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How Does the Local Electrostatic Field Influence Emitted Wavelengths and Bioluminescent Intensities of Modified Heteroaromatic Luciferins?

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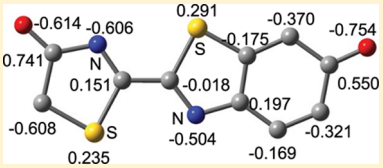
Supporting Information

ABSTRACT: The firefly chromophore, oxyluciferin, is in the pocket of the firefly luciferase and is surrounded by the side-chains of some amino acid residues. The charged residues produce the local electrostatic field (LEF) around the oxyluciferin. The emitted wavelengths and intensities of the oxyluciferin and its heterocyclic analogs under the LEF are examined. The common overlapping volumes of the HOMO and LUMO explain why the oscillator strengths vary under the LEF. Three average E_x change rates of the first excited energy are introduced to measure what luciferins are more sensitive to the LEF. The first excited energies and intensities in two enzymatic-like microenvironments are simulated via the LEF. The oscillator strengths and the net electric charges of the O6′ and the O4 are applied to explain the experimental bioluminescent intensities.



INTRODUCTION

Convenient, reliable, sensitive, and noninvasive bioluminescence (BL) imaging has become a powerful tool in cancer research due to its ability to monitor gene expression, enzyme activity, protein−protein interaction, cell trafficking networks, drug delivery, and tumor growth.[1] Firefly BL consists of two step reactions of firefly luciferin (LH_2), ATP, O_2, and Mg^{2+} catalyzed by firefly luciferase.[2] The luciferyl-adenylate and pyrophosphate are first produced, then the excited-state of the oxyluciferin ($OxyLH_2$) is generated, and its subsequent relaxation to the ground state releases photons of yellow-green light. The BL technology for multicomponent imaging needs more light-emitting luciferins and catalytic luciferases to provide different colors of bioluminescent light. Several natural luciferases are available in BL,[3] and nearly all utilize the same substrate, LH_2. Many experiments have focused on mutating luciferase enzymes, which can be carried out by the site-directed mutagenesis method.[4−8] Some efforts have been directed to produce caged luciferins,[9−14] for example, an aminoluciferin Cy5 conjugate,[12] but this type of modification changes the cellular uptake of the substrate and its biodistribution *in vivo*.[15]

Recently, several groups have synthesized heterocyclic analogs of LH_2,[15−17] and these analogs emit different colors of light with the firefly *Photinus pyralis* luciferase (PpyLuc)[18] and other luciferases.[3] Owing to a lack of rapid and reliable syntheses for these functionalized molecules, it is not easy to produce the heterocyclic analogs of the LH_2 experimentally,[16] so it is desirable to study more new analogs of the LH_2 computationally.[19−22] The firefly chromophore, LH_2, is in the pocket of the firefly luciferase and is surrounded by the side-chains of some amino acid residues. The charged residues produce a local electrostatic field (LEF) around the LH_2.[23−26] In BL, the mutant luciferases can cause the color modulation of the LH_2 by inducing a different LEF.[23−26] Because the absorption and scattering of light by tissue result in strong attenuation of BL signals emitted below 600 nm, one needs emitters that can emanate red or near-infrared (RNIR) light with measurable intensity. To search for new heterocyclic analogs of the LH_2 emitting a RNIR light with detectable intensity, we need to examine luciferins in two aspects. The first is how the LEF shifts the emission colors of the LH_2 and its analogs toward RNIR direction and what luciferines are more sensitive to the LEF. With the LEF sensitive luciferins, one can get a RNIR emitting light by site-directed mutated luciferase easily. The second is what features of luciferins can cause high intensity of the emitting light and whether the LEF can increase the photon flux of light. By replacement of N3, S1, S1′, and N3′ at X_1, X_2, X_3 and X_4 position (Figure 1) by various elements, heterocyclic analogs of the LH_2 can be obtained.[15−17] The LEF

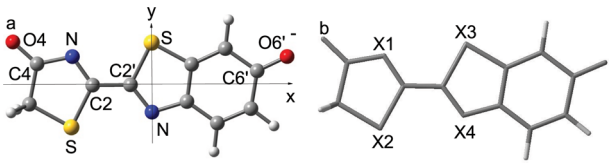


Figure 1. (a) The structure of the $OxyLH_2$. (b) When X_1 = N, X_2 = S, $OxyLH_2$, X_3 = S, X_4 = N; $OxyBiLH_2$, X_3 = NH, X_4 = N; $OxyBoLH_2$, X_3 = O, X_4 = N; $OxyBtLH_2$, X_3 = S, X_4 = CH.

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simulation can determine what amino acid residues in the PpyLuc should be substituted to produce the designed light colors. The net electrical charge distributions of luciferins are discussed in order to determine what features affect the bioluminescent intensities. The modulated emission spectra can be realized by modifying both luciferins and (latent) luciferases,[27,28] and one can get the optimal combination of the analog of the LH_2 and the mutant PpyLuc for the required light color and intensity. So far, the emitted wavelengths and intensities of the heterocyclic analogs of the $OxyLH_2$ under the LEF have not yet been examined. The linear response of the adsorption wavelengths to the LEF along the long-axis direction for the $OxyLH_2$ (Figure 1a) was discussed recently.[23,26] It would be interesting to check whether such linearity exists in the emission spectra.

In this contribution, we inspect the emission spectra of four luciferins under the LEF computationally. They are the $OxyLH_2$ and three others obtained by replacing the benzothiazole of the $OxyLH_2$ with the benzimidazole ($OxyBiLH_2$), benzoxazole ($OxyBoLH_2$), and benzothiophene ($OxyBtLH_2$), respectively (Figure 1). The geometries of the first excited states for the above four compounds under the LEF are optimized, and the responses of the first excited energies and oscillator strengths to the LEF along the *x*-, *y*-, and *z*-direction are examined. The energies of the highest occupied molecular orbits (HOMO) and lowest unoccupied molecular orbits (LUMO) are computed to interpret the changes of the first excited energies under the LEF. The common overlapping volumes of the HOMO and LUMO orbits are examined to explain why the oscillator strengths vary under the LEF. Three average E_x change rates of the first excited energy are introduced to measure which luciferins are more sensitive to the LEF. The average LEFs induced by the PpyLuc and the mutant R218Q PpyLuc are calculated to simulate two different enzymatic-like microenvironments. The first excited energies under these two LEF are evaluated and match the experimental results.[5,16] The net electric charges of the O6′ and the O4 under the LEF are computed in order to explain the bioluminescent intensities.

COMPUTATIONAL METHODS

The geometries of the ground states with the LEF were calculated by density functional theory at the B3LYP/6-311+G(d,p) level. The structures of the first excited states with the LEF were optimized by the TDDFT method.[29] Four density functionals (B3LYP,[30] CAM-B3LYP,[31] M06-2X,[32] and HCTH[33]) have been tested with the 6-311+G(d,p) basis set. To refine the first excited state energies, the SAC-CI method[34] with the basis set of 6-31G* was applied to calculate the emission energies for the optimized geometries obtained from the TDDFT. The HCTH functional is selected for the structure optimization of the first excited states because combined with the SAC-CI, the results of the HCTH functional best fit the experimental data.[15−17] The average LEFs induced by the PpyLuc and mutant R218Q PpyLuc were calculated to simulate the enzymatic-like microenvironment, and the emission spectra of the four compounds under two LEFs were computed.[35] To verify the methods used in the calculation, the solvant effect of DMSO was simulated by the conductor-like polarizable continuum model[36] with dielectric constant of 46.8, and the computational wavelength of the $OxyLH_2$ agreed with the experimental value[37] within 2.1%. The calculations were carried out using Gaussian 09.[38]

RESULTS AND DISCUSSION

As we know, the $OxyLH_2$ can take the keto and enol form, and the deprotonated keto(−1) form is one of the important light emitters in BL,[39,40] so we mainly focus on the keto(−1) form of the $OxyLH_2$ with the thiazoline and benzothiazole ring linked by the C2−C2′ bond (Figure 1). The analogs can be produced by replacing the benzothiazole ring by benzimidazole ($OxyBiLH_2$), benzoxazole ($OxyBoLH_2$), benzothiophene ($OxyBtLH_2$), benzofuran ($OxyBfLH_2$), and indole ($OxyInLH_2$).[16,17] When X1 = X4 = N, other analogs can be made by replacing X2 with N and X3 with NH.[17] If S1 in the thiazoline ring of the $OxyLH_2$ is substituted with Se, a red-shifted light emitter is synthesized.[15] Here we present the results of the $OxyLH_2$, $OxyBiLH_2$, $OxyBoLH_2$, and $OxyBtLH_2$. The $OxyLH_2$ (or its analogs) is in the pocket of the PpyLuc and is surrounded by the side chains of some amino acid residues. The charged residues create the LEF around the $OxyLH_2$. This protein pocket LEF can be treated as an effective external electric field imposed on the $OxyLH_2$.[23−26] Based on the structure of the PpyLuc (represent by the Arg218, His245, Phe247, Gly341, Leu342, Ser347, Ala348, and HOH45) and the AMP around the keto(−1) $OxyLH_2$,[7,40] we calculate the LEFs induced by the PpyLuc and AMP on the points that spread over the region where the oxyluciferin previously occupied (the $OxyLH_2$ is taken away). After a series of the LEFs on these points have been obtained, then we average the LEFs over these points. The resulting average LEF is (−134.7, 148.1, −180.2 mV/Å). Similarly, the average LEF induced by the mutant R218Q enzyme and AMP is (−36.7, 131.2, −182.9 mV/Å). Relative to the PpyLuc, the mutant R218Q luciferase introduces an extra positive electric field roughly along the *x*-direction (+98.0 mV/Å), which is consistent with the fact that the Arg218 carries a positive charge, while its substitute, glutamine, is neutral and sits on the right side of the O6′. From the value of the LEF induced by the microenvironment of the $OxyLH_2$, the emission spectra will be calculated under the LEF from −150 to 150 mV/Å along the *x*-, *y*-, and *z*-direction. This range of the protein pocket LEF does not conflict with the dielectric breakdown field of cell membranes.[41,42]

First, we optimize the structures of the first excited state under the LEF along the *x*-direction (long-axis of the molecule, see Figure 1) for the $OxyLH_2$, $OxyBiLH_2$, $OxyBoLH_2$, and $OxyBtLH_2$. The variation of the geometries under E_x can be reflected by the C2−C2′ bond length. When E_x varies between −150 and 150 mV/Å, this bond length decreases from 1.45 to 1.42 Å for the $OxyLH_2$ and $OxyBoLH_2$ and from 1.44 to 1.41 Å for the $OxyBiLH_2$ and $OxyBtLH_2$. For the $OxyLH_2$ and $OxyBoLH_2$, the first excited energies decrease when E_x runs from −150 mV/Å to 0, but they vary very little between 0 and 150 mV/Å (Figure 2a). For the $OxyBiLH_2$ and $OxyBtLH_2$, they descrease when E_x goes from −150 to 150 mV/Å. For the four compounds, their oscillator strengths, however, increase along the *x*-direction (Figure 2b). Because of the relaxation of the geometries of the first excited state, the first excited energies and oscillator strengths are not linearly dependent on the electric field strength along the *x*-direction, which is different from the linear adsorption response for the $OxyLH_2$.[23,26] Figure 2c,d shows that the first excited energies and the oscillator strengths along the *y*-direction change less in magnitude than those along the *x*-direction (the changes along *z*-direction are much less than along *x*-direction), so the most sensitive direction of the LEF is along the *x*-axis. This observation

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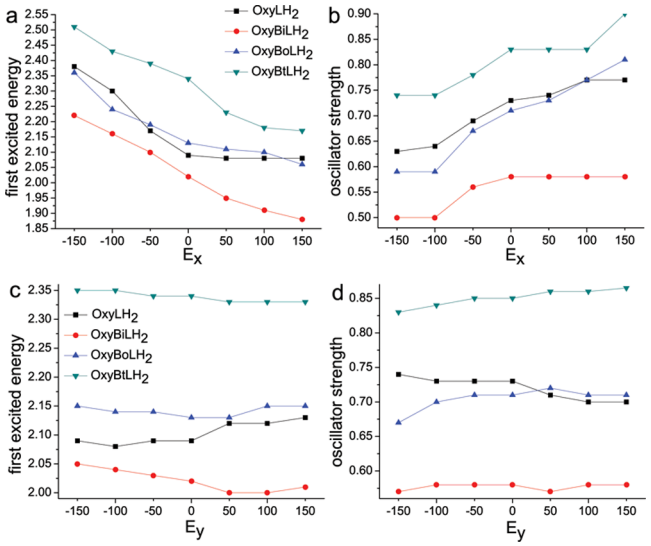

Figure 2. (a) The first excited energies (eV) as functions of E_x (mV/Å). (b) The oscillator strengths as functions of E_x. (c) The first excited energies as functions of E_y (mV/Å). (d) The oscillator strengths as functions of E_y.

indicates that when tuning light colors via mutagenesis, it would be more efficient to replace the amino acid residues that can produce the electric field mainly along the long-axis direction of the luciferins. To avoid the attenuation of the bioluminescent signals transmitting through tissue for *in vivo* BL imaging, red-shifted bioluminescent emission is required, so the mutant luciferase should provide a less negative LEF along the *x*-direction for the $OxyLH_2$. The mutant R218Q luciferase does add an extra positive electric field along the *x*-direction to that of the PpyLuc, and results in red-shifted light.[5] Figure 2a predicts that the $OxyBiLH_2$ can emit a near-infrared light when an extra positive LEF is added along the *x*-axis. Recent studies showed that the enol form of the $OxyLH_2$ play some roles in the BL.[26,43,44] The first excited energies and oscillator strengths of the $OxyLH_2$ in the keto(−1) and enol(−1) form as functions of E_x are compared in Figure 3. For both the keto and enol

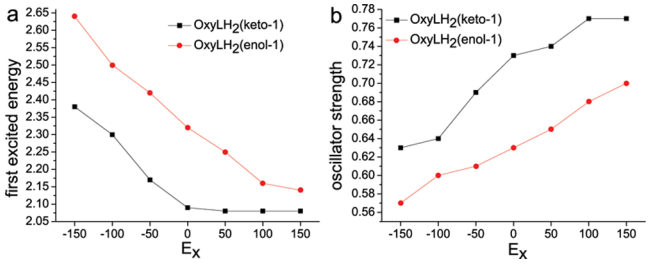

Figure 3. (a) The first excited energies (eV) of the $OxyLH_2$ in the keto(−1) and enol(−1) form as functions of E_x (mV/Å). (b) The corresponding oscillator strengths as functions of E_x.

form, the first excited energies decrease when E_x runs from −150 mV/Å to 0. The emission energy of the keto form changes very little, but that of the enol form decreases as E_x goes from 0 to 150 mV/Å. The emission spectrum of the phenolate-enol (−1) form (Figure 3a) indicates that it could be the intermediate state of the phenolate−enolate $OxyLH_2$.[43]

To estimate how sensitive to E_x the first excited energies are, we introduce the average E_x change rates of the first excited energy along the positive, negative, and whole *x*-direction defined as $\left.\frac{\Delta E_{ex}}{\Delta E_x}\right|_+ = \frac{E_{ex}(0) - E_{ex}(150)}{150}$, $\left.\frac{\Delta E_{ex}}{\Delta E_x}\right|_- = \frac{E_{ex}(-150) - E_{ex}(0)}{150}$, and $\frac{\Delta E_{ex}}{\Delta E_x} = \frac{E_{ex}(-150) - E_{ex}(150)}{300}$, where $E_{ex}(-150)$, $E_{ex}(0)$, and $E_{ex}(150)$ are the first excited energy at −150, 0, and 150 mV/Å. The values of $\left.\frac{\Delta E_{ex}}{\Delta E_x}\right|_+$, $\left.\frac{\Delta E_{ex}}{\Delta E_x}\right|_-$, and $\frac{\Delta E_{ex}}{\Delta E_x}$ for four compounds are listed in Table 1, where the maximum values are underlined.

Table 1. $\left.\frac{\Delta E_{ex}}{\Delta E_x}\right|_+$, $\left.\frac{\Delta E_{ex}}{\Delta E_x}\right|_-$ and $\frac{\Delta E_{ex}}{\Delta E_x}$ (eV·Å/mV) for Four Compounds

	$OxyLH_2$	$OxyBiLH_2$	$OxyBoLH_2$	$OxyBtLH_2$
$\left.\frac{\Delta E_{ex}}{\Delta E_x}\right\|_+$	6.67×10^{-5}	$\underline{9.34 \times 10^{-4}}$	4.67×10^{-4}	9.31×10^{-4}
$\left.\frac{\Delta E_{ex}}{\Delta E_x}\right\|_-$	$\underline{1.87 \times 10^{-3}}$	1.33×10^{-3}	1.60×10^{-3}	1.13×10^{-3}
$\frac{\Delta E_{ex}}{\Delta E_x}$	9.67×10^{-4}	$\underline{1.13 \times 10^{-3}}$	1.03×10^{-3}	1.03×10^{-3}

The average E_x change rates of the first excited energy along the positive *x*- and whole *x*-direction for the $OxyBiLH_2$ are the greatest. When E_x is along the negative *x*-direction, the average E_x change rate of the first excited energy for the $OxyLH_2$ is maximum. The benzimidazole ring in the $OxyBiLH_2$ undergoes rapid N−H isomerization (between X_3 = NH, X_4 = N and X_3 = N, X_4 = NH), which results in broadened ^1H and ^{13}C NMR signals.[16,17] Our calculation shows that the emission spectrum of the isomer of the $OxyBiLH_2$ (X_3 = N, X_4 = NH) is out of the visible light range, so it is inactive in visible light production. The rapid tautomerization suppresses the fluorescent quantum yield of the $OxyBiLH_2$. Due to the rapid tautomerization of the $OxyBiLH_2$, even though it is sensitive to the E_x, its bioluminescent intensity is smaller than that of the $OxyLH_2$. In the range of negative E_x, $OxyLH_2$ is the most sensitive to the LEF. The induced E_x by the PpyLuc is −134.7 mV/Å, and the corresponding E_x by the mutant R218Q luciferase is −36.7 mV/Å, which explains the red shift of the emitting light caused by the mutant R218Q luciferase.[5] When searching for new analogs of the $OxyLH_2$, one needs to find some analogs with larger values of $\left.\frac{\Delta E_{ex}}{\Delta E_x}\right|_+$, $\left.\frac{\Delta E_{ex}}{\Delta E_x}\right|_-$, or $\frac{\Delta E_{ex}}{\Delta E_x}$ (depending on the electric field range) to control the emitted wavelength feasibly. So with the help of these indices, one can get the optimal combination of a luciferin and a luciferase for the required emitting light colors.

As is well-known, the emission energies vary with changes in the energy levels of both HOMO and LUMO, and the emission intensity is controlled by the overlapping volume of HOMO and LUMO.[19,45−47] To understand how the first excited energies change with the electric field, the energy levels of the HOMO and LUMO for the four compounds in the presence of E_x are calculated and illustrated in Figure 4. When E_x increases, the energies of the HOMO increase at faster rates than those of the LUMO. To elucidate why the oscillator strengths change with the LEF, we consider the HOMO and LUMO orbitals of the $OxyLH_2$ in the presence of E_x. The HOMO and LUMO of the $OxyLH_2$ at E_x = 0 and E_x = −150 mV/Å are depicted in Figure 5. Comparing Figure 5, part a with part b (E_x = 0), one finds that the primary common overlapping volume of the HOMO and LUMO is the part of C2−C2′ region, which contributes to the oscillator strength. When the negative

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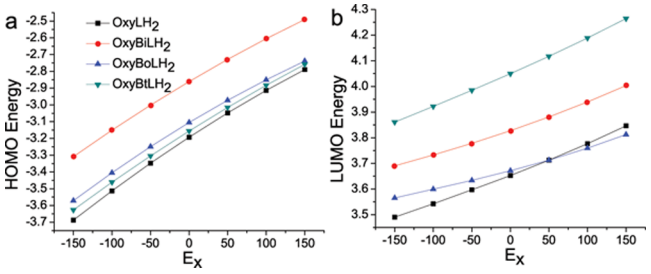

Figure 4. (a) HOMO energies (eV) as functions of E_x. (b) LUMO energies as functions of E_x.

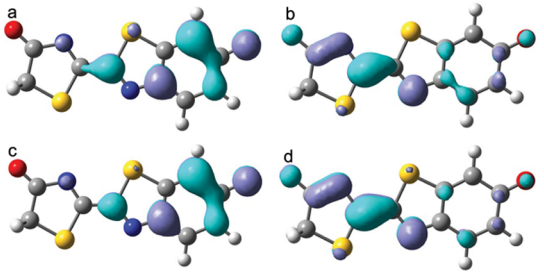

Figure 5. (a) HOMO of $OxyLH_2$ at $E_x = 0$. (b) LUMO of $OxyLH_2$ at $E_x = 0$. (c) HOMO of $OxyLH_2$ at $E_x = -150$ mV/Å. (d) LUMO of $OxyLH_2$ at $E_x = -150$ mV/Å.

electric field along x-axis is added, the electrons tend to move to $+x$-direction, and the part of the HOMO around the C2−C2′ bond shrinks (Figure 5c). Comparing Figure 5, part c with part d ($E_x = -150$ mV/Å), one can see that the overlapping volume of the HOMO and LUMO becomes smaller, so the oscillator strength at $E_x = -150$ mV/Å is less than that at $E_x = 0$, which is consistent with the observation that when E_x decreases, the oscillator strengths also decrease (Figure 2b).

In order to check whether the above results are reliable, the emitted excited energies, wavelengths, and relative fluorescent intensities for the $OxyLH_2$ and its analogs under the electric fields induced by the PpyLuc and the mutant R218Q enzyme are evaluated and listed in Table 2. In the microenvironment of the PpyLuc enzyme, the first excited energy differences between the computational and experimental values are 0.05 eV ($OxyLH_2$), −0.02 eV ($OxyBiLH_2$), 0.01 eV ($OxyBoLH_2$), and 0.06 eV ($OxyBtLH_2$) respectively,[16,17] which indicates that computational and experimental results match well and the above LEF simulation is reliable. In the microenvironment of the mutant R218Q luciferase, the difference between the computational and experimental excitation energy for the $OxyLH_2$ is 0.04 eV.[5] Again, the computational result is consistent with the experimental one. For the $OxyBiLH_2$, $OxyBoLH_2$, and $OxyBtLH_2$, we predict that their maximum wavelengths in the microenvironment of the R218Q enzyme are 641, 612, and 554 nm, respectively. It would be interesting for experimental groups to verify these computational results.

Table 2. First Excited Energies (eV), Emitted Wavelengths (nm) and Relative Intensities in the Microenvironment of the PpyLuc and the Mutant R218Q Enzyme

	$OxyLH_2$	$OxyBiLH_2$	$OxyBoLH_2$	$OxyBtLH_2$
$E^{theor}(R)$	2.28	2.14	2.24	2.43
$E^{exp}(R)$	2.23	2.16	2.23	2.37
$\lambda^{theor}(R)$	545	581	554	511
$\lambda^{exp}(R)^a$	557	574	557	523
$I_{Fl}^{theor}(R)$ (%)	100	84	84	87
$I_{Bl}^{exp}(R)$ (%)	100	14	0.9	70
$E^{theor}(Q)$	2.08	1.94	2.03	2.24
$E^{exp}(Q)$	2.04			
$\lambda^{theor}(Q)$	598	641	612	554
$\lambda^{exp}(Q)^b$	608			

[a]For the PpyLuc (R), the data are from ref 16. [b]For the mutant R218Q enzyme (Q), the data are from ref 5.

Table 2 reveals that the computational fluorescent intensities of four luciferins (I_{Fl}^{theor}) are close to each other. The experimental data show that the fluorescent intensities for the $OxyLH_2$, $OxyBoLH_2$, and $OxyBtLH_2$ are at the same order but that of the $OxyBiLH_2$ is about 28-fold lower than that of the $OxyLH_2$.[16,17] The big difference between the computational and experimental fluorescent intensity of the $OxyBiLH_2$ can be explained by the tautomerization. The rapid tautomerization suppresses the fluorescent quantum yield of the $OxyBiLH_2$.[16,17] The experimental bioluminescent intensities of the $OxyLH_2$, $OxyBiLH_2$, $OxyBoLH_2$, and $OxyBtLH_2$ (I_{Bl}^{exp}) are 100%, 14%, 0.9%, and 70%.[16] The difference between the computational fluorescent and experimental bioluminescent intensity comes from the fact that I_{Fl}^{theor} is directly related to the fluorescent quantum yield of the $OxyLH_2$ or its analogs (Φ_{Fl}) and I_{Bl}^{exp} corresponds to the bioluminescent quantum yield (Φ_{Bl}). Their relation is $\Phi_{Bl} = \Phi_{Oxy}\Phi_{ES}\Phi_{Fl}$, where Φ_{Oxy} is the yield of the $OxyLH_2$ or its analogs, Φ_{ES} is the formation of the first excited state, and Φ_{Fl} is the fluorescent quantum yield. In BL, besides Φ_{Fl}, one has to consider Φ_{Oxy} and Φ_{ES}, which are closely related to the interaction between the luciferins and the luciferase. In other words, the bioluminescent intensity is determined by the fluorescent intensity of the luciferin as well as the interaction between the luciferin and the luciferase.

To see how the bioluminescent intensity is affected by the interaction between the luciferin and the luciferase, the net electric charge distributions are computed and displayed in Figure 6. The amino acid residue Arg218 in the PpyLuc is at

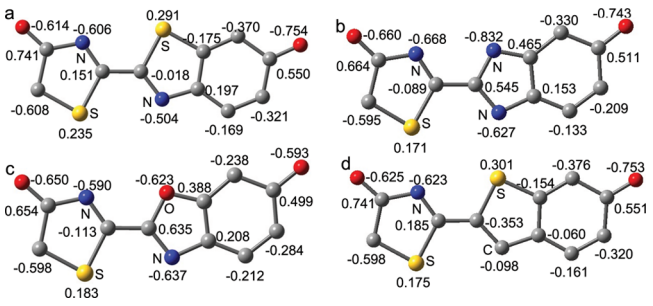

Figure 6. Electric charge distributions for (a) $OxyLH_2$, (b) $OxyBiLH_2$, (c) $OxyBoLH_2$, and (d) $OxyBtLH_2$, where the hydrogen atoms are omitted for clarity.

the bottom of the luciferin binding pocket and in close proximity to the substrate's O6′ (Figure 7).[5] The positively charged side chain of the Arg218 anchors the benzothiazole ring of the $OxyLH_2$ via an electrostatic interaction with the O6′.[5,40,48−50] If the O6′ carries more negative charge, the positively charged side chain of the Arg218 pulls the $OxyLH_2$ or its analogs through the O6′ more tightly. Figure 6 shows that the negative charge of the O6′ (Mulliken charge) is −0.754 ($OxyLH_2$), −0.743 ($OxyBiLH_2$), −0.593 ($OxyBoLH_2$), and −0.753 ($OxyBtLH_2$), respectively. Here the O6′ of the

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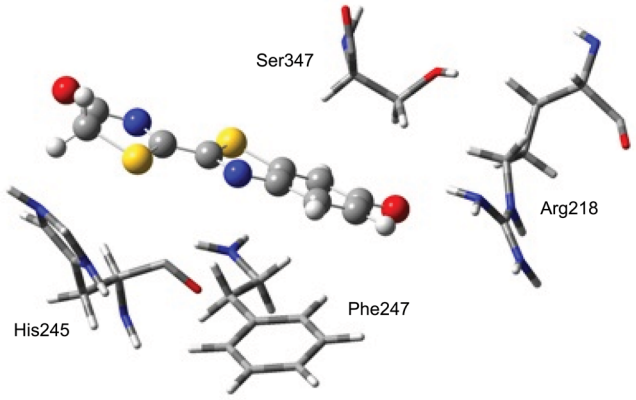

Figure 7. Amino acid residues close to the O6′ and O4.

$OxyBoLH_2$ carries much less negative charge than the other three compounds. Comparing the computed negative charges of the O6′ with the experimental bioluminescent intensities of the $OxyLH_2$ (100%), $OxyBiLH_2$ (14%), $OxyBoLH_2$ (0.9%), and $OxyBtLH_2$ (70%),[16] one can see that more negative charge of the O6′, that is, stronger interaction between the O6′ and the Arg218, yields higher bioluminescent intensity. This observation is supported by the study of the mutant luciferases R218K, R218Q, and R218A, where the stronger interaction between the hydroxyl group and K218, Q218, or A218 results in higher specific activity.[5] Recently, some work suggested that neither luciferin, luciferyl-adenylate, nor oxyluciferin should have a deprotonated benzothiazole hydroxyl group.[26,51] The amounts of the negative charges carried by the whole hydroxyl group OH have also been calculated, and their order is the same as that of the negative charges carried by the O6′. Thus, the negative charge of the O6′ plays the same role as that of the whole hydroxyl group OH. The structure and chemical differences among the four compounds come from the replacement of the benzothiazole ring of the $OxyLH_2$ by its counterparts. We point out that the $OxyBiLH_2$ has much lower fluorescent quantum yield (Φ_{Fl}) due to tautomerization, and the $OxyBoLH_2$ has much less negative charge of the O6′, which explains why the $OxyBiLH_2$ and $OxyBoLH_2$ have lower experimental bioluminescent intensities.

The side-chain imidazole of the His245 in the PpyLuc is at the opening of the luciferin binding pocket in close proximity to the substrate's O4 (Figure 7).[6] The negative charges of the O4 are −0.614 ($OxyLH_2$), −0.660 ($OxyBiLH_2$), −0.650 ($OxyBoLH_2$), and −0.625 ($OxyBtLH_2$), respectively. If the O4 carries less negative charge, the His245 pulls the luciferin more loosely. By comparing experimental bioluminescent intensities with the negative charges of the O4, one infers that $\Phi_{Oxy}\Phi_{ES}$ tends to be greater when the interaction between the O4 and the His245 is weaker. This picture is supported by the mutagenesis study of the His245, where the bioluminescent intensity becomes weaker when the interaction between the substitute amino acid residue (A245, N245, F245, Q245, D245, and R245) and the O4 tends to be greater.[4] Like the Gly315, the His245 provides conformational flexibility to adenylation and oxidation of luciferins.[6] Because the negative charge of the O6′ for the $OxyBiLH_2$ (−0.743) is much greater than that for the $OxyBoLH_2$ (−0.593), the bioluminescent intensity of the $OxyBiLH_2$ is stronger than that of the $OxyBoLH_2$ (even the O4 of the $OxyBoLH_2$ carries slightly less negative charge than that of the $OxyBiLH_2$). We conclude that if the Arg218 in the PpyLuc holds the benzothiazole ring (or its counterpart) more tightly, and the His245 holds the thiazoline ring (or its counterpart) more loosely, $\Phi_{Oxy}\Phi_{ES}$ becomes greater. Figure 6 and Figure S2 show that when an extra negative electric field is applied along the x-direction, the O6′ of the $OxyLH_2$ carries more negative charge (−0.754 → −0.796) and the O4 has less negative charge (−0.614 → −0.373). The above mechanism indicates that $\Phi_{Oxy}\Phi_{ES}$ gets greater. Figure 2b reveals that its fluorescent quantum yield decreases slightly. As a whole, the bioluminescent intensity would become stronger. On the other hand, when an extra positive electric field is applied along the x-direction, the O6′ carries less negative charge and the O4 has more negative charge (Figure S2). Then, the corresponding bioluminescent intensity would decrease. When the luciferin is in the R218Q mutant luciferase, from the electric field point of view, an extra positive electric field is added, so the intensity reduces. The experimental bioluminescent intensity of the LH_2 in the R218Q mutant luciferase is only 5.1% of that in the PpyLuc,[5] which supports the mechanism proposed here.

CONCLUSION

The emitted wavelengths and intensities of the $OxyLH_2$, $OxyBtLH_2$, $OxyBiLH_2$, and $OxyBoLH_2$ under the LEF have been investigated by the combination of the time-dependent density functional theory (TDDFT) and the symmetry-adapted cluster-configuration interaction (SAC-CI) approach. Three average E_x change rates of the first excited energy have been introduced to measure which luciferins are more sensitive to the LEF. The common overlapping volumes of the HOMO and LUMO have been checked to explain why the oscillator strengths vary under the LEF. The first excited energies and intensities under two LEFs induced by the PpyLuc and the mutant R218Q luciferase have been evaluated, which match the experimental values. The net electric charges of the O6′ and the O4 have been employed to describe the interaction between the modified heteroaromatic luciferins and the luciferases. By combining with oscillator strengths, we have found that if the Arg218 in the PpyLuc holds the benzothiazole ring (or its counterpart) more tightly and the His245 holds the thiazoline ring (or its counterpart) more loosely, $\Phi_{Oxy}\Phi_{ES}$ becomes greater. When we search new heterocyclic analogs of the LH_2, we should design analogs that have greater average E_x change rates of the first excited energy to the LEF in order to shift their wavelengths to the RNIR direction easily by mutant luciferases. Since the negative charge of the O6′ plays an important role for $\Phi_{Oxy}\Phi_{ES}$, the O6′ of new heterocyclic analogs of the LH_2 should carry more negative charge in order to get more efficient bioluminescent emission. Because the Lys443 and the Lys529 are far away from the benzothiazole ring (or its counterpart) of the luciferin and play key roles in the oxidation and adenylation process, respectively,[8] we are in the process of exploring whether there is a long-range correlation between the Lys443 (or Lys529) and the benzothiazole ring or its counterpart to explain how the Lys443 and the Lys529 affect the bioluminescent emitting wavelength and intensity.[52]

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jpcb.5b03827.

Cartesian coordinates of four molecules, two step reactions of firefly luciferin, and the electric charge

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distributions for four types of the heteroaromatic luciferins at E_x = −150 and 150 mV/Å (PDF)

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Notes

The authors declare no competing financial interest.

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REFERENCES

(1) Dragulescu-Andrasi, A.; Chan, C. T.; De, A.; Massoud, T. F.; Gambhir, S. S. Bioluminescence resonance energy transfer (BRET) imaging of protein-protein interactions within deep tissues of living subjects. *Proc. Natl. Acad. Sci. U. S. A.* **2011**, *108*, 12060−12065.

(2) Fraga, H. Firefly luminescence: A historical perspective and recent developments. *Photochem. Photobiol. Sci.* **2008**, *7*, 146−158.

(3) Nakatsu, T.; Ichiyama, S.; Hiratake, J.; Saldanha, A.; Kobashi, N.; Sakata, K.; Kato, H. Structural basis for the spectral difference in luciferase bioluminescence. *Nature* **2006**, *440*, 372−376.

(4) Branchini, B. R.; Magyar, R. A.; Murtiashaw, M. H.; Anderson, S. M.; Helgerson, L. C.; Zimmer, M. Site-directed mutagenesis of firefly luciferase active site amino acids: a proposed model for bioluminescence color. *Biochemistry* **1999**, *38*, 13223−13230.

(5) Branchini, B. R.; Magyar, R. A.; Murtiashaw, M. H.; Portier, N. C. The role of active site residue arginine 218 in firefly luciferase bioluminescence. *Biochemistry* **2001**, *40*, 2410−2418.

(6) Branchini, B. R.; Southworth, T. L.; Murtiashaw, M. H.; Boije, H.; Fleet, S. E. A mutagenesis study of the putative luciferin binding site residues of firefly luciferase. *Biochemistry* **2003**, *42*, 10429−10436.

(7) Branchini, B. R.; Southworth, T. L.; Murtiashaw, M. H.; Magyar, R. A.; Gonzalez, R. A.; Ruggiero, M. C.; Stroh, J. G. An alternative mechanism of bioluminescence color determination in firefly luciferase. *Biochemistry* **2004**, *43*, 7255−7262.

(8) Branchini, B. R.; Southworth, T. L.; Murtiashaw, M. H.; Wilkinson, S. R.; Khattak, N. F.; Rosenberg, J. C.; Zimmer, M. Mutagenesis evidence that the partial reactions of firefly bioluminescence are catalyzed by different conformations of the luciferase C-terminal domain. *Biochemistry* **2005**, *44*, 1385−1393.

(9) Woodroofe, C. C.; Shultz, J. W.; Wood, M. G.; Osterman, J.; Cali, J. J.; Daily, W. J.; Meisenheimer, P. L.; Klaubert, D. H. N-Alkylated 6′-aminoluciferins are bioluminescent substrates for Ultra-Glo and QuantiLum luciferase: New potential scaffolds for bioluminescent assays. *Biochemistry* **2008**, *47*, 10383−10393.

(10) Branching, B. R.; Hayward, M. M.; Bamford, S.; Brennan, P. M.; Lajiness, E. J. Naphthyl- and quinolylluciferin: Green and red light emitting firefly luciferin analogues. *Photochem. Photobiol.* **1989**, *49*, 689−695.

(11) Reddy, G. R.; Thompson, W. C.; Miller, S. C. Robust light emission from cyclic alkylaminoluciferin substrates for firefly luciferase. *J. Am. Chem. Soc.* **2010**, *132*, 13586−13587.

(12) Takakura, H.; Kojima, R.; Urano, Y.; Terai, T.; Hanaoka, K.; Nagano, T. Aminoluciferins as Functional bioluminogenic substrates of firefly luciferase. *Chem. - Asian J.* **2011**, *6*, 1800−1810.

(13) Kojima, R.; Takakura, H.; Ozawa, T.; Tada, Y.; Nagano, T.; Urano, T. Rational design and development of near-infrared-emitting firefly luciferins available in vivo. *Angew. Chem., Int. Ed.* **2013**, *52*, 1175−1179.

(14) Iwano, S.; Obata, R.; Miura, C.; Kiyama, M.; Hama, K.; Nakamura, M.; Amano, Y.; Kojima, S.; Hirano, T.; Maki, S.; et al. Development of simple firefly luciferin analogs emitting blue, green, red, and near-infrared biological window light. *Tetrahedron* **2013**, *69*, 3847−3856.

(15) Conley, N. R.; Dragulescu-Andrasi, A.; Rao, J.; Moerner, W. W. A selenium analogue of firefly D-luciferin with red-shifted bioluminescence emission. *Angew. Chem., Int. Ed.* **2012**, *51*, 3350−3353.

(16) Woodroofe, C. C.; Meisenheimer, P. L.; Klaubert, D. H.; Kovic, Y.; Rosenberg, J. C.; Behney, C. E.; Southworth, T. L.; Branchini, B. R. Novel heterocyclic analogues of firefly luciferin. *Biochemistry* **2012**, *51*, 9807−9813.

(17) McCutcheon, D. C.; Paley, M. A.; Steinhardt, R. C.; Prescher, J. A. Expedient synthesis of electronically modified luciferins for bioluminescence imaging. *J. Am. Chem. Soc.* **2012**, *134*, 7604−7607.

(18) Wilson, T.; Hastings, J. W. Bioluminescence. *Annu. Rev. Cell Dev. Biol.* **1998**, *14*, 197−230.

(19) Pinto da Silva, L.; Esteves da Silva, J. C. Theoretical analysis of the color tuning mechanism of oxyluciferin and 5-hydroxyoxyluciferin. *Comput. Theor. Chem.* **2012**, *988*, 56−62.

(20) Milne, B. F. Red-shifting the optical response of firefly oxyluciferin with group 15/16 substitutions. *Phys. Chem. Chem. Phys.* **2014**, *16*, 24971−24977.

(21) Cheng, Y.; Zhu, J.; Liu, Y. Theoretical tuning of the firefly bioluminescence spectra by the modification of oxyluciferin. *Chem. Phys. Lett.* **2014**, *591*, 156−160.

(22) Ran, X. Q.; Zhou, X.; Goddard, J. D. The spectral-structural relationship of a series of oxyluciferin derivatives. *ChemPhysChem* **2015**, *16*, 396−402.

(23) Cai, D.; Marques, M. A.; Nogueira, F. Accurate color tuning of firefly chromophore by modulation of local polarization electrostatic fields. *J. Phys. Chem. B* **2011**, *115*, 329−332.

(24) Cai, D.; Marques, M. A.; Nogueira, F. Full Color Modulation of Firefly Luciferase through Engineering with Unified Stark Effect. *J. Phys. Chem. B* **2013**, *117*, 13725−13730.

(25) Pinto da Silva, L.; Esteves da Silva, J. C. Quantum/molecular mechanics study of firefly bioluminescence on luciferase oxidative conformation. *Chem. Phys. Lett.* **2014**, *608*, 45−49.

(26) Pinto da Silva, L.; Esteves da Silva, J. C. Chemiexcitation induced proton transfer: Enolate oxyluciferin as the firefly bioluminophore. *J. Phys. Chem. B* **2015**, *119*, 2140−2148.

(27) Mofford, D. M.; Reddy, G. R.; Miller, S. C. Latent luciferase activity in the fruit fly revealed by a synthetic luciferin. *Proc. Natl. Acad. Sci. U. S. A.* **2014**, *111*, 4443−4448.

(28) Mofford, D. M.; Reddy, G. R.; Miller, S. C. Aminoluciferins extend firefly luciferase bioluminescence into the near-infraed and can be preferred substrates over D-luciferin. *J. Am. Chem. Soc.* **2014**, *136*, 13277−13282.

(29) Bauernschmitt, R.; Ahlrichs, R. Treatment of electronic excitations within the adiabatic approximation of time dependent density functional theory. *Chem. Phys. Lett.* **1996**, *256*, 454−464.

(30) Becke, A. D. Density-functional thermochemistry III. The role of exact exchange. *J. Chem. Phys.* **1993**, *98*, 5648−5652.

(31) Yanai, T.; Tew, D. P.; Handy, N. C. A new hybrid exchange correlation functional using the Coulomb-attenuating method(CAM-B3LYP). *Chem. Phys. Lett.* **2004**, *393*, 51−57.

(32) Zhao, Y.; Truhlar, D. G. The M06 suite of density functionals for main group thermochemistry, thermochemical kinetics, noncovalent interactions, excited states, and transition elements: two new functionals and systematic testing of four M06-class functionals and 12 other functionals. *Theor. Chem. Acc.* **2008**, *120*, 215−241.

(33) Hamprecht, F. A.; Cohen, A.; Tozer, D. J.; Handy, N. C. Development and assessment of new exchange-correlation functionals. *J. Chem. Phys.* **1998**, *109*, 6264−6271.

(34) Nakatsuji, H.; Hirao, K. Cluster expansion of the wavefunction: Symmetry-adapted-cluster expansion, its variational determination, and extension of open-shell orbital theory. *J. Chem. Phys.* **1978**, *68*, 2053−2065.

DOI: 10.1021/acs.jpcb.5b03827
J. Phys. Chem. B 2015, 119, 10399−10405

(35) Zhou, J. G.; Williams, Q. L.; Wu, R. Thioglycolic acid on the gold (111) surface and Raman vibrational spectra. *J. Chem. Phys.* **2010**, *132*, 064702.
(36) Cossi, M.; Rega, N.; Scalmani, G.; Barone, V. Energies, structures, and electronic properties of molecules in solution with the C-PCM solvation model. *J. Comput. Chem.* **2003**, *24*, 669−681.
(37) Naumov, P.; Ozawa, Y.; Ohkubo, K.; Fukuzumi, S. Structure and spectroscopy of oxyluciferin, the light emitter of the firefly bioluminescence. *J. Am. Chem. Soc.* **2009**, *131*, 11590−11605.
(38) Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Scalmani, G.; Barone, V.; Mennucci, B.; Petersson, G. A.; Nakatsuji, H.; Caricato, M.; Li, X.; Hratchian, H. P.; Izmaylov, A. F.; Bloino, J.; Zheng, G.; Sonnenberg, J. L.; Hada, M.; Ehara, M.; Toyota, K.; Fukuda, R.; Hasegawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Vreven, T.; Montgomery, J. A., Jr.; Peralta, J. E.; Ogliaro, F.; Bearpark, M.; Heyd, J. J.; Brothers, E.; Kudin, K. N.; Staroverov, V. N.; Kobayashi, R.; Normand, J.; Raghavachari, K.; Rendell, A.; Burant, J. C.; Iyengar, S. S.; Tomasi, J.; Cossi, M.; Rega, N.; Millam, J. M.; Klene, M.; Knox, J. E.; Cross, J. B.; Bakken, V.; Adamo, C.; Jaramillo, J.; Gomperts, R.; Stratmann, R. E.; Yazyev, O.; Austin, A. J.; Cammi, R.; Pomelli, C.; Ochterski, J. W.; Martin, R. L.; Morokuma, K.; Zakrzewski, V. G.; Voth, G. A.; Salvador, P.; Dannenberg, J. J.; Dapprich, S.; Daniels, A. D.; Farkas, O.; Foresman, J. B.; Ortiz, J. V.; Cioslowski, J.; Fox, D. J. *Gaussian 09*, Revision D.01; Gaussian, Inc.: Wallingford, CT, 2009.
(39) White, E. H.; Rapaport, E.; Hopkins, T. A.; Seliger, H. H. Chemi- and bioluminescence of firefly luciferin. *J. Am. Chem. Soc.* **1969**, *91*, 2178−2180.
(40) Nakatani, N.; Hasegawa, J.; Nakatsuji, H. Red light in chemiluminescence and yellow-green light in bioluminescence: color-tuning mechanism of firefly, Photinus pyralis, studied by the symmetry-adapted cluster-configuration interaction method. *J. Am. Chem. Soc.* **2007**, *129*, 8756−8765.
(41) Lockhart, D. J.; Kim, P. S. Internal Stark effect measurement of the electric field at the amino terminus of an alpha-helix. *Science* **1992**, *257*, 947−951.
(42) Zimmermann, U.; Pilwat, G.; Riemann, F. Dielectric breakdown of cell membranes. *Biophys. J.* **1974**, *14*, 881−899.
(43) Solntsev, K. M.; Laptenok, S. P.; Naumov, P. Photoinduced dynamics of oxyluciferin analogues: unusual enol "super" photoacidity and evidence for keto-enol isomerization. *J. Am. Chem. Soc.* **2012**, *134*, 16452−16455.
(44) Modestova, Y.; Koksharov, M. I.; Ugarova, N. N. Point mutations in firefly luciferase C-domain demonstrate its significance in green color of bioluminescence. *Biochim. Biophys. Acta, Proteins Proteomics* **2014**, *1844*, 1463−1471.
(45) Cai, D.; Marques, M. A. L.; Milne, B. F.; Nogueira, F. Bioheterojunction effect on fluorescence origin and efficiency improvement of firefly chromophores. *J. Phys. Chem. Lett.* **2010**, *1*, 2781−2787.
(46) Pinto da Silva, L.; Esteves da Silva, J. C. TD-DFT/molecular mechanics study of the photinus pyralis bioluminescence system. *J. Phys. Chem. B* **2012**, *116*, 2008−2013.
(47) Pinto da Silva, L.; Esteves da Silva, J. C. Theoretical fingerprinting of the photophysical properties of four firefly bioluminophores. *Photochem. Photobiol. Sci.* **2013**, *12*, 2028−2035.
(48) Orlova, G.; Goddard, J. D.; Brovko, L. Y. Theoretical study of the amazing firefly bioluminescence: the formation and structures of the light emitters. *J. Am. Chem. Soc.* **2003**, *125*, 6962−6971.
(49) Pinto da Silva, L.; Simkovitch, R.; Huppert, D.; Esteves da Silva, J. C. Oxyluciferin photoacidity: the missing element for solving the keto-enol mystery? *ChemPhysChem* **2013**, *14*, 3441−3446.
(50) Min, C. G.; Ren, A. M.; Guo, J. F.; Zou, L.-Y.; Goddard, J. D.; Sun, C. C. Theoretical investigation on the origin of yellow-green firefly bioluminescence by time-dependent density functional theory. *ChemPhysChem* **2010**, *11*, 2199−2204.
(51) Pinto da Silva, L.; Santos, A. M.; Esteves da Silva, J. C. Efficient firefly chemi/bioluminescence: evidence for chemiexcitation resulting from the decomposition of a neutral firefly dioxetanone molecule. *J. Phys. Chem. A* **2013**, *117*, 94−100.
(52) Branchini, B. R.; Rosenberg, J. C.; Fontaine, D. M.; Southworth, T. L.; Behney, C. E.; Uzasci, L. Bioluminescence is produced from a trapped firefly luciferase conformation predicted by the domain alternation mechanism. *J. Am. Chem. Soc.* **2011**, *133*, 11088−11091.

DOI: 10.1021/acs.jpcb.5b03827
J. Phys. Chem. B 2015, 119, 10399−10405