



Theoretical tuning of the firefly bioluminescence spectra by the modification of oxyluciferin



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ABSTRACT

Extending the firefly bioluminescence is of practical significance for the improved visualization of living cells and the development of a multicolor reporter. Tuning the color of bioluminescence in fireflies mainly involves the modification of luciferase and luciferin. In this Letter, we theoretically studied the emission spectra of 9 firefly oxyluciferin analogs in the gas phase and in solutions. Three density functionals, including B3LYP, CAM-B3LYP and M06-2X, were employed to theoretically predict the efficiently luminescent analogs. The reliable functionals for calculating the targeted systems were suggested. The luminescence efficiency, solvent effects, and substituent effects are discussed based on the calculated results.

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1. Introduction

The bioluminescence of fireflies is produced by luciferase catalyzing the oxidation of luciferin in the presence of ATP, Mg^{2+} and O_2 [1]. The light emitter of the firefly is the excited-state oxyluciferin (see Figure 1) [2,3], which has been extensively studied experimentally [4,5] and theoretically [4,6–10]. The bioluminescence of fireflies has been widely applied in various fields, such as biochemical analysis, gene expression, gene regulation, environmental monitoring [11–13] and especially in molecular imaging *in vivo* [14,15], which is of critical importance for understanding the underlying mechanisms of health, injury, aging and disease [16]. To detect signals from deep tissues [17,18], develop multicolor reporters [19], and concurrently detect more than one biochemical process [16], it is highly desired to tune the emission wavelength of the bioluminescence of fireflies.

To tune the color of firefly bioluminescence, modifications to both luciferase [12,19–26] and luciferin [4,17,18,27–33] have been made. Examples of luciferase modifications include the four luciferase mutants reported by Shapiro et al., two of which emit red light and two of which emit orange light. However, the four mutants' emission intensities, especially those of the mutants that emit orange light, are decreased compared to the intensity of the original luciferase [26]. Branchini et al. reported several types of mutated luciferases with luminescence maxima between 546 and 617 nm [19,24,25]. As an example of luciferin modification, Reddy et al. synthesized cyclic alkylaminoluciferins and compared their fluorescence wavelengths with the wavelength of the naturally bioluminescent luciferin [29]. Conley

et al. synthesized a luciferin analog by replacing the S atom of the thiazoline with an Se atom and observed a red-shift emission with regards to the naturally bioluminescent luciferin [18]. McCutcheon et al. synthesized two types of luciferin analogs by replacing benzothiazole and thiazoline with benzimidazole and imidazoline, respectively [32]. In addition, some engineered luciferin substrates have been recently reviewed [31,34]. Li et al. theoretically investigated oxyluciferin analogs bearing an amino group and replacing the S and N atoms of the benzothiazole [30,33]. Da Silva et al. considered the novel emitter 5-hydroxyoxyluciferin [35]. There are two reasons for the current theoretical study: first, to design new efficient oxyluciferin analogs based on the newly experimental syntheses [29,32], and second, to propose reliable functionals of density functional theory (DFT) for the targeted systems. The previously employed computational method, Becke's three-parameter hybrid functional [36] with the nonlocal correlation of Lee-Yang-Parr [37] (B3LYP), which does not perform well for charge-transfer excited states [38–41].

In this Letter, according to the structures of the synthesized firefly luciferin analogs [42], we designed corresponding oxyluciferin analogs in which the hydroxyl groups, the S atoms of the benzothiazole and thiazolone of the oxyluciferin were substituted (see Figure 2), and we theoretically studied their absorption and fluorescence spectra in the gas phase and in solutions using the time-dependent DFT (TD DFT) [43] with three functionals, including B3LYP, a long range corrected version of B3LYP using the Coulomb-attenuating method (CAM-B3LYP) [44] and a high-nonlocality hybrid functional with double nonlocal exchanges (M06-2X) [45]. We explored the influences of the substituents and solvent effects on the fluorescence wavelength and simultaneously considered the fluorescence efficiency.

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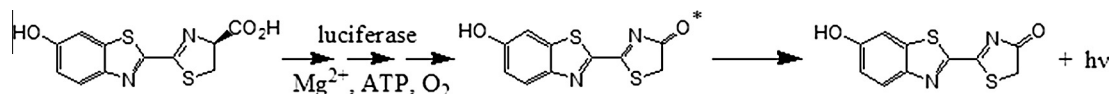


Figure 1. The brief process by which D-luciferin releases light.

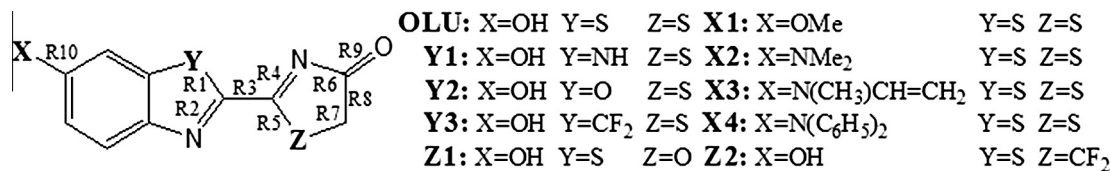


Figure 2. Structures of oxyluciferin and oxyluciferin analogs and the labels of the main bond.

2. Computational details

For comparison, the geometries of the ground and excited states, absorption maxima (λ_{\max}) and fluorescence wavelengths (λ_{F}) were optimized or calculated using three functionals: B3LYP, CAM-B3LYP and M06-2X. The vibrational analyses for the ground states at the same computational level have indicated that the currently located geometries are minima. B3LYP, which has been generally employed in previous calculations for oxyluciferin [30,33], underestimates the charge-transfer excitation energies [46,47]. CAM-B3LYP, a new long-range-corrected hybrid exchange-correlation functional proposed by Yanai et al. in 2004, performs well in predicting the charge-transfer excitations [44]. M06-2X, a new hybrid meta exchange-correlation functional presented in 2008, was recommended for applications involving main-group thermochemistry, kinetics, noncovalent interactions, and electronic excitation energies to the valence and Rydberg states [45].

The λ_{\max} and λ_{F} of **Y1** were predicted in the gas phase using TD CAM-B3LYP with the 6-31G**, 6-31+G**, 6-31++G** and 6-311++G** basis sets, respectively. The results indicate that the size of the basis set hardly affects the λ_{\max} and λ_{F} (see Table S1 in the Supplementary information). Considering that oxyluciferin analogs are conjugated systems and we focused on their excited states in this Letter, it is necessary to include a diffusion function [48] in the calculations. Hereafter, the predictions were performed with the 6-31+G** basis set.

Based on the calculated results for **OLU**, **X2** and **Y1**, M06-2X and CAM-B3LYP performed well, while B3LYP red-shifted the λ_{\max} and λ_{F} values, as expected. Therefore, the calculations on the remaining compounds were only performed using the CAM-B3LYP functional. Parts of the compounds were also studied in solutions. The solvent effects of DMSO, water and ethanol were modeled by the conductor-like polarizable continuum model (CPCM) [49] with dielectric constants of 46.8, 78.4 and 24.9, respectively. All of these calculations were performed using the GAUSSIAN09 program package [50].

3. Results and discussion

3.1. Ground and excited state geometries

In order to propose reliable DFT functionals for the targeted systems, the CAM-B3LYP, B3LYP and M06-2X functionals were employed. The optimized geometries of the targeted molecules by the three functionals are similar, and the solvent (DMSO, water or ethanol) does not significantly affect the optimized geometries in the gas phase (see Tables S2–S8 in the Supplementary information). The optimized geometries of the ground and excited states for the ten compounds at the CAM-B3LYP/6-31+G** and TD

CAM-B3LYP/6-31+G** computational levels in DMSO are summarized in Table 1. As shown in Table 1, all of the geometries are planar. Due to the differences in the radii of the substituent atoms, the R_1 values of **Y1**, **Y2**, and **Y3** and the R_5 and R_7 values of **Z1** and **Z2** are significantly different from the other values in the table. The variations in the geometries between the ground state and the excited state are very regular. For the ten compounds in the ground state, the R_3 value, which ranges from 1.450 to 1.461 Å, is clearly shorter than the conventional C–C single-bond length (1.540 Å). In the excited state, R_3 and R_6 are shortened and R_2 and R_4 are lengthened. These results indicate that excitation is related to R_2 , R_3 , R_4 , and R_6 , which is also confirmed by the molecular orbital analysis in the next section. The dipole moment (DM) of the ground and excited state are much different for the ten compounds, which indicates that the first excited states of these oxyluciferin analogs are strong charge-transfer states.

3.2. Molecular orbital analysis

The calculated frontier molecular orbitals are very similar at the B3LYP/6-31+G**, CAM-B3LYP/6-31+G** and M06-2X/6-31+G** levels and are also similar in the gas phase and in solutions (DMSO, water, and ethanol) (see Figures S1–S7 in the Supplementary information). The CAM-B3LYP calculated results are shown in Figure 3. According to Figure 3, a large overlap of the HOMO and LUMO is indicative of a high luminous efficiency. Due to the conjugation effect, R_3 and R_6 become shorter, while R_2 and R_4 become longer in the excited states.

Compared with the gas phase, the HOMO energy increases and the LUMO energy decreases in DMSO (see Figure S1 in the Supplementary information), which indicates that the energy gaps of the ten compounds are smaller in DMSO than in the gas phase. The substituents at the Y position affect both the HOMO and LUMO energies, those at the X position mainly affect only the HOMO energies, and those at the Z position mainly affect only the LUMO energies. The order of the ten compounds' energy gaps is **X4** > **X2**, **X3** > **Y3**, **Z2** > **X1** > **Y1** > **OLU** > **Y2** > **Z1**.

3.3. Absorption spectra in the gas phase and in solution

The λ_{\max} values and oscillator strengths (f) of **OLU**, **X2**, and **Y1** were calculated by TD B3LYP, TD CAM-B3LYP and TD M06-2X with 6-31+G** in the gas phase and in DMSO. The λ_{\max} and f values of the remaining compounds were calculated by TD CAM-B3LYP in the gas phase. As shown in Table 2, the λ_{\max} of **OLU** in DMSO is 371.4 nm, which is similar to the experimental value (377 nm). The TD CAM-B3LYP and TD M06-2X calculations predicted approximately the same λ_{\max} values, whereas, when TD B3LYP was used, the λ_{\max} values were red-shifted and the f values were decreased

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