QM/MM Simulations of the Fluorescence Quenching of *H*-Type Homodimers of Fluorescein and Tetramethyl Rhodamine

Paul A. Johns[†], Patrik R. Callis[‡], and Pedro L. Muíño[†]

[†]Department of Chemistry, Mathematics and Phys. Sci., Saint Francis University, Loretto, PA 15940

[‡]Department of Chemistry and Biochemistry, Montana State University, Bozeman, MT 59717

ABSTRACT

Fluorescent xanthene dyes used as probes in biological systems tend to undergo fluorescence quenching when at a high concentration in an aqueous environment. This phenomenon is believed to be caused by the formation of dye aggregates, and results in an often drastic decrease in fluorescence signal. Although exciton splitting, which creates a quasi forbidden fluorescing state, is most often invoked as the root of the loss of fluorescence, it is unclear that this effect could lead to the extreme quenching usually seen. Vibronic coupling to the higher strongly allowed exciton state should provide considerable emitting strength. Here, using hybrid quantum mechanical-molecular mechanical (QM-MM) models of explicitly solvated sandwich homo dimers of fluorescein and tetramethyl rhodamine (TMR), we investigate the possibility that fluctuations in the water environment could stabilize preferential electron transfer between seemingly identical molecules.

This method has previously been successfully applied, in this laboratory, to fluorescence quenching of tryptophan. During 50 ps molecular dynamics trajectories in which the charge density was simulating the fluorescent state, we found that the local solvation almost always favored electron transfer to one of the identical molecules. Although the mean separation between S_1 and the lowest charge transfer (CT) state was 5000 cm^{-1} (0.62 eV), large fluctuations in CT state energy suggested that the two states could become degenerate, thereby facilitating electron transfer. Simulation of a CT event by moving an electron from one molecule to another caused the CT state to be stabilized by 2 eV within 100 fs. This suggests that quenching would be complete. At this point in our study, electron transfer appears to be a likely mechanism for concentration quenching. The effect is more pronounced for fluorescein when compared to TMR.

Exciton Theory & Charge Transfer Theory

Exciton theory says that the lowest state in which excitation is shared by the two molecules has canceling transition dipoles and leads to a forbidden emission process. (*E'* in Figure 1)[1]. While theoretically forbidden, transitions from these energy states are allowed under certain circumstances, including degeneracy. This would lead to reduced, but not total absence of, fluorescence, if other non-radiative pathways are not reduced by the dimer formation.

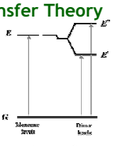


Figure 1

Electron transfer is a mechanism that involves one molecule with a very high occupied molecular orbital (the quencher) and a fluorescent molecule (the quenched molecule). In the fluorescent molecule, an electron becomes excited to a higher molecular orbital, leaving a hole in its place. By leaving this hole, an electron from the quencher is transferred to the lower energy orbital. Since the excited electron is unable to return to its ground state, fluorescence is not emitted, and the fluorescence is quenched, see Figure 2.

Why does electron transfer quench fluorescence?

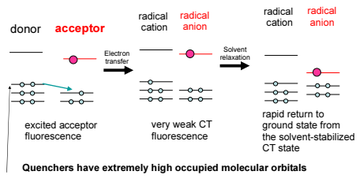


Figure 2

Why would electron transfer occur in homodimers?

If the molecular orbitals of the dyes are exactly the same, it would be expected that electron transfer could not occur.

Is the random fluctuation of the water molecules enough to promote electron transfer?

METHODS

- Create dimers spaced 5.00 Å and 3.75 Å as reported by Millié[2], and solvate in 20.0 Å of water (see Figure 3).
- Hybrid Molecular Dynamics (CHARMM) – ZINDO/S method
- Dye bond lengths are fixed at Charge Transfer (CT) state geometry. Solvent motion simulated using CHARMM
- T = 300 K, time interval 1 fs.
- Every 10 fs, coordinates are input in ZINDO/S, which then calculates the vertical transition energy, taking into account electrostatic effects
- Two excited states are tracked, the S_1 and the charge transfer (CT) state
- Information from ZINDO/S is then used in the next iteration
- For the first 50 ps of simulation, the charges in the dye molecules are those of the S_1 state. At t=50 ps, the values are switched, instantaneously, to those of the CT state

Solvated Tetramethyl Rhodamine Dimer

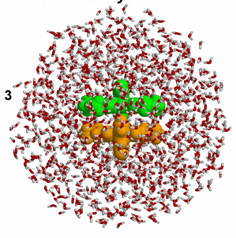


Figure 3

RESULTS & DISCUSSION

Figures 4 and 5 show the 60 ps trajectories that were generated for the fluorescein dimers in fixed parallel conformations located 5.00 and 3.75 Å apart respectively.

As discussed in the Methods section, the first 50 ps of simulation correspond to dimers in their S_1 state. At t=50 ps, the atomic charges are switched to those of the CT state, thereby simulating the charge transfer event.

Comparison of Figures 4 and 5 to Figures 10 and 11 shows that the average size of the fluctuations does not depend on the separation between the component parts of the homodimers. However, the overall energy of the CT state is lower when the two molecules are spaced closer together. This is due to the lower energy that is needed for an electron to transfer a shorter distance.

In addition, the closer molecules result in a larger number of mixed states, as is shown by the large deviations (~10,000 cm^{-1}) from the average band of the CT state. The actual values for the energy of the mixed states lie within the average band of values.

The key factor in the stabilization of the CT state is the orientation of the water molecules. For any particular moment in time when the CT state is at a low point, one molecule in the dimer had a predominance of water hydrogen atoms pointed toward it, while the other was predominantly close to oxygen atoms. When this occurred, it allowed an electron to be transferred to the molecule that would be stabilized by a positive electric field generated by the partial charges in the water. (Figure 6).

This was also shown to be true even during unusual orientations of water (Figure 9). In this case, with the water forming a sheet around the electron donor, the most stabilizing waters were those that were further away. In any case, the orientation of the water molecules was still consistent with what had previously been noticed.

Figure 7 shows the 60 ps trajectory generated for fluorescein dimers that are free to move.

Comparing Figures 4 and 7, it can be seen that the fluctuations in the transition energy are much greater during the unfixed trajectories. (See Tables 1 and 2) It is thought that this occurs because the molecules are able to interact with each other more freely than when they were fixed. The fluctuations themselves occur due to the differences in the solvation coordinate over time. The lowest energy cases have more water molecules that stabilize the CT state, thus driving the energy lower.

Along the unfixed trajectories, there are points in which the dye molecules separate a great deal. This is obvious form Figure 8, which shows the relative position of the two monomers at the times indicated in Figure 7. The energy of the CT state increases monotonically with increased distance.

Figure 9 shows the fluorescein dianion dimer and selected solvent molecules. The picture on the left shows the location of the waters that are the largest contributors to the stabilization of the charge transfer state. This compares to the picture on the right, which shows the closest water molecules.

Figures 10 and 11 show the 60 ps trajectories for tetramethyl rhodamine dimers (again at separations of 5.00 and 3.75 Å.

The general findings regarding the S_1 – CT energy gap and the magnitude of the energy fluctuations are similar to those of the fluorescein dimers.

Vertical Transition Energy for the Fixed Dianion Fluorescein Dimer, 5.00 Å

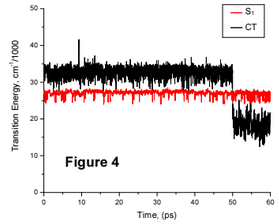


Figure 4

Vertical Transition Energy for the Fixed Dianion Fluorescein Dimer, 3.75 Å

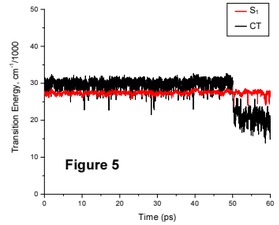


Figure 5

Fixed Fluorescein Dimer, 5.00 Å

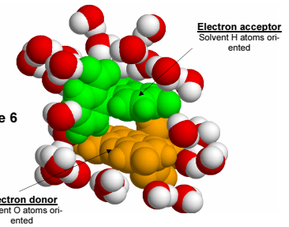


Figure 6

Vertical Transition Energy for the Unfixed Dianion Fluorescein Dimer, 5.00 Å

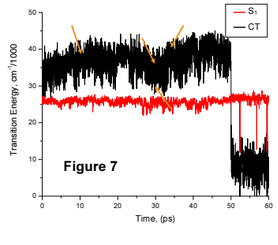


Figure 7

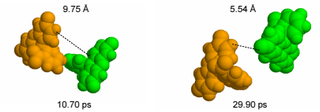
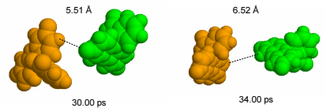


Figure 8



Unfixed Dianion, 5.00 Å, 59.6 ps

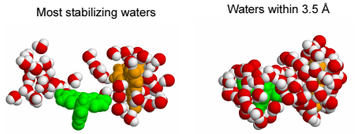


Figure 9

Fluorescein	Charge
Orange	-3
Green	-1

Vertical Transition Energy for the Fixed Zwitterion Tetramethyl Rhodamine Dimer, 5.00 Å

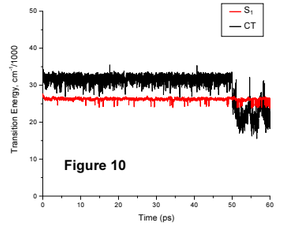


Figure 10

Vertical Transition Energy for the Fixed Zwitterion Tetramethyl Rhodamine Dimer, 3.75 Å

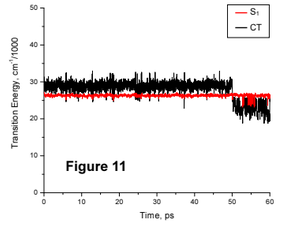


Figure 11

Table 1. Transition Energies and Relaxation Time for Fluorescein and Tetramethyl Rhodamine Dimers
(5.00 Å) (From Figures 4, 7, and 10)

		Fluorescein		Tetramethyl Rhodamine	
		Fixed	Unfixed	Fixed	Unfixed
$<E_{CT}>$ ($x1000cm^{-1}$)	S_1 charges	32.8	37.0	31.7	29.7
	CT charges	18.5	8.1	22.2	12.1
$<E_{S_1}>$ ($x1000cm^{-1}$)		27.1	25.8	26.2	24.3
$<\Delta E_{CT}>$ $S_1 \rightarrow CT$ charges	($x1000cm^{-1}$)	-14.3	-28.8	-9.5	-17.6
	kcal/mol	-41	-82	-27	-50
$<E_{CT}-E_{S_1}>$ kcal/mol	S_1 charges	16	32	16	15
	CT charges	-25	-51	-11	-35
Relaxation time (fs)		30	20	200	30

Table 2. Transition Energies and Relaxation Time for Fluorescein and Tetramethyl Rhodamine Dimers
(3.75 Å) (From Figures 5 and 11)

		Fluorescein		Tetramethyl Rhodamine	
		Fixed	Unfixed	Fixed	Unfixed
$<E_{CT}>$ ($x1000cm^{-1}$)	S_1 charges	30.0	35.3	29.1	27.1
	CT charges	21.2	6.5	24.2	17.6
$<E_{S_1}>$ ($x1000cm^{-1}$)		27.4	25.6	26.3	24.8
$<\Delta E_{CT}>$ $S_1 \rightarrow CT$ charges	($x1000cm^{-1}$)	-8.8	-28.8	-4.9	-9.5
	kcal/mol	-25	-82	-14	-27
$<E_{CT}-E_{S_1}>$ kcal/mol	S_1 charges	8	28	8	7
	CT charges	-18	-54	-8	-21
Relaxation time (fs)		40	20	160	80

Conclusions

- This is the first study, to our knowledge, that demonstrates that in aqueous solution, electron transfer as a quenching mechanism between ***identical*** dye molecules is physically possible
- **Random (thermal) fluctuations in the solvent field are responsible for the stabilization of the CT state that is key to the electron transfer quenching process.**
- This finding is not contradictory to exciton theory. Exciton effects are always present in dimers but are not expected to cause complete quenching that is often reported for dimers

Future Work

- Improve the topology and parameter files used for this study; those that were used were only rudimentary in the specifications for the molecules involved.
- Vary the initial angle at which the two molecules interact.

Acknowledgements

The authors thank the NSF REU program and Montana State University for providing funds for research and guest services.

We also thank Ryan Hutcheson for providing the fluorescein topology and parameter files and the initial optimized structure for fluorescein. He also assisted in creating the topology and parameter files for tetramethyl rhodamine.

References

- Bagh, S.; Paige, M. F. *J. Phys. Chem. A.* **2006**, *110*, 7057.
- Daré-Doyen, S.; Doizi, D.; Guilbaud, Ph.; Djedaïni-Pilard, F.; Perly, B.; Millié, Ph. *J. Phys. Chem. B.* **2003**, *107*, 13803.
- Ilich, P.; Mishra, P. K.; Macura, S.; Burhardt, T. P. *Spectrochim. Acta A* **1996**, *52*, 1323.
- Ajtai, K.; Burghardt, T. P. *Biochemistry* **1995**, *34*, 15943.
- Penzkofer, A.; Leupacher, W. *J. Lumin.* **1987**, *37*, 61
- Callis P. R.; Liu, T. *J. Phys. Chem. B.* **2004**, *108*, 4248.
- Ridley, J.; Zerner, M. *Theor. Chim. Acta.* **1973**, *32*, 111.
- MacKerell, A. D., Jr.; Bashford, D.; Bellott, M.; et al. *J. Phys. Chem. B.* **1998**, *102*, 3586.