fold}$ = 6.1 was determined using eq 1 and the low concentration data (<1 mM, Figure 1a).[10] Similarly, $K_{fold}$ = 6.2 was estimated from the intensity ratio of 0→0 and 0→1 transitions from the electronic absorption spectra (Figure 1b).

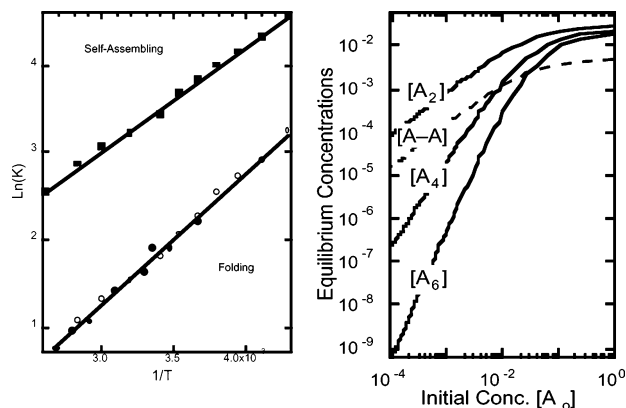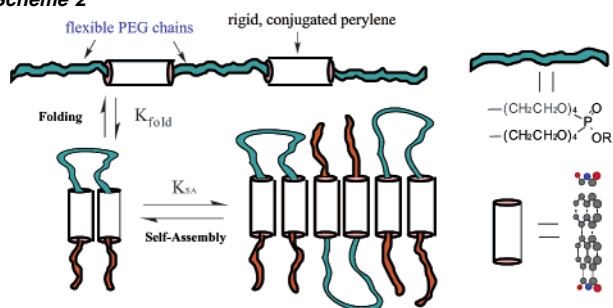$$K_{fold} = \frac{\delta_1 - \delta_{obs}}{\delta_{obs} - \delta_2} \qquad (1)$$

**Figure 2.** (a) Plot of experimental folding $K_{fold}$ (open circles: NMR data; solid circles: UV−vis data) and self-assembling $K_{SA}$ (solid squares) constants (TCE) against $1/T$, which yields comparable enthalpies. (b) Theoretical prediction of unfolded dimer [A−A], folded dimer [A$_2$], and dimer-assembled tetramer [A$_2$:A$_2$] and hexamer [A$_2$:A$_2$:A$_2$].

**Scheme 2**



As the oligomer concentrations increase ([A$_o$] > 1 mM), the Ha and Hb resonance peaks shift further upfield. The same forces that drive the oligomer to fold now drive aggregation into larger nanostructures (Scheme 2). Therefore, we expect these two processes to have similar enthalpies, and the difference between folding and self-organization should be largely due to entropic contributions.

The self-assembly formation constant ($K_{SA}$) can be determined by solving an oversimplified equal-$K_{SA}$ power series using observed chemical shift ($\delta_{obs}$) at various concentrations (Figure 1a).[11] Fitting this power series (solid lines in Figure 1a) to the experimental data, we obtained $K_{SA} = 31$ M$^{-1}$ in TCE and $K_{SA} = 90$ M$^{-1}$ in CHCl$_3$ at 20 °C. Figure 2 shows the van't Hoff plots [ln K = $\Delta S°/R -\Delta H°/(RT)$] of the variable temperature UV−vis and $^1$H NMR data for both the folding ($K_{fold}$) and the self-assembling ($K_{SA}$) processes. As expected, enthalpies for folding and self-assembling are comparable with $\Delta H°_{fold} = -3.01 \pm 0.06$ kcal/mol and $\Delta H°_{SA}$ (dimer) $= -2.44 \pm 0.12$ kcal/mol. This indicates both folding and self-assembling of HPTD molecules are exothermic processes in organic solvents. Self-organization requires more ordering; therefore, it should have a larger entropy change than that of folding into similar final structures. Experimentally, we observed $\Delta S°_{fold} = -6.60 \pm 0.40$ cal/mol·K and $\Delta S°_{SA}$(mono) $= -17.5 \pm 0.7$ cal/mol·K,[11] indicating that folding is favored by entropy contribution.[12]

As the foldable oligomers become larger and absorption blue-shifts to 500 nm (0→1), the photoluminescence favors emissions

to higher vibronic ground states (0 → v, v = 0, 1, 2, 3), thereby red-shifting the spectra. As the concentration increases (>1mM), the folded molecular structure further assembles and intensifies the red emissions (0→3) at the expenses of decreasing green (0→0) and yellow (0→1) emissions. Although spectroscopic features may vary in detail, the trimer or higher oligomers predominantly emit red color (see the graphic in the Table of Contents).

In summary, folding dominates at low concentrations (<~1 mM); it precedes self-assembling at slightly higher concentrations (~1−100 mM). Although both are driven by the same enthalpy effect, the difference is due to "high" local concentration of foldable units within an oligomer. At very high concentrations, our model predicts that self-assembling could precede folding, giving rise to a completely different aggregate morphology; however, one cannot investigate this due to solubility limitations.

**Supporting Information Available:** Synthetic procedures of **1**−**6**, $^1$H NMR shifts, fluorescence spectra, and UV−vis model for $K_{fold}$ (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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(10) Here, $\delta_1$ is the chemical shift of the free monomer or unfolded dimer, $\delta_2$ is the chemical shift of the folded dimer, $\delta_{obs}$ is the observed chemical shift in rapid exchange between folded dimer and unfolded dimer.

(11) The theoretical model is described, and the entropy is calculated in Wang, W.; Li, L.-S. Han, J. J.; Wang, L−Q.; Li, A. D. Q. Manuscript submitted.

(12) Folding processes: A−A ↔ A$_2$, $\Delta S°_{fold} = S(A_2) - S(A−A)$; SA processes: A + A ↔ A: A, $\Delta S°_{SA} = S(A:A) - 2S(A)$. Assume $S(A_2) \approx S(A:A)$, then $\Delta S°_{fold} - \Delta S°_{SA} = 2S(A) - S(A−A) > 0$. Thus, $\Delta S°_{fold} > \Delta S°_{SA}$.

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