Reviewer 1

The work by Garcia-Iriepa et al. tries to investigate the influence of the protein environment and the pH on the emission spectra of the oxyluciferin by means of MD simulations and QM/MM calculations. The authors considered two different protein conformations and two protonation states of AMP for both enol and keto form oxyluciferin. This study shows that the protein conformation and the pH values could influence the hydrogen-bond interactions and the protonation state of AMP so that could affect the emission of fireflies’ bioluminescence. The conclusions gave out a reasonable explanation on the multicolor of bioluminescent system. Thus I basically think that this work is potentially interesting to the general readership of the journal.

I have only one question about this article: the authors attributed the pH influence mainly on the different protonated states of AMP under different pH value. According to this article, the protein conformation and the forms of oxyluciferin (enol or keto) also strongly affects the emission spectrum. I wondering that whether or not the pH will influence the protein conformation and the forms of oxyluciferin. The authors could illustrate this in the paper.

We agree with the referee that changes on the pH could lead to several modifications in the fireflies’ bioluminescent system such as the protein conformation, the chemical nature of the light emitter and the protonation state of AMP among others. In this work, a partial study of the effect of pH on the emission has been done by analyzing only the influence of the different protonation states of AMP. We have modified some sentences in the manuscript to clarify this point showing that we focus only on one factor related to the pH effect (AMP protonation/deprotonation).

Regarding the influence on the protein conformation, an ongoing project is being performed in collaboration with other theoretical group to do molecular dynamics at constant pH values and to analyze the influence on the emission spectra.

Considering the chemical form of oxyluciferin it has been already demonstrated that the emission comes mainly from a phenolate form, although not basic media is used (10.1021/ja017400m, 10.1021/ja3045212). For this reason, in this article we simulate the emission spectra of the most probable light emitters in fireflies, the phenolate-keto and phenolate-enol forms.

Moreover, I thought the molecule orbitals of enol forms had better been put in the Supplementary Materials, too.

As suggested by the referee, the molecular orbitals of the enol form have been added in Figure S2 in the Supplementary Materials.