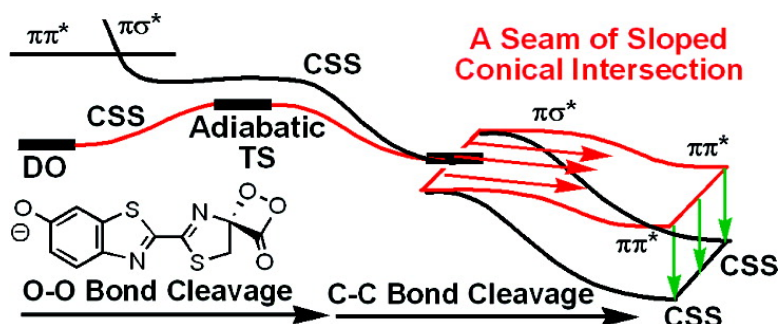


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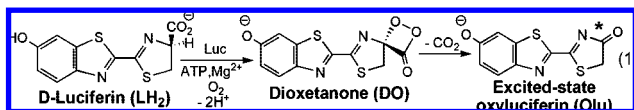
## Mechanism of Efficient Firefly Bioluminescence via Adiabatic Transition State and Seam of Sloped Conical Intersection

Lung Wa Chung,<sup>†</sup> Shigehiko Hayashi,<sup>‡</sup> Marcus Lundberg,<sup>†</sup> Toru Nakatsu,<sup>§</sup> Hiroaki Kato,<sup>§</sup> and Keiji Morokuma<sup>\*,†</sup>

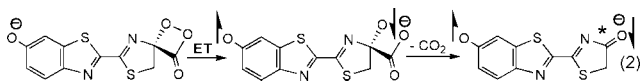
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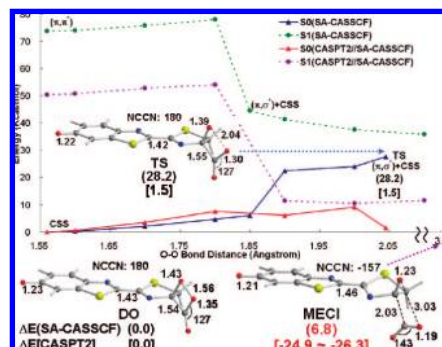
Firefly emission is a well-known efficient bioluminescence.<sup>1</sup> The recently revised quantum yield ( $\Phi_{\text{bi}}$ ) is 0.41.<sup>2</sup> Recently, X-ray crystal structures of Japanese firefly Luciferase (Luc) containing a high-energy intermediate analogue or oxyluciferin product were obtained by two of the authors.<sup>3</sup> The accepted course of firefly bioluminescence involves reaction of D-luciferin, ATP, and O<sub>2</sub> to give oxyluciferin in a singlet excited state, the assumed emitter, via the formation of D-luciferyl adenylate (not shown) and high-energy dioxetanone intermediate (DO) in the Luc (eq 1).<sup>1</sup>



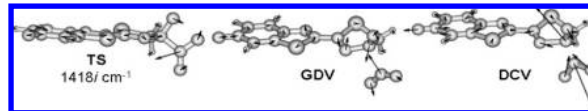
To account for chemiexcitation process in firefly, intramolecular chemically initiated electron-exchange luminescence (CIEEL) was proposed (eq 2).<sup>4a-c</sup> The CIEEL mechanism involving full electron-transfer has been questioned. A similar mechanism via charge-transfer was proposed.<sup>4d</sup> Recently, phenoxide-substituted dioxetanones were used to study theoretically the reaction mechanism of chemiluminescence and support charge-transfer mechanism by MRMP2//B3LYP and CASSCF//B3LYP methods.<sup>5</sup> In contrast, decomposition of simple dioxetanes or dioxetanones yield carbonyl compounds predominantly in a triplet excited state.<sup>6</sup> For these simpler systems, a diradical mechanism or, later, “merged” mechanism initiating with homolytic O–O bond cleavage was proposed<sup>6</sup> and supported by theoretical studies.<sup>7,8</sup> Although a sloped conical intersection (CI) is suggested to be a key to access the excited-state from the ground state,<sup>5,8,9</sup> the mysterious channel for the efficient thermal generation of singlet excited-state in firefly and nature of the channel are still unclear at the quantum level.



In this Communication, we report the first theoretical study of firefly bioluminescence from DO by using SA-CASSCF(12e,12o)/6-31G\* and CASPT2(12e,12o)/6-31G\*\*//SA-CASSCF(12e,12o)/6-31G\* methods.<sup>10</sup> The active space includes the reacting O–O and C–C  $\sigma/\sigma^*$  orbitals (4e/4o) and high-lying occupied  $\pi$  and low-lying unoccupied  $\pi^*$  orbitals (8e/8o) (Figures S1–S3 in Supporting Information (SI)). All calculations were performed by Molpro and Gaussian.<sup>10</sup> The computational details are given in SI. We propose that the adiabatic transition state (TS) for the O–O bond cleavage and unique topology around MECI (a minimum energy on the seam



**Figure 1.** Potential energy profile for the O–O cleavage on the S<sub>0</sub> and S<sub>1</sub> surfaces. Structures of DO, adiabatic transition state (TS), and minimum energy conical intersection (MECI) by the SA-CASSCF method are also shown, with distances, angles, and relative energies given in Å, deg, and kcal/mol, respectively.



**Figure 2.** Transition vector of TS, gradient difference, and derivative coupling vectors (GDV and DCV) of MECI.

of sloped conical intersection) play crucial roles in the efficient firefly bioluminescence.

As shown in Figures 1 and S4–S6, decomposition of the high-energy anionic intermediate DO starts with the O–O bond cleavage via TS. When the O–O bond is significantly elongated, the S<sub>0</sub> and S<sub>1</sub> surfaces become close in energy and the two electronic configurations, closed-shell singlet (CSS) and  $\pi, \sigma^*$ , are mixed (Figures S1–S2), indicating occurrence of an avoided crossing. Therefore, the O–O bond cleavage via TS is an adiabatic process on S<sub>0</sub>. Formally,  $\pi, \sigma^*$  configuration represents intramolecular electron-transfer from the  $\pi$ -conjugation moiety to the O–O  $\sigma^*$  orbital of the dioxetanone moiety (i.e., intramolecular CIEEL). Mulliken charge analysis shows that, from DO to TS, about 0.19 e is transferred from the phenoxide moiety to the dioxetanone moiety (Figure S7).<sup>4d,5</sup> Inclusion of dynamic electron correlation strongly stabilizes charge transfer. The relative energy of TS is about 1.5 kcal/mol at the CASPT2 method.<sup>11</sup> The reaction coordinate at TS involves the O–O bond cleavage coupled with one O–C bond formation and O–C–O bending (Figure 2).

When the C–C bond is considerably elongated, S<sub>0</sub> crosses with S<sub>1</sub>. CI plays a key role in photophysics and photochemistry.<sup>6a,8,12</sup> The MECI of the firefly dioxetanone DO was located and characterized for the first time (Figures 1, 3 and S3). The relative energy of MECI is about 6.8 and –24.9 to ca. –26.3 kcal/mol by

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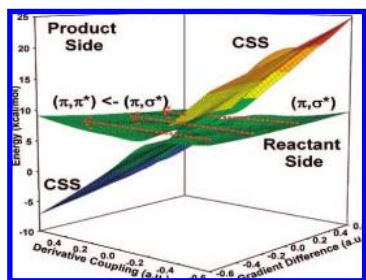


Figure 3. Topology around MECI.

the CASSCF and CASPT2//CASSCF methods, respectively. At MECI, the O–O bond is completely broken, the C–C bond is considerably elongated, and the central C2–C2' bond become slightly twisted.<sup>13</sup>

Gradients on  $S_0$  and  $S_1$ , and the two branching space coordinates of MECI (i.e., gradient difference vector (GDV) and derivative coupling vector (DCV)) are shown in Figures 2 and S8. Interestingly, these vectors essentially follow along intrinsic reaction pathway of the second step (the C–C bond stretching and O–C–O bending). Since the final reaction and the surface crossing are qualitatively in the same direction, the molecule should have high probability to encounter MECI without barrier (Figure S9). Similar gradients on  $S_0$  and  $S_1$  give rise to a sloped topology<sup>9</sup> for MECI as shown in Figure 3. The sloped topology of MECI is essential to efficiently access the electronic excited-state from the ground state.<sup>8,9</sup> Importantly, the two surfaces along one of the branching space coordinates (GDV) are very close in energy.<sup>14</sup> Thus, MECI can be regarded as a seam of sloped conical intersection (N-1-dimensional sloped seam instead of N-2-dimensional point).<sup>9a</sup> Therefore, during the C–C cleavage process of the firefly dioxetanones, a seam of sloped conical intersection emerges to provide a widely extended channel to diabatically access the excited-state surface from the ground-state surface, when the molecules have enough velocity<sup>15</sup> ( $\Delta E_{TS} - \Delta E_{MECI}$ : 20.7 and 26.4–27.8 kcal/mol by CASSCF and CASPT2//CASSCF methods, respectively). In comparison, the C–C cleavage process in  $S_0$  has a larger energy gap between  $S_0$  and  $S_1$ , and is lower in energy than chemiexcitation via MECI (Figure S10). Conformation change was also found in  $S_0$ ,<sup>5</sup> in which the oxyluciferin part becomes planar and the closed-shell singlet state is dominated. Hence, it should be a potential nonradiative decay channel in the gas phase, and should be partly suppressed by the protein.

Mulliken charges of DO and MECI in  $S_0$  indicate that more charge (0.45 e) is transferred to the dioxetanone moiety from the phenoxide moiety (0.30 e) in MECI (Figure S7), while charge transfer becomes modest in  $S_1$ . Similar to TS, the carboxylic group bears more negative charge in MECI.<sup>5,8</sup> Intramolecular charge transfer to the 4-m-r dioxetanone part not only reduces the barrier of the O–O bond cleavage,<sup>4a–c,5</sup> but also could render C–C bond cleavage along the  $S_0$  and  $S_1$  surface barrierless (Figures S10,11). Intramolecular charge transfer presumably results in similar gradients at the two states and small amplitude of GDV. The C–C bond cleavage process in  $S_0$  and  $S_1$  should, thus, be very fast so that the spin remains conserved. In contrast, for the case of the simple dioxetanes<sup>7</sup> or dioxetanone (Figures S12,S13), the C–C bond cleavage transition states for  $S_1$  and  $T_1$  can be located. The higher preference for the formation of triplet carbonyl compound over singlet excited-state product can be attributed to a higher energy of the C–C cleavage transition state for  $S_1$  than for  $T_1$ .<sup>7a,b,d</sup>

Potential surface along the C–C bond in  $S_1$  shows the energy difference between the two surfaces are quite similar

( $\Delta E_{CASPT2//CASSCF} = 48.0\text{--}48.7$  kcal/mol), when the C–C bond is almost broken (2.3–2.9 Å, Figure S11). It suggests that effect of closeness of CO<sub>2</sub> on the emission<sup>16</sup> in protein should be small.

In summary, we reported the first CASSCF and CASPT2//CASSCF study on the mechanism of the efficient firefly bioluminescence. Adiabatic transition state and unique sloped conical intersection seam, determined in the present work, are proposed to provide an efficient pathway to access the excited-state for the firefly bioluminescence. Effects of protein and excited-state molecular dynamics simulations are under study.

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**Supporting Information Available:** Complete ref 10, Figures S1–S14, computation details, results for the simplest dioxetanone, Cartesian coordinates, and absolute energies. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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