This manuscript studied the effect of protein conformation and AMP protonation on the bioluminescence of firefly via MD and QM/MM calculations. Please the authors consider below comments:

1. The article claimed that the emission spectra of oxyluciferin is simulated. Actually, only the vertical emission was calculated. Although the emission spectra were simulated by a convolution of gaussian functions, there is no more information than vertical emission. In figure 7, the emission intensity is the same for all spectra. What are the oscillator strength of each vertical emission? They should not be the same.

The simulated emission spectra have been built considering the emission energies and oscillator strength computed at the QM/MM level for 200 snapshots of each system under study. The spectra given in the manuscript have been normalized. We agree with the referee that the information of the oscillator strength could be interesting to analyze further differences between the systems.

For this aim, first the intensity of the emission maximum simulated for all the systems have been analyzed, finding that the largest one corresponds to phenolate-enol-4G37-AMP. This intensity has been taken as the reference to compute the relative intensity of the other spectra as shown in Figure 7. A sentence has been added in the manuscript to analyze the differences found between the relative intensities of the systems.

Moreover, as there is not a unique value of oscillator strength, 200 snapshots have been considered, a histogram with the oscillator strengths of the emission transition for all the systems under study is presented in Figures S4 and S5.

2. 4G36 is the adenylate-forming conformations of luciferase, which is the protein environment of the first catalytic reaction in Scheme 1. Right? In this reaction, oxyluciferin has not been produced. It is unreasonable to calculate emission energy of excited-state oxyluciferin in 4G36 conformation. If I am wrong, please correct me.

In fact it is still not clear which is the conformation of the protein when the light is emitted. Branchini et al. designed the 4G37 conformation aimed by the fact that luciferase belongs to the superfamily of adenylating enzymes, characterized by the domain alternation mechanism. However, the 4G37 is an engineered luciferase as a link between two amino acids should be included to keep the protein in that conformation. Not many experimental evidences support the domain alternation mechanism in fireflies. So, as the protein conformation during light emission is not clear, we have considered both the 4G36 and 4G37 for the emission spectra simulation.

Moreover, leaving aside the domain alternation mechanism, we consider the 4G36 and 4G37 structures as two models with quite different active site conformation with respect to oxyluciferin. As explained in the introduction, the 4G36 is open whereas the 4G37 is closed. Hence, we would like to analyze if this fact would affect the hydrogen-bond pattern around oxyluciferin (facilitating or not the water entrance) and so the emission energy.

3. The Cartesian coordinates of QM/MM optimized geometries should be put in the Supporting Information.

As suggested by the referee we have included the geometries of the QM region for a representative snapshot (near the emission maximum) for the eight systems under study.

4. In the subsection of QM/MM calculations. The QM method should be TD B3LYP not B3LYP. I have to say B3LYP functional really has problem when handling H-bond systems
and ESPT. At TD DFT computational level, the emission of 529,536 and 540 nm have no
difference. Once different functional is employed, their order could change quietly possible,
sometimes, even there is 50 nm difference among them.

*We agree with the referee that the election of the DFT functional is crucial. For computing the emission of oxyluciferin we have chosen the B3LYP functional as has been previously shown its suitability for this system (see references in the computational methods of the manuscript). Regarding this work, we don’t study the ESPT process and no H-bonds are considered at the QM level as oxyluciferin leads to hydrogen-bond interactions with the protein active site, AMP or water molecules which are considered at the MM level.*

Moreover, we agree that for some systems under study, the energy difference between the maximum emission spectra is quite small, being lower than 0.1 eV (within the TD DFT error). In order to clarify this point we have added some sentences along the manuscript.

*Finally, the emission energy of one representative snapshot for each system (eight in total) have been computed using the CAM-B3LYP and M062X functional, following the same procedure used for the B3LYP one (Table S1). Similar trends have been observed although the absolute values are red shifted compared to the ones obtained with the B3LYP functional. In fact, the emission values computed with the B3LYP functional are the closest ones to the experiment.*