1. INTRODUCTION

Motivation & Background

• Dye aggregates, which are most readily identified by spectral shifts in their absorption spectrum relative to the isolated dye,[1-3] can exhibit interesting photophysics including small Stokes shifts, superradiance, and long-range coherent energy transport.[4-6]

• Templated assembly of dye aggregates via covalent attachment of dye molecules to a DNA scaffold (DNA templating) is a promising strategy for controlled dye aggregation.[7-11]

• We studied the excited state dynamics of three DNA templated Cy5 structures: a monomer, a dimer, and a tetramer (See Figure 1).[12]

• In our primary poster, we report the extracted fluorescence emission spectrum of the J-dimer species, as well as the contribution of nonradiative decay to the overall relaxation dynamics of the aggregate structures.

• This poster presents additional details of the emission spectrum extraction, which accounts for the presence of two subpopulations in the J-dimer solution.

• Additional detail regarding the estimation of nonradiative decay in the aggregates is also presented.

• In both cases, the expected photophysical properties of small dye aggregates is leveraged to place bounded estimates on photophysical quantities which cannot be directly measured.

2. IDENTIFICATION OF MONOMER SUBPOPULATION

Figure 2. (Left) Fluorescence emission spectra (solid lines) and visible absorption spectra (dashed lines) of the Cy5 monomer (green), J-dimer (red), and H-tetramer (blue) solutions. (Right) Fluorescence excitation spectra of the aggregate (green), J-dimer (red), and H-tetramer (blue) solutions. (Middle) The J-dimer results from the hybridization of the monomer structure with its Cy5 labeled complement. (Right) The H-tetramer forms at high dye concentration through the association of two J-dimer structures.[9]

• The emission spectra of the Cy5 aggregate solutions (Figure 2, left) resemble the Cy5 monomer emission spectrum and lack mirror symmetry with their respective absorption spectra, possibly suggesting a monomer subpopulation.

• A Cy5 monomer subpopulation was confirmed through fluorescence excitation measurements of the aggregate solutions (Figure 2, right).

• The J-dimer and H-tetramer fluorescence excitation spectra strongly resemble the monomer absorption spectrum, indicating that the Cy5 monomer is the primary emissive species in the aggregate solutions despite their low concentration.

3. IDENTIFICATION OF H-AGGREGATE SUBPOPULATION IN J-DIMER SOLUTION

Figure 3. (Left) Absorption spectra collected on a J-dimer solution (red) above the denaturation temperature, and just below the denaturation temperature (black). (Right) Absorption spectra of the J-dimer collected at temperatures between -5 °C (dark blue) and 70 °C (red). Reproduced from ref [44,49,52] with permission.

• A solution of J-dimer was heated to 70 °C to denature the duplex.

• Upon cooling, an absorption feature at 597 nm appears just below denaturation temperature (54 °C), while the strongest absorption feature at 666 nm of the room temperature J-dimer solution is absent.

• Below the DNA denaturation temperature, the 597 nm and 666 nm absorption peaks change counter to one another under varied temperature.

• The spectra collected below the denaturation temperature exhibit isosbestic points at 604.5 and 610 nm.

• The presence of a new band at 597 nm and the presence of isosbestic points indicate the presence of an additional small H-aggregate subpopulation in the solution.

4. J-DIMER EMISSION SPECTRUM EXTRACTION

Figure 4. The emission spectrum which resulted from the extraction procedure. The half width at half max (HWHM) of the J-dimer origin band was used along with the constraint that the whole spectrum must be positive to fix the value of $\Phi_{\text{J}}$.

• The spectrum collected below the denaturation temperature exhibit isosbestic points at 604.5 and 610 nm.

• The presence of a new band at 597 nm and the presence of isosbestic points indicate the presence of an additional small H-aggregate subpopulation in the solution.

• For the three-component J-dimer solution, the equation for fluorescence quantum yield, $\Phi_{\text{TOT}}(\lambda)$, has three terms:

$$\Phi_{\text{TOT}}(\lambda) = \Phi_{\text{M}}(\lambda) + \Phi_{\text{J}}(\lambda) + \Phi_{\text{H}}(\lambda)\Phi_{\text{F,J}}$$

Equation 1. Expression for the fluorescence quantum yield of the three-component J-dimer solution. $\Phi_{\text{TOT}}(\lambda)$ is the absorbance at wavelength $\lambda$ of solution component n; $\Phi_{\text{TOT}}(\lambda)$ is the total absorbance of the solution at excitation wavelength, $A_{\text{ex}}$; $\Phi_{\text{M}}(\lambda)$ is the fluorescence quantum yield of solution component m; and $\Phi_{\text{F,J}}$ is the fluorescence quantum yield of the solution at excitation wavelength, $A_{\text{ex}}$.

• By making the assumption that the H-aggregate component is nonfluorescent ($\Phi_{\text{H}} = 0$), the expression above can be solved with a single fitting parameter, $A_{\text{J}}$.

• Because $\Phi_{\text{M}}$ and $\Phi_{\text{J}}$ can be measured directly, a narrow bounded approximation of $\Phi_{\text{H}}$ can be made based on the monomer radiative rate (see part 5).

• $A_{\text{J}}$ was allowed to float in order to solve for $A_{\text{J,which}}$ which was used to scale and subtract a monomer emission spectrum from the J-dimer solution emission spectrum.

• A realistic J-dimer emission spectrum (all positive, similar peak width to J-dimer absorption spectrum) emerges from the subtraction when $A_{\text{J}}$ is 49% of $A_{\text{TOT}}(\lambda)$.

5. ESTIMATION OF $K_{\text{NR}}$ AND $K_{\text{F,J}}$ FOR J-DIMER

Figure 5. Measured (solid red) and expected (dashed black) excited state kinetics for the J-dimer. The Cy5 monomer kinetic (solid green) is approximated by time resolved photon counting (TRPC) forms the upper bound of the expected J-dimer kinetics. The lower bound (red dashed line) with a time constant of 1 ns represents the background kinetics similar to superradiance only.

• More rapid decay beyond the shaded region in Figure 5 can only be accounted for by an increase in the nonradiative relaxation rate relative to the Cy5 monomer upon aggregation.

• The ground state recovery rate of the J-dimer was nearly 100-fold faster than expected, indicating that its relaxation is almost entirely nonradiative, a conclusion that is further supported by the low fluorescence measured from J-dimer solutions relative to the monomer.

• Table 1 provides a summary of the photophysical properties of the monomer, J-Dimer, and H-Tetramer, including a quantitative evaluation of the extent to which nonradiative decay contributes to the overall decay.

Table 1. Fluorescence Quantum Yields, Overall Lifetimes, and Overall, Radiative, and Nonradiative Decay Rates for Cy5 Monomer, J-Dimers, and H-tetramers

<table>
<thead>
<tr>
<th>Sample</th>
<th>$\Phi_1$</th>
<th>$\tau_{\text{M}}$ (ns)</th>
<th>$\Phi_2$ (s⁻¹)</th>
<th>$\tau_{\text{2}}$ (ns)</th>
<th>$\Phi_3$ (s⁻¹)</th>
<th>$\tau_{\text{3}}$ (ns)</th>
<th>$\Phi_{\text{NR}}$ (%)</th>
</tr>
</thead>
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<tr>
<td>Monomer</td>
<td>0.29</td>
<td>1.3</td>
<td>7.69 × 10⁴</td>
<td>2.23 × 10³</td>
<td>5.46 × 10⁵</td>
<td>71.99</td>
<td>0.0</td>
</tr>
<tr>
<td>J-dimer (N = 1.5)</td>
<td>N/A</td>
<td>0.011</td>
<td>9.09 × 10⁴</td>
<td>3.34 × 10³</td>
<td>9.06 × 10⁵</td>
<td>99.65</td>
<td>0.0</td>
</tr>
<tr>
<td>H-tetramer</td>
<td>N/A</td>
<td>0.035</td>
<td>2.86 × 10⁴</td>
<td>1.12 × 10³</td>
<td>2.86 × 10⁴</td>
<td>99.96</td>
<td>0.0</td>
</tr>
</tbody>
</table>

6. REFERENCES & ACKNOWLEDGMENTS

References


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