Electronic Supplementary Material (ESI) for Physical Chemistry Chemical Physics.

SUPPLEMENTARY INFORMATIONS

QM/MM calculations on a new synthesised oxyluciferin substrate: new insights on the conformational effect

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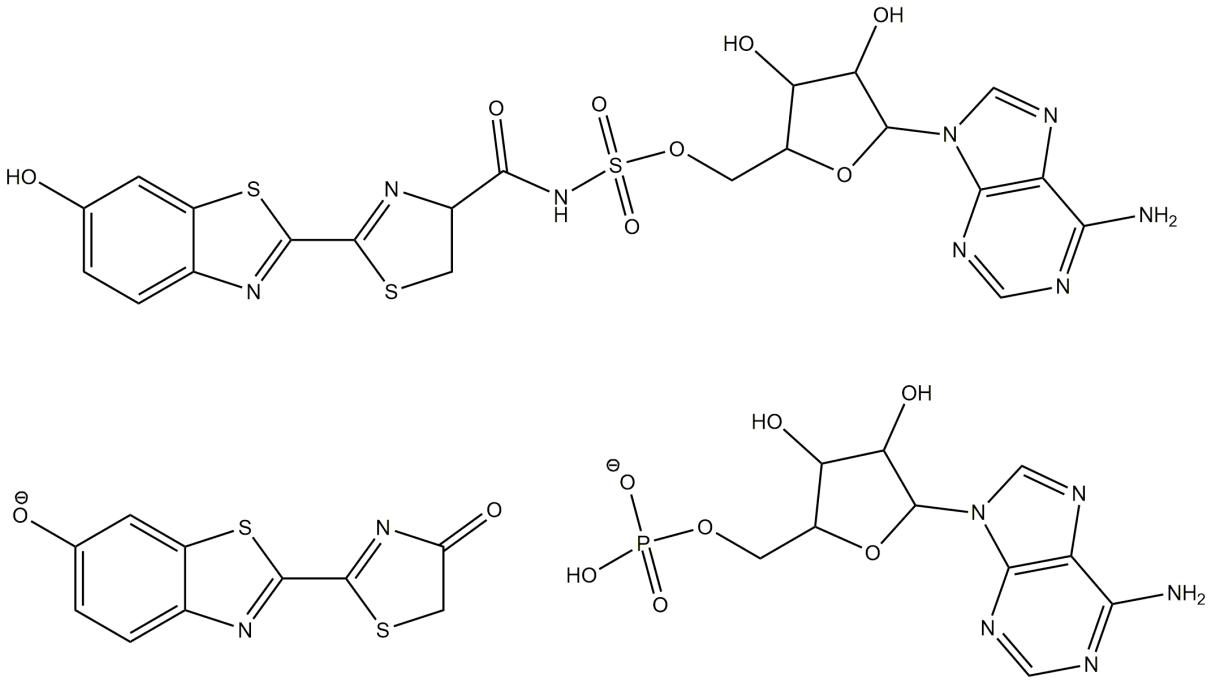

Figure S1. Graphical representation of the molecule DLSA (top) and the complex $OxyLH_2$ + AMPH (bottom).

Table S1. TD-DFT/MM electronic transition between S1 and S0 (T_e) with a small 6-31G(d,p) basis set and associated oscillator strength of models after dynamic and optimisation. For each case, T_e is given in eV, the corresponding wavelength in bracket in nm and the and associated oscillator strength after "f=".

Model	CAM-B3LYP	B3LYP	Exp.
Ser_$OxyLH_2$	2.54 (488) f=0.59	2.20 (562) f=0.34	2.22 (558)
Ser_$OxyiLH_2$-1	2.25 (551) f=1.13	Not converged f=0.00	1.85 (670)
Ser_$OxyiLH_2$-2	2.13 (582) f=0.89	1.90 (651) f=0.48	
Thr_$OxyLH_2$	2.46 (505) f=0.60	2.17 (573) f=0.34	2.04 (605)
Thr_$OxyiLH_2$-1	2.26 (548) f=1.12	1.69 (735)[a] f=0.00	1.75 (706)
Thr_$OxyiLH_2$-2	2.17 (572) f=0.87	1.90 (653) f=0.47	

[a] forbidden transition between HOMO-1 and LUMO

Table S2. TD-DFT/MM electronic transitions between S1 and S0 (T_e) (and associated oscillator strengths), with a 6-311G(2d,p) basis set for different functionals, of models obtained after classical dynamic and QM/MM optimisation using the same functional, with associated oscillator strengths.

Model	Ser_OxyLH_2	Ser_OxyiLH_2-1	Ser_OxyiLH_2-2
B3LYP	2.20 (562) f=0.34	2.00 (616) f= 0.53	1.87 (663) f=0.53
M06	2.28 (544) f=0.37	2.04 (607) f=0.64	1.91 (651) f=0.52
CAM-B3LYP	2.54 (488) f=0.59	2.23 (554) f=1.13	2.10 (590) f=1.13
M062X	2.53 (490) f=0.57	2.21 (559) f=1.11	2.07 (597) f=0.83
ωB97XD	2.54 (487) f=0.57	2.2 (563) f=1.11	2.13 (581) f=0.81
Exp. eV (nm)	2.22 (558)	1.85 (670)	

Table S3. Electronic transitions between S1 and S0 (T_e) with a 6-311G(2d,p) basis set and CAM-B3LYP or B3LYP functionals in different solvents, with associated oscillator strengths.

Model	CAM-B3LYP	B3LYP
	eV (nm)	eV (nm)
in water (PCM)		
$OxyLH_2$	2.24 (558) f=0.53	2.09 (592) f=0.89
$OxyiLH_2$-1	1.90 (651) f=1.70	1.84 (672) f=1.38
$OxyiLH_2$-2	1.83 (678) f=1.53	1.77 (700) f=1.23
in protein (QM/MM)		
Ser_$OxyLH_2$	2.53 (490) f=0.57	2.20 (564) f=0.33
Ser_$OxyiLH_2$-1	2.23 (554) f=1.13	2.00 (616) f=0.53
Ser_$OxyiLH_2$-2	2.10 (590) f=1.13	1.87 (663) f=0.53
in chloroform (PCM)		
$OxyLH_2$	2.34 (529) f=1.01	2.23 (554) f=1.15
$OxyiLH_2$-1	2.05 (605) f=1.56	1.92 (643) f=1.21
$OxyiLH_2$-2	1.97 (630) f=1.40	1.85 (670) f=1.08
in vacuum		
$OxyLH_2$	2.52 (482) f=0.67	2.27 (547) f=0.41
$OxyiLH_2$-1	2.36 (525) f=1.11	2.13 (580) f=0.76
$OxyiLH_2$-2	2.26 (547) f=1.02	2.03 (608) f=0.65

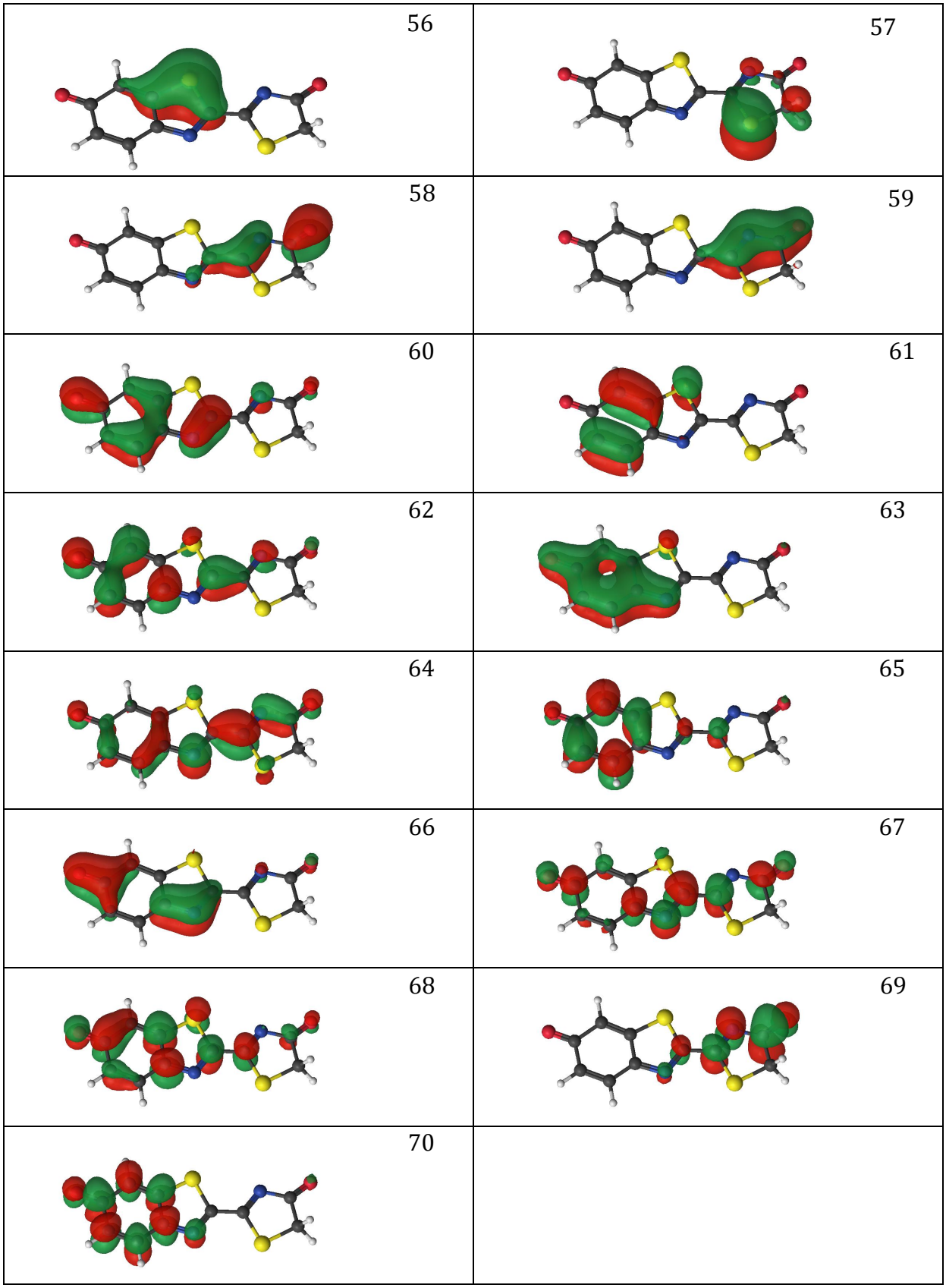

Figure S2. The selected 15 active CASSCF orbitals of $OxyLH_2$ molecule, HOMO orbital is number 62, LUMO 64. The removal of the orbital 57 gives the 16-in-14 active space while the removal of both number 56 and 57 give the 14-in-13 active space.

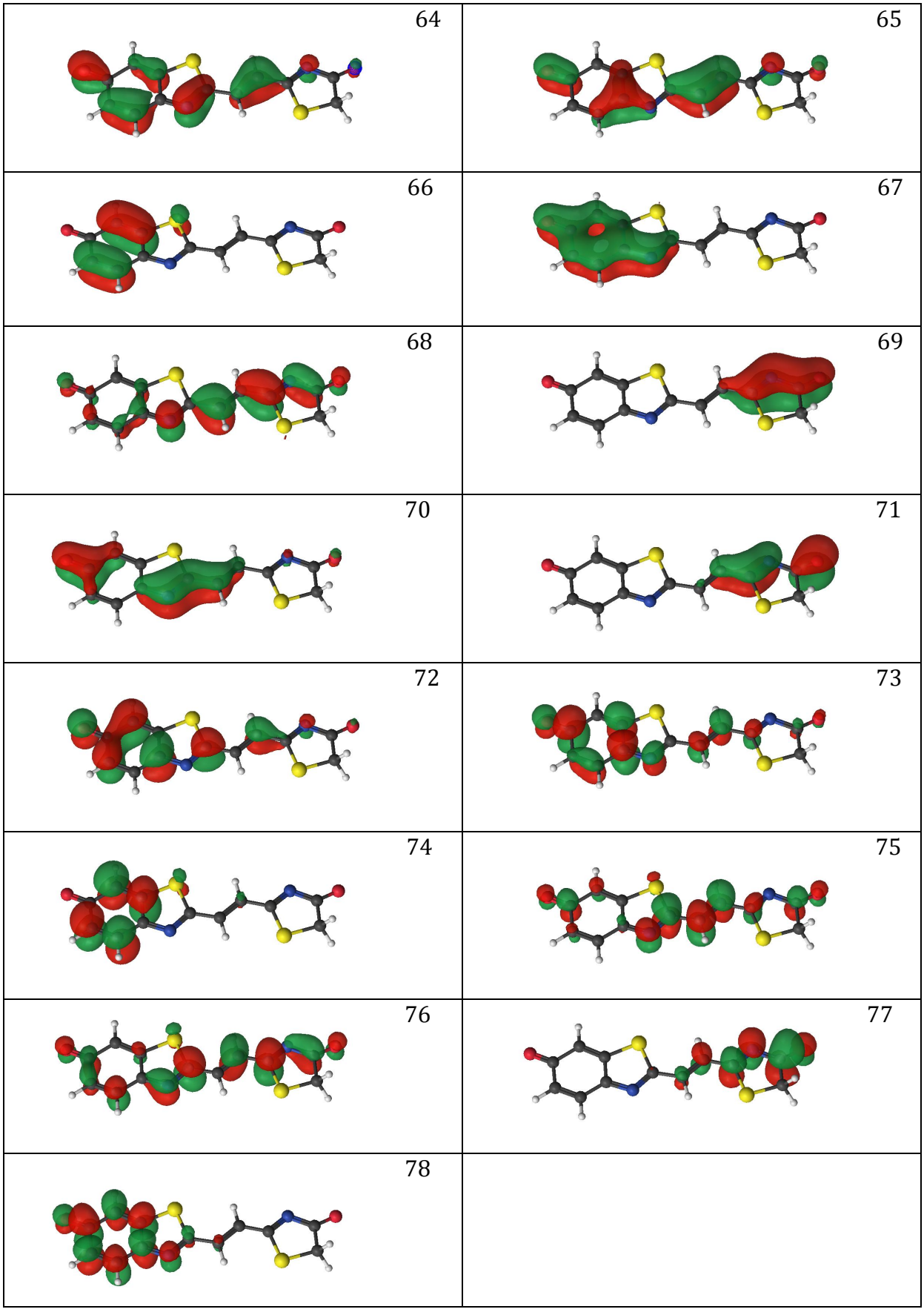

Figure S3. The selected 15 active CASSCF orbitals of OxyiLH$_2$ molecule, HOMO orbital is number 72, LUMO 68.

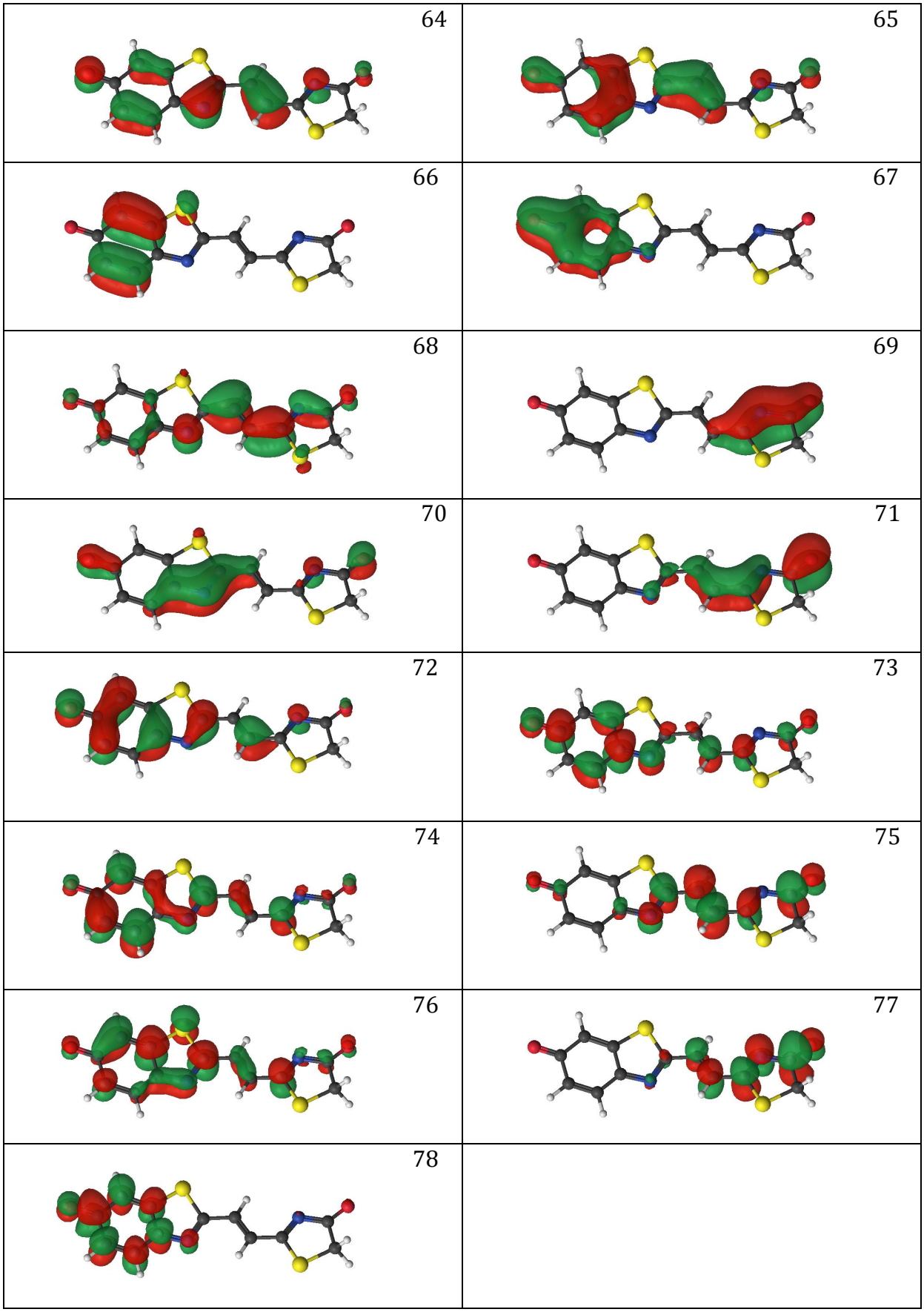

Figure S4. The selected 15 active CASSCF orbitals of OxyiLH$_2$-rot molecule, HOMO orbital is number 72, LUMO 68.

Table S4. MS-CASPT2/MM electronic transition between S1 and SO (T_e) with ANO-RCC-VTZP basis set with 2 states and a 0.1 level shift.

Model	Active Space	MS-CASPT2 in eV (nm) IPEA= 0.25	MS-CASPT2 in eV (nm) IPEA= 0.1	Exp. eV (nm)
Ser_OxyLH$_2$	14/13 16/14 18/15	2.41 (513) 2.30 (531) 2.37 (523)	2.24 (554) 2.14 (579) /	2.22 (558)
Ser_OxyiLH$_2$-1 Ser_OxyiLH$_2$-2	16/15 16/15	2.35 (527) 2.06 (601)	2.19 (566) 1.92 (645)	1.85 (670)
Thr_OxyLH$_2$	14/13 16/14 18/15	2.39 (518) 2.32 (534) 2.32 (532)	2.07 (598) 2.15 (577) /	2.04 (605)
Thr_OxyiLH$_2$-1 Thr_OxyiLH$_2$-2	16/15 16/15	2.35 (526) 2.09 (592)	2.15 (578) 1.93 (643)	1.75 (706)
vacuum OxyLH$_2$	14/13 16/14 18/15	2.49 (498) 2.38 (519) 2.42 (512)	2.33 (532) 2.25 (550) /	
vacuum OxyiLH$_2$-1	16/15	2.16 (572)	2.02 (614)	
vacuum OxyiLH$_2$-2	16/15	2.07 (597)	1.93 (642)	

Table S5. MS-CASPT2/MM electronic transition between S1 and SO (T_e) with ANO-RCC-VTZP basis set with an IPEA shift = 0.25.

Model	Active Space	MS-CASPT2 in eV (nm) 2 roots	MS-CASPT2 in eV (nm) 4 roots	Exp. eV (nm)
Ser_OxyLH$_2$	14/13 16/14 18/15	2.41 (513)[a] 2.30 (531)[a] 2.37 (523)[a]	2.41 (512)[b] 2.32 (532)[b] 2.32 (532)[b]	2.22 (558)
Ser_OxyiLH$_2$-1 Ser_OxyiLH$_2$-2	16/15 16/15	2.35 (527)[a] 2.06 (601)[a]	2.35 (527)[b] 2.04 (606)[b]	1.85 (670)
Thr_OxyLH$_2$	14/13 16/14 18/15	2.39 (518)[a] 2.32 (534)[a] 2.32 (532)[a]	2.39 (518)[b] 2.32 (534)[b] 2.33 (533)[b]	2.04 (605)
Thr_OxyiLH$_2$-1 Thr_OxyiLH$_2$-2	16/15 16/15	2.35 (526)[a] 2.09 (592)[a]	2.36 (524)[b] 2.05 (605)[a]	1.75 (706)
vacuum OxyLH$_2$	14/13 16/14 18/15	2.49 (498)[a] 2.38 (519)[a] 2.42 (512)[a]	2.46 (504)[b] 2.38 (520)[b] 2.42 (512)[a]	
vacuum OxyiLH$_2$-1	16/15	2.16 (572)[a]		
vacuum OxyiLH$_2$-2	16/15	2.07 (597)[a]		

[a] with a 0.2 level shift

[b] with a 0.1 level shift

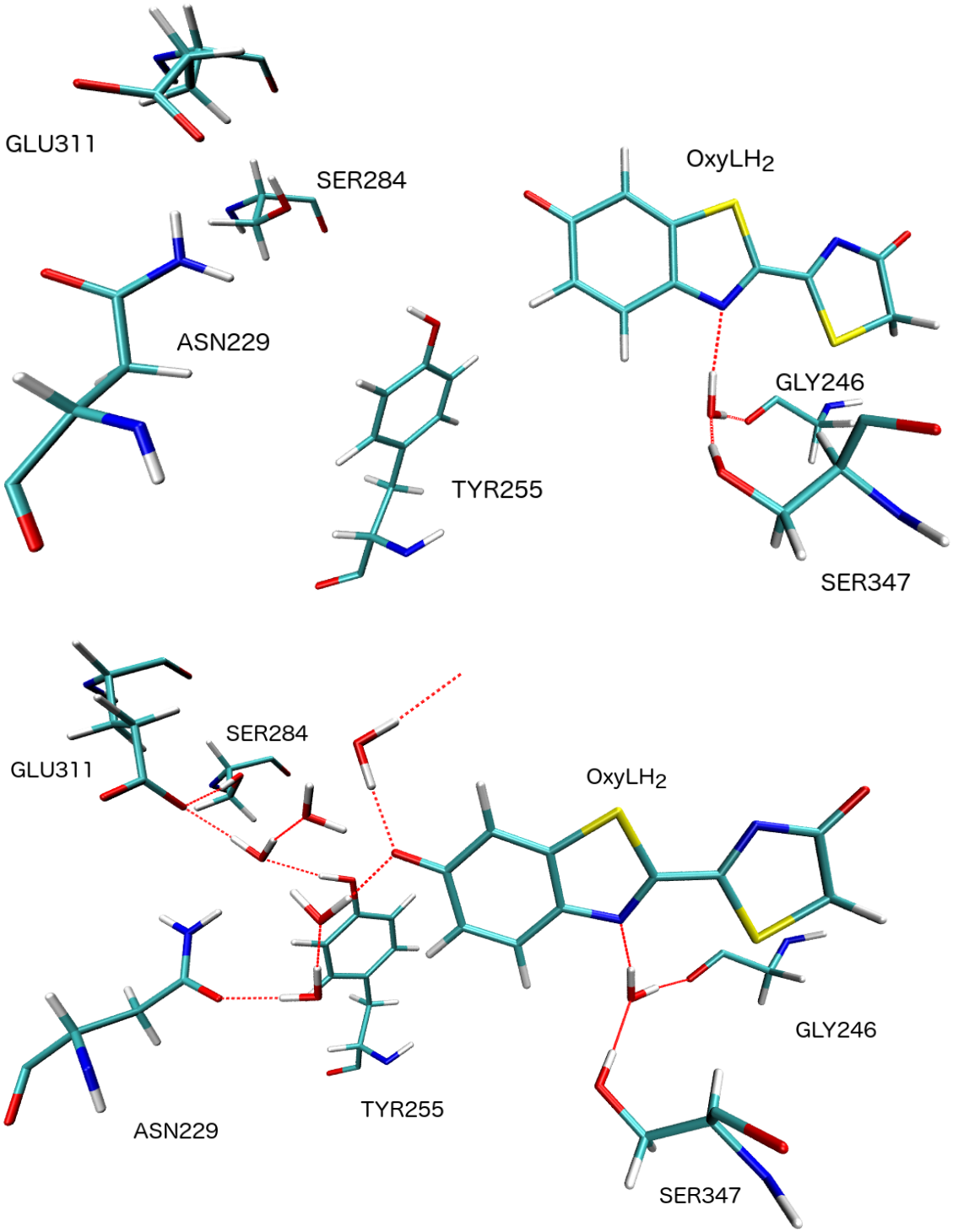

Figure S5. Representation of the H-bond network of the model Ser_OxyLH$_2$ before classical molecular dynamics (MD) (top figure) and Ser_OxyLH$_2$ after MD (bottom figure). The residues or part of the residues represented are involved in the H-bond network. Atoms are colours-coded as O red, H white, C cyan, N blue, S yellow, and P tan.

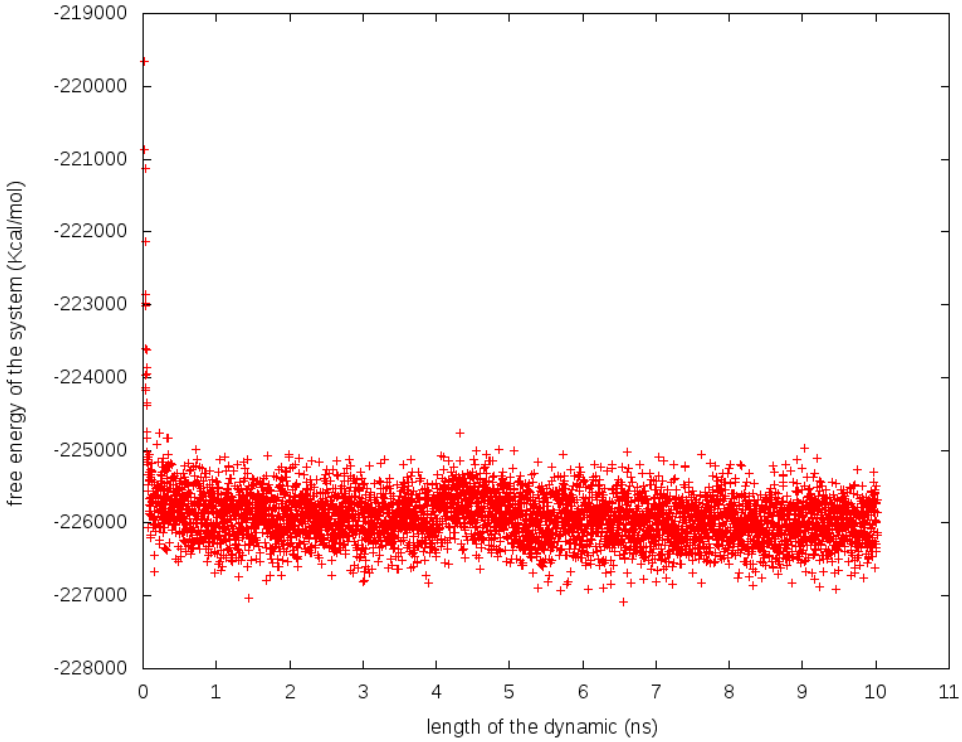

Figure S6. Graphical representation of the energy of the model Ser_OxyLH$_2$ during the classical molecular dynamics simulation (MD).

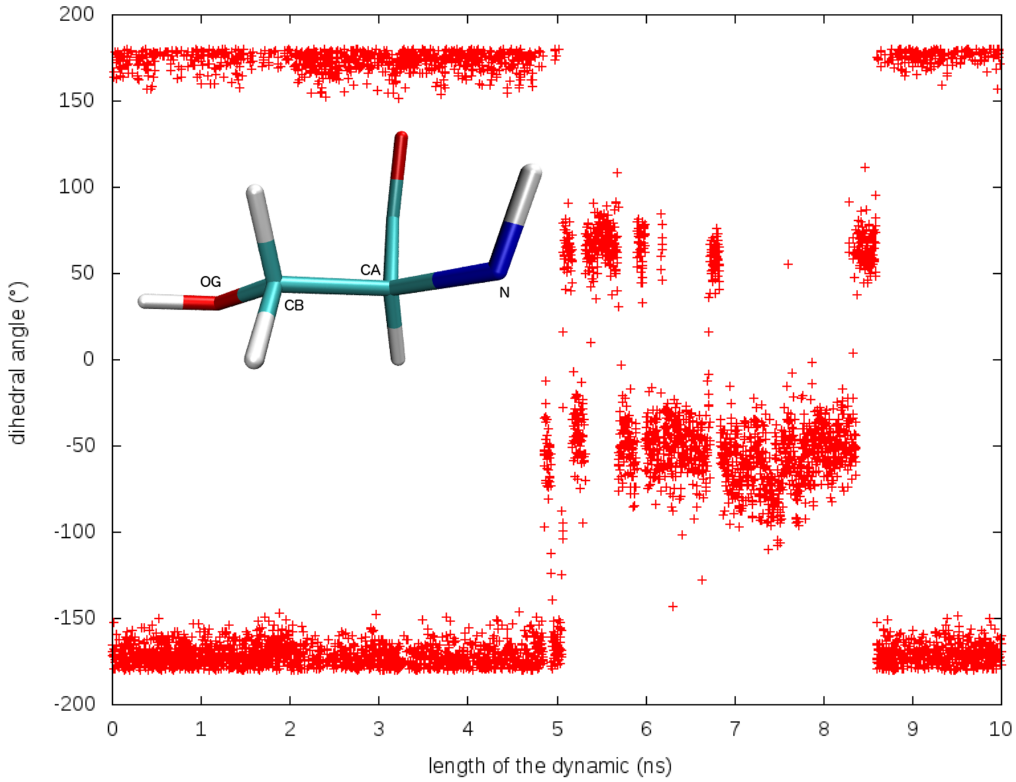

Figure S7. Graphical representation of the dihedral angle N-CA-CB-OG of the residue Serine 284 in the model Ser_OxyLH$_2$ during the classical molecular dynamics simulation (MD).

Table S6. QM/MM electronic transition between S1 and S0 (T_e) in eV (nm). Comparison of the model Ser_OxyLH_2 before and after classical molecular dynamics (MD). TD-DFT/MM calculations are optimised with the corresponding functional (either CAM-B3LYP or B3LYP) and with the 6-31G(d,p) basis set. MS-CASPT2/MM calculations are computed from the CAM-B3LYP 6-31G(d,p) optimized geometry and with the ANO-RCC-VTZP basis set with an IPEA shift = 0.25.

Model	Ser_OxyLH_2	Ser_OxyLH_2	Exp.
	Before MD	After MD	
CAM-B3LYP	2.28 (544) f=0.56	2.54 (488) f=0.59	2.22 (558)
B3LYP	1.98 (623)[a] f=0.00	2.20 (562) f=0.34	
MS-CASPT2 13-in-14	2.31 (536)	2.41 (513)	
MS-CASPT2 16-in-14	2.21 (557)	2.33 (531)	

[a] forbidden transition between HOMO-1 and LUMO

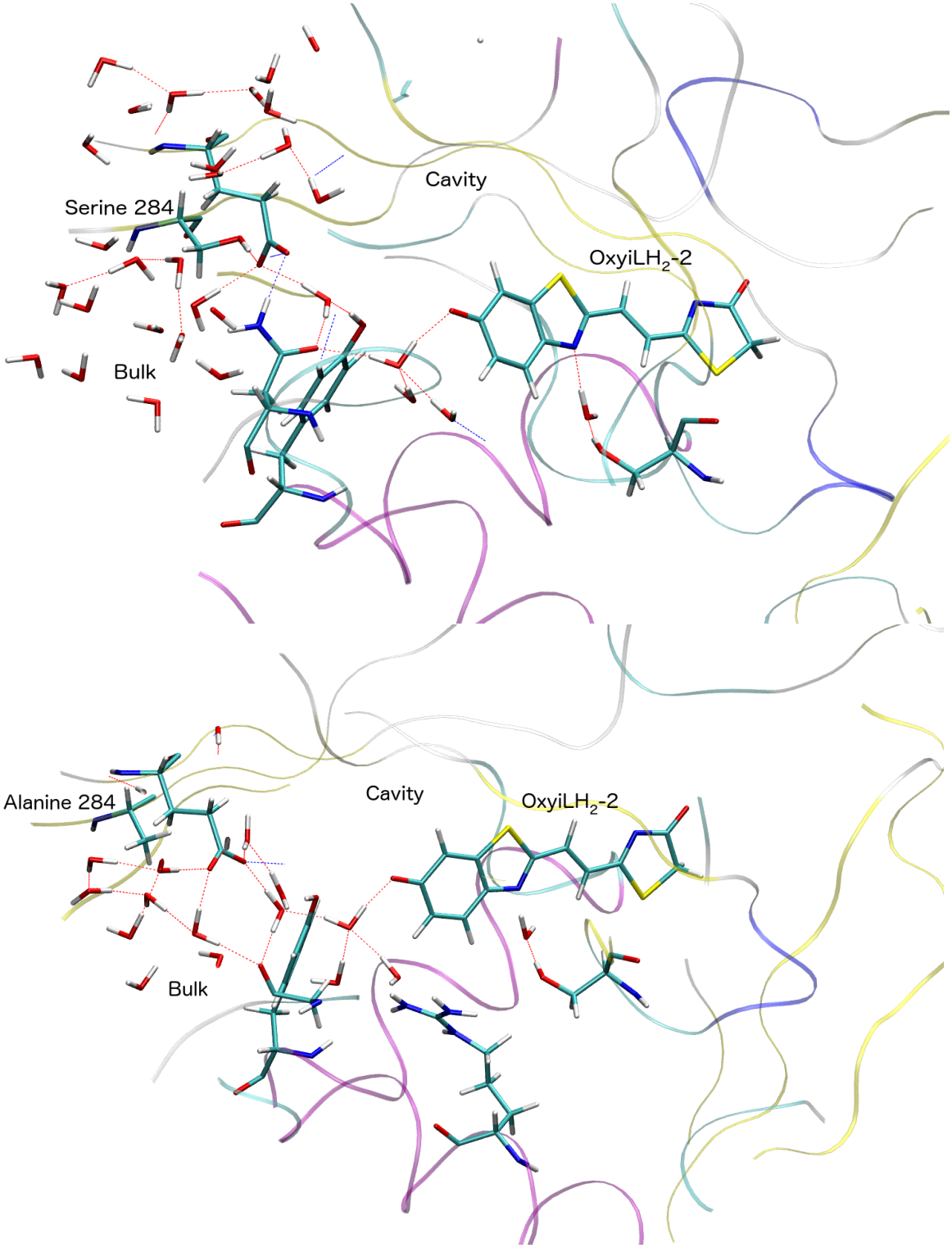

Figure S8. Representation of the H-bond network of the model Ser_OxyiLH$_2$-2 (top figure) and Ala_OxyiLH$_2$-2 (bottom figure). The residues or part of the residues represented are involved in the H-bond network. Atoms are colours-coded as O red, H white, C cyan, N blue, S yellow, and P tan.

When the residue 284 is a polar one (Serine), it can interact with the H-bond network in the cavity of the substrate. However when the residue 284 is non-polar (Alanine), it does not participate in the H-bond network. Moreover, the water bulk is linked to the residue in the cavity as well as the H-bond network through the Glutamic acid 311.

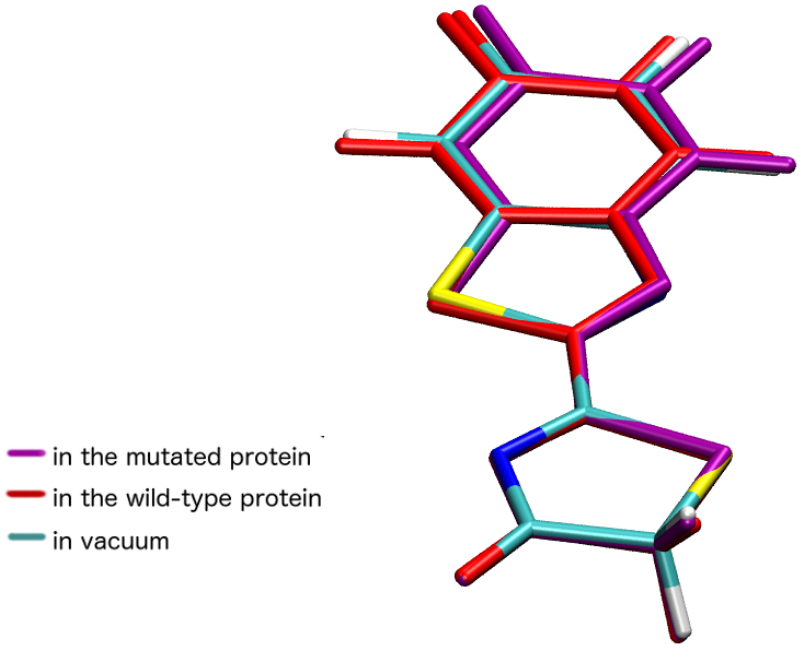

Figure 9. Comparison of the structures of $OxyLH_2$. Molecules are colour-coded as: in vacuum cyan; in wild-type protein red; in mutated protein purple.

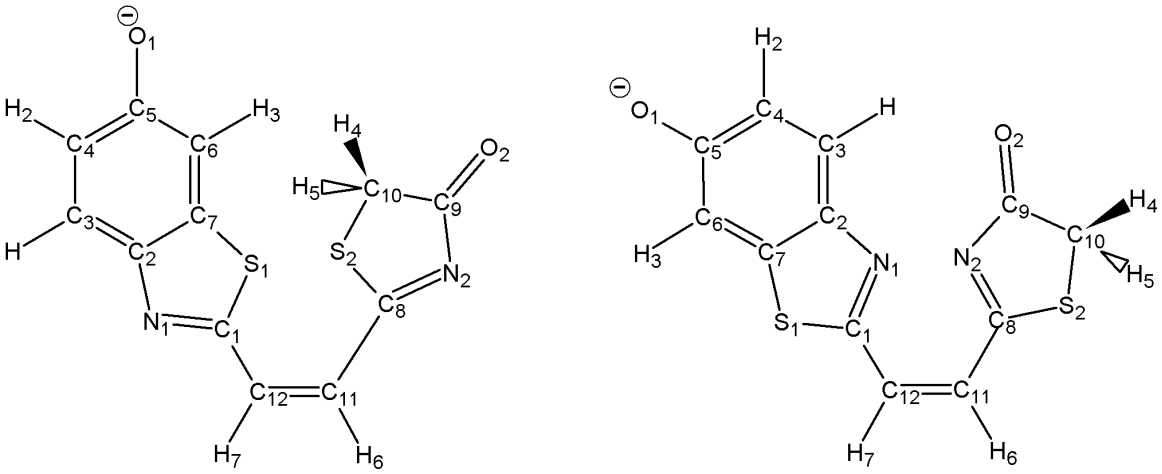

Figure S10. Structures of $OxyiLH_2$-Z-1 (left) and $OxyiLH_2$-Z-2 (right).

Table S7. TD-DFT/MM electronic transition between S1 and S0 (T_e) in eV (nm). with 6-311G(2d,p) basis set and associated oscillator strength.

Model	CAM-B3LYP	B3LYP	Exp.
Ser_OxyiLH$_2$-Z-1	2.13 (579) f=0.67	1.88 (657) f=0.33	1.85 (670)
Ser_OxyiLH$_2$-Z-2	2.18 (567)	1.89 (654) f=0.36	
Thr_OxyiLH$_2$-Z-1	/	/	1.75 (706)
Thr_OxyiLH$_2$-Z-2	2.20 (564) f=0.72	1.97 (627) f=0.41	

Table S8. Cartesian coordinates of the TD-DFT-B3LYP 6-311G(2d,p) optimized S1 State of OxyiLH$_2$-1 (in Å) in vacuum

O	0.191066	-0.000494	-0.089039
C	0.110142	-0.000200	1.125968
N	1.165616	-0.000159	2.006728
C	-1.276230	0.000157	1.821125
H	-1.837061	-0.886242	1.523640
H	-1.836794	0.886597	1.523257
S	-0.958894	0.000496	3.610122
C	0.816964	0.000170	3.268107
C	2.410258	0.000709	6.757306
N	2.079987	0.001051	8.018068
C	3.191775	0.001096	8.847656
C	3.192671	0.001365	10.255913
H	2.235285	0.001551	10.767671
C	4.371810	0.001379	10.964850
H	4.369953	0.001563	12.048769
C	5.672904	0.001202	10.324262
O	6.757851	0.000953	10.947799
C	5.650866	0.000742	8.865388
H	6.604551	0.000465	8.352451
C	4.435295	0.000766	8.185566
S	4.173804	0.000366	6.459207
C	1.765645	0.000273	4.335043
H	2.798272	0.000089	4.000343
C	1.469321	0.000579	5.661302
H	0.434297	0.000769	5.989345

Table S9. Cartesian coordinates of the TD-DFT-B3LYP 6-311G(2d,p) optimized S1 State of OxyiLH$_2$-2 (in Å) in vacuum

O	5.829365	-1.817143	-0.000010
C	5.073015	-0.864358	-0.000004
N	3.701929	-0.916724	0.000000
C	5.639993	0.583255	-0.000002

H	6.256594	0.733708	0.886390
H	6.256588	0.733713	-0.886399
S	4.206143	1.695368	0.000005
C	3.115696	0.253027	0.000005
C	-0.633178	-0.276950	0.000015
B	-1.250579	0.869977	0.000023
C	-2.629280	0.717522	0.000017
C	-3.594025	1.742388	0.000017
H	-3.245585	2.770618	0.000028
C	-4.941010	1.455696	0.000000
H	-5.678466	2.250039	-0.000002
C	-5.454959	0.100619	-0.000020
O	-6.672311	-0.188356	-0.000027
C	-4.441223	-0.949462	-0.000012
H	-4.787321	-1.975572	-0.000018
C	-3.086999	-0.616710	0.000004
S	-1.719998	-1.693778	0.000027
C	1.710999	0.479991	0.000010
H	1.361745	1.506239	0.000013
C	0.787124	-0.524587	0.000011
H	1.126846	-1.552627	0.000009

Table S10. Cartesian coordinates of the TD-DFT-B3LYP 6-311G(2d,p) optimized S1 State of Ser_OxyiLH_2-1 (in Å)

N	-0.120065	-0.891792	-0.888164
C	0.042011	0.143958	-0.040576
O	-0.781373	1.052368	0.148458
C	1.376347	0.149894	0.719873
H	1.884833	1.104701	0.595366
H	1.192646	-0.004046	1.781851
S	2.344469	-1.223936	0.036031
C	0.935162	-1.687007	-0.992039
C	0.991921	-2.798363	-1.854390
H	0.070089	-2.995037	-2.390595
C	2.105785	-3.574177	-2.084350
H	3.029713	-3.338755	-1.563477
C	2.233463	-4.658960	-2.994618
N	3.327379	-5.367659	-3.209888
C	3.172011	-6.254994	-4.249603
C	4.156566	-7.082909	-4.819601
H	5.153098	-7.074810	-4.385234
C	3.892526	-7.863622	-5.924471
H	4.663847	-8.486306	-6.355816
C	2.599215	-7.891627	-6.570184
O	2.327938	-8.564111	-7.583670
C	1.562502	-7.058680	-5.949359
H	0.569898	-7.068693	-6.382693
C	1.883306	-6.269264	-4.846765

S	0.858709	-5.135174	-4.041087

Table S11. Cartesian coordinates of the TD-DFT-B3LYP 6-311G(2d,p) optimized S1 State of Ser_OxyiLH_2-2 (in Å)

N	0.497955	-0.904990	-0.735253
C	-0.043263	0.091420	0.008222
O	-1.025177	0.756981	-0.295640
C	0.673532	0.355732	1.343069
H	0.919296	1.408198	1.460982
H	-0.011139	0.070762	2.134595
S	2.145538	-0.700820	1.345096
C	1.596618	-1.408721	-0.213349
C	2.359057	-2.445024	-0.778665
H	3.229459	-2.784668	-0.232609
C	2.032599	-3.070776	-1.954812
H	1.146588	-2.759566	-2.491266
C	2.811111	-4.146178	-2.467439
N	3.973678	-4.565175	-2.009773
C	4.334503	-5.769708	-2.583773
C	5.431713	-6.574661	-2.248328
H	6.082472	-6.244590	-1.444324
C	5.661586	-7.765955	-2.898932
H	6.500027	-8.387093	-2.630585
C	4.824238	-8.258343	-3.966041
O	5.061972	-9.291876	-4.631131
C	3.637862	-7.458225	-4.249218
H	2.948253	-7.811661	-5.004485
C	3.440722	-6.254371	-3.568184
S	2.115796	-5.163694	-3.736155

Table S12. Cartesian coordinates of the TD-DFT-B3LYP 6-311G(2d,p) optimized S1 State of Thr_OxyiLH_2-1 (in Å)

O	-0.872348	0.960084	0.110241
C	-0.009400	0.071345	0.053979
N	0.045515	-0.908078	-0.871717
C	1.120289	0.035802	1.095703
H	1.628471	0.997743	1.139848
H	0.700896	-0.170370	2.079984
S	2.240193	-1.289916	0.577295
C	1.110565	-1.688211	-0.775842
C	1.383318	-2.755931	-1.651138
H	0.625689	-2.923255	-2.408809
C	2.516410	-3.530912	-1.609349
H	3.280029	-3.308356	-0.869382
C	2.838664	-4.645095	-2.432347
N	3.911300	-5.399170	-2.318422

C	3.929448	-6.411043	-3.255486
C	4.879523	-7.433700	-3.394072
H	5.695593	-7.485876	-2.680988
C	4.793318	-8.365828	-4.409691
H	5.557928	-9.123253	-4.529426
C	3.737240	-8.350960	-5.385314
O	3.698621	-9.086355	-6.402513
C	2.694838	-7.354257	-5.181700
H	1.868458	-7.324748	-5.879939
C	2.835531	-6.415300	-4.157729
S	1.739914	-5.142359	-3.756187

Table S13. Cartesian coordinates of the TD-DFT-B3LYP 6-311G(2d,p) optimized S1 State of Thr_OxyiLH$_2$-2 (in Å)

O	-1.220400	0.471796	0.006066
C	-0.071084	0.036997	0.087369
N	0.410968	-1.008200	-0.615895
C	0.953606	0.695105	1.037967
H	1.125560	1.738831	0.773774
H	0.563737	0.665714	2.051072
S	2.481810	-0.256252	0.875737
C	1.670219	-1.278860	-0.343793
C	2.405276	-2.317759	-0.948606
H	3.436795	-2.445582	-0.648025
C	1.881941	-3.163990	-1.883583
H	0.849006	-3.057850	-2.186497
C	2.691386	-4.195245	-2.460855
N	3.983918	-4.340077	-2.302892
C	4.435299	-5.523623	-2.867712
C	5.749136	-6.012925	-2.872757
H	6.513817	-5.422323	-2.377847
C	6.063345	-7.196392	-3.510040
H	7.080371	-7.563242	-3.543410
C	5.084932	-7.989886	-4.199715
O	5.340581	-9.064187	-4.807867
C	3.719656	-7.492456	-4.164106
H	2.951142	-8.074824	-4.655066
C	3.441554	-6.285263	-3.512889
S	1.912431	-5.485508	-3.389430