## **Supporting Information for**

## Emission's colour modulation of oxyluciferin synthetic analogues by QM and QM/MM approaches

Madjid Zemmouche<sup>a</sup>, Cristina García-Iriepa<sup>ab</sup> and Isabelle Navizet<sup>\*a</sup>

<sup>a</sup>Université Paris-Est, Laboratoire Modélisation et Simulation Multi Échelle, MSME UMR 8208 CNRS, UPEM, 5 bd Descartes, 77454 Marne-la-Vallée, France

b Departamento de Química Analítica, Química Física e Ingeniería Química, Universidad de Alcalá, E-28871 Alcalá de Henares, Madrid, Spain

		benzothiophene_Oxy	dihydropyrrolone_Oxy Natural_Oxy		allylbenzothiazole_Oxy	
Absorption						
	HOMO (au)	-0.063	-0.061	-0.067	-0.068	
gas phase	LUMO (au)	0.034	0.032	0.024	0.023	
	ΔE <sub>HOMO-LUMO</sub> (eV)	2.64	2.53	2.48	2.48	
Water (PCM)	HOMO (au)	-0.179	-0.180	-0.183	-0.182	
	LUMO (au)	-0.079	-0.084	-0.090	-0.090	
	ΔE <sub>HOMO-LUMO</sub> (eV)	2.72	2.61	2.53	2.50	
Emission						
	HOMO (au)	-0.059	-0.058	-0.064	-0.065	
gas phase	LUMO (au)	0.028	0.025	0.018	0.016	
	ΔE <sub>HOMO-LUMO</sub> (eV)	2.37	2.26	2.22	2.20	
	HOMO (au)	-0.178	-0.180	-0.183	-0.181	
Water (PCM)	LUMO (au)	-0.083	-0.090	-0.095	-0.095	
	ΔE <sub>HOMO-LUMO</sub> (eV)	2.58	2.45	2.39	2.34	

**Table S1** Frontier molecular orbital energies ( $E_{HOMO}$  and  $E_{LUMO}$  (au)) and their energy difference (eV), computed at  $S_0 \rightarrow S_1$  (absorption) and  $S_1 \rightarrow S_0$  (emission) transitions for all the structures in gas phase and in water (PCM).

**Table S2** Mulliken charges of benzothiazole and thiazolone moieties at ground ( $S_0$ ) and first ( $S_1$ ) excited states computed at the equilibrium structure of  $S_0$  (for absorption) and equilibrium structure of  $S_1$  (for emission) in water (PCM). Charge transfer (CT) character calculated as the difference of the thiazolone moiety charges between the final state  $q_{S_1}^{thiaz}$  and initial state  $q_{S_1}^{thiaz}$ . For absorption ( $S_0 \rightarrow S_1$ ),  $CT = q_{S_1}^{thiaz} - q_{S_0}^{thiaz}$  with the charges taken at the equilibrium structure of  $S_0$  in water (PCM). For emission ( $S_1 \rightarrow S_0$ ),  $CT = q_{S_0}^{thiaz} - q_{S_1}^{thiaz}$  with the charges taken at the equilibrium structure of  $S_1$ in water (PCM). Negative CT values mean that there exists a fraction of the electronic charge transferred from benzothiazole to thiazolone moiety during the transition.

	benzothiophene_Oxy	dihydropyrrolone_Oxy	Natural_Oxy	allylbenzothiazole_Oxy		
	Absorption					
$q_{S_0}^{thiaz}$ -0.296		-0.259	-0.308	-0.306		
$q_{S_0}^{benzo}$	-0.704	-0.741	-0.692	-0.694		
$q_{S_1}^{thiaz}$	-0.433	-0.405	-0.454	-0.462		
$q_{S_1}^{benzo}$	-0.567	-0.595	-0.546	-0.538		
$CT=\!\Delta q_{S_1-S_0}^{thiaz} \ (e)$	-0.137	-0.146	-0.146	-0.156		
	Emission					
$q_{S_0}^{thiaz}$	-0.279	-0.254	-0.294	-0.296		
$q_{S_0}^{benzo}$	-0.721	-0.746	-0.706	-0.704		
$q_{S_1}^{thiaz}$	-0.438	-0.409	-0.456	-0.468		
$q_{S_1}^{benzo}$	-0.562	-0.591	-0.544	-0.532		
$CT=\!\Delta q_{S_0-S_1}^{thiaz} \ (e)$	0.159	0.155	0.162	0.172		



**Figure S1** Natural transition orbital (NTO) of Natural\_Oxy and its analogues computed at the equilibrium geometry of  $S_0$  corresponding to  $S_0 \rightarrow S_1$  electronic transition (absorption) in gas phase, in water (PCM) and in protein. For the compounds in protein, the selected snapshots have been taken from the MD simulations and correspond to the maximum absorption wavelength. The NTOs are plotted using Avogadro package.



**Figure S2** Natural transition orbital (NTO) of Natural\_Oxy and its analogues computed at the equilibrium geometry of  $S_1$  corresponding to  $S_1$ ->  $S_0$  electronic transition (emission) in gas phase, in water (PCM) and in protein. For the compounds in the protein, the selected snapshots have been taken from the MD simulations and correspond to the maximum emission wavelength. The NTOs are plotted using Avogadro package.

H-bond	benzothiophene_Oxy	dihydropyrrolone_Oxy	Natural_Oxy	allylbenzothiazole_Oxy		
H <sub>2</sub> O – O10	1.61	1.33	1.18	0.63		
H <sub>2</sub> O – N2	x	0.67	0.74	0.60		
H <sub>2</sub> O – O11	0.21	0.15	0.29	0.51		
H2 of ARG337 – O10	0.10	0.89	0.78	0.87		
H1 of AMPH – O11	0.67	0.76	0.42	0.17		
Atom numbering	H2 010 C7 S2 N1 011 ARG337 N2 S1 H1					

 Table S3 Average number of H-bonds involving specific atoms of the target molecule in protein during MD simulation in the excited state.

Oscillator strengths for the 100 vertical  $S_1 \rightarrow S_0$  transitions of the four compounds under study have been computed (Figure S3 and S4). For all the compounds, the vertical transition energies are located in narrow range of energy except for the ones of allylbenzothiazole\_Oxy, which are found in a larger range. Hence, a gaussian functions convolution of the vertical transition emission energies of these compounds gives a simulated allylbenzothiazole\_Oxy emission spectrum shape to be relatively boarder than the others. This can be explained by the fact that the allylbenzothiazole\_Oxy has an allyl group, which rotates during the MD simulation (Figure S5 shows a large dihedral angle fluctuation between 40 and - 160°).

By comparison, the experimental emission spectrum of allylbenzothiazole\_Oxy seems to be as large as the ones of the other compounds (Figure 3) but it does not comes from the same experiments. Therefore, it is difficult to conclude about the width of the spectra.



**Figure S3** Oscillator strengths against wavelengths for the 100 vertical  $S_1 \rightarrow S_0$  transitions of Natural\_Oxy and its analogues simulated with QM/MM methods used to simulate the emission spectra. *a*) benzothiophene\_Oxy, *b*) dihydropyrrolone\_Oxy, *c*) Natural\_Oxy, and *d*) allylbenzothiazole\_Oxy. Under figure d, three geometries of allylbenzothiazole\_Oxy along the MD simulation with different allyl group rotation are represented.



Figure S4 Oscillator strengths against transition energies for the 100 vertical  $S_1 \rightarrow S_0$  transitions of Natural\_Oxy and its analogues simulated with QM/MM methods used to simulate the emission spectra. *a*) benzothiophene\_Oxy, *b*) dihydropyrrolone\_Oxy, *c*) Natural\_Oxy, and *d*) allylbenzothiazole\_Oxy.



**Figure S5** Dihedral angle fluctuation obtained with CPPTRAJ program defined by the four atoms C6, C7, C8 and C9 of allylbenzothiazole\_Oxy along the MD simulation in the excited state.



**Figure S6** Absorption spectra of the Natural\_Oxy and its analogues simulated with QM/MM methods considering 100 statistical MD snapshots (HWHM of 0.20 eV).



**Figure S7** *a***)** Distance (Å) between the atoms O10 and O11 and carbon (CX) atom of amino acids (or the phosphate (P) atom of AMPH) computed from MD simulations in the excited state of the Natural\_Oxy and its analogues. *b***)** Atom numbering and the initial distances (Å) between (O10 – CX) and (O11 – P) before MD simulation of Natural\_Oxy are given. *c***)** Distance (Å) between the atoms O10 and CX of amino acid ARG337 computed from two MD simulations of the benzothiophene\_Oxy.



**Figure S8** H-bonds networks formed during MD simulations in the excited state of Natural\_Oxy (in green) and benzothiophene\_Oxy (in blue) at the excited state  $S_1$ . The extracted snapshots correspond to the maximum emission wavelength.



**Figure S9** H-bonds networks formed during MD simulations in the excited state of Natural\_Oxy (in green) and dihydropyrrolone\_Oxy (in light-blue) at the excited state  $S_1$ . The extracted snapshots correspond to the maximum emission wavelength.



**Figure S10** H-bonds networks formed during MD simulations in the excited state of Natural\_Oxy (in green) and allylbenzothiazole\_Oxy (in red) at the excited state  $S_1$ . The extracted snapshots correspond to the maximum emission wavelength.



**Figure S11** Root mean square deviation (RMSD) analysis obtained from the MD simulations in the excited state of the compounds under study. The calculated mass-weighted RMSD are obtained using all non-hydrogen atoms in compounds under study and the structure of the first trajectory frame is taken as reference.



**Figure S12** Statistics of water molecules found around 5 Å of the atom C7 (see atom numbers in Table S3) of Natural\_Oxy (in green) and allylbenzothiazole\_Oxy (in red) along the MD simulations in the excited state.

Thanks to the present study, we can propose one oxyluciferin analogue in two conformations, Red\_1\_Oxy and Red\_2\_Oxy (Table S4), which could be good candidates to emit light in the red and near-IR spectral window. This analogue has been designed by introducing an allyl group on the phenol moiety and a double bond between the benzothiazole and thiazolone moieties of the oxyluciferin.

The computed vertical transition energies, the corresponding wavelengths ( $\lambda_{emi}$ ) and oscillator strengths (f) of the emission of the Red\_1\_Oxy and the Red\_2\_Oxy in gas phase and in implicit water at the QM level are reported in Table S4. The predicted results of both conformations in gas phase and in PCM (water) indicate that the emission wavelengths are red-shifted regarding the Natural\_Oxy and red-shifted comparing to the allylbenzothiaozole\_Oxy. For instance, for Red\_1\_Oxy the emission wavelengths are red-shifted by 54 nm and 62 nm compared to the Natural\_Oxy, respectively in gas phase and in PCM. While, for Red\_2\_Oxy the emission wavelengths are red-shifted by 83 nm and 89 nm, respectively in gas phase and in PCM. In general, the presence of the protein environment redshifts the emission wavelength. Therefore, the Red\_1\_Oxy and Red\_2\_Oxy forms of this analogue could be good bioluminescent candidates emitting in the near-IR spectral window. Furthermore, the NTOs involved in the emission transitions for both structures in gas phase and in PCM (Figure S13) show a similar electron density as in the other analogues in both gas phase and PCM.

**Table S4** Vertical transition energies ( $T_e$ ), the corresponding wavelengths ( $\lambda_{emi}$ ) and oscillator strengths (f) of the emission of Red\_1\_Oxy and Red\_2\_Oxy analogues in gas phase and in implicit solvent (water) at the QM level.

compounds						
	Red_1_Oxy			Red_2_Oxy		
	T <sub>e</sub> (eV)	λ <sub>emi</sub> (nm)	f	T <sub>e</sub> (eV)	λ <sub>emi</sub> (nm)	f
Gas phase	2.07	599	0.67	1.97	628	0.59
PCM (water)	2.1	595	0.97	2.0	622	0.86



**Figure S13** Natural transition orbitals (NTOs) of Red\_1\_Oxy and Red\_2\_Oxy analogues computed at the equilibrium geometry of S1 corresponding to S1-> S0 electronic transition (emission) in gas phase, in water (PCM) and in protein. The NTOs are plotted using Avogadro package.