Electronic Supplementary Material (ESI) for RSC Advances.

Electronic Supplementary Information

Unraveling how the Gly526Ser mutation arrests prostaglandin formation from arachidonic acid catalyzed by cyclooxygenase-2: A combined molecular dynamics and QM/MM study.

Adrián Suñer-Rubio,[†] Anna Cebrián-Prats, [†] Àngels González-Lafont[†,‡] and José M. Lluch[†,‡ *]

[†]Departament de Química and [‡]Institut de Biotecnologia i de Biomedicina (IBB), Universitat Autònoma de Barcelona, 08193 Bellaterra, Barcelona, Spain

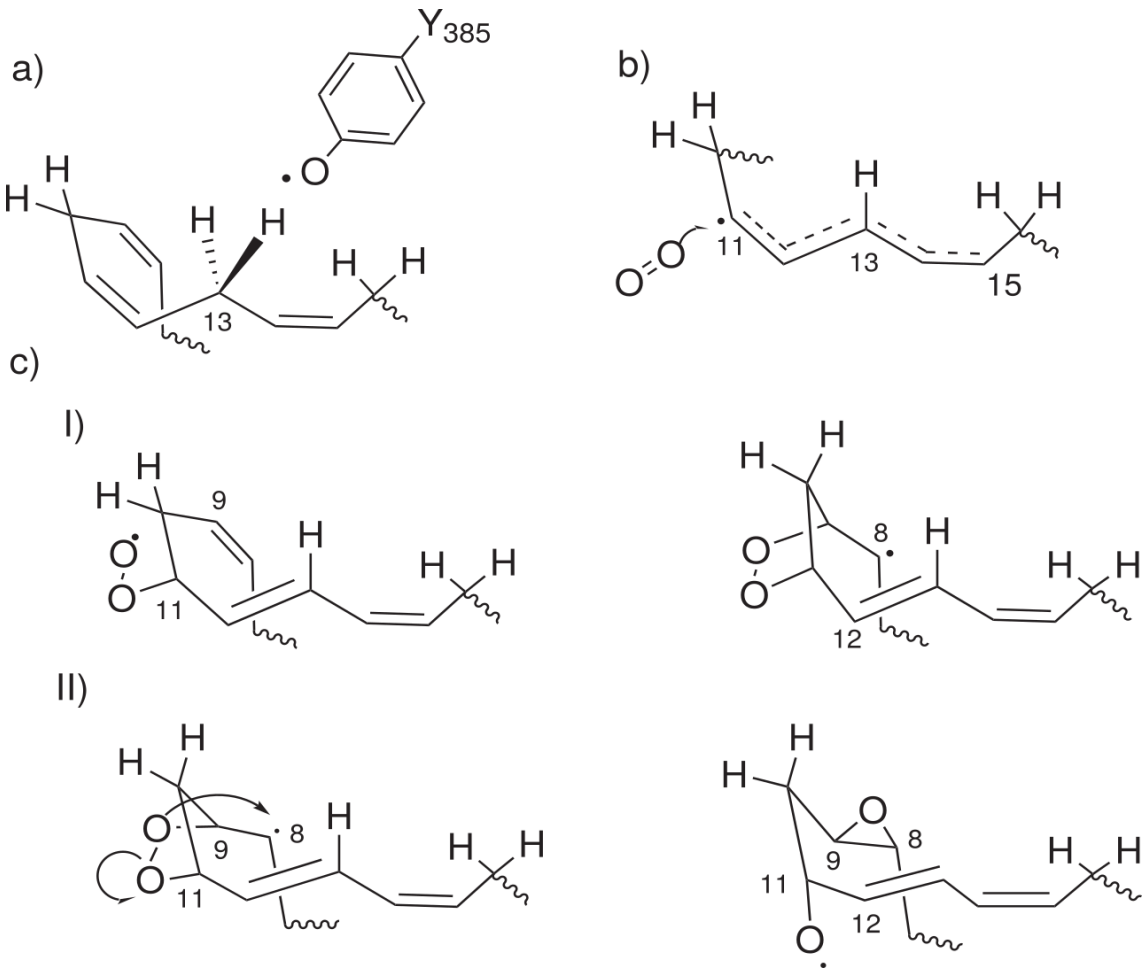

Figure S1. QM region of each particular reaction step. a) 38 atoms for the H-abstraction. b) 18 atoms for the O_2 addition at C_{11}. c) 25 atoms for: I) both cyclizations, the formation of the endoperoxide bridge and the cyclopentane ring and II) both epoxidations, the formation of 8,9-epoxide and 11,12-epoxide.

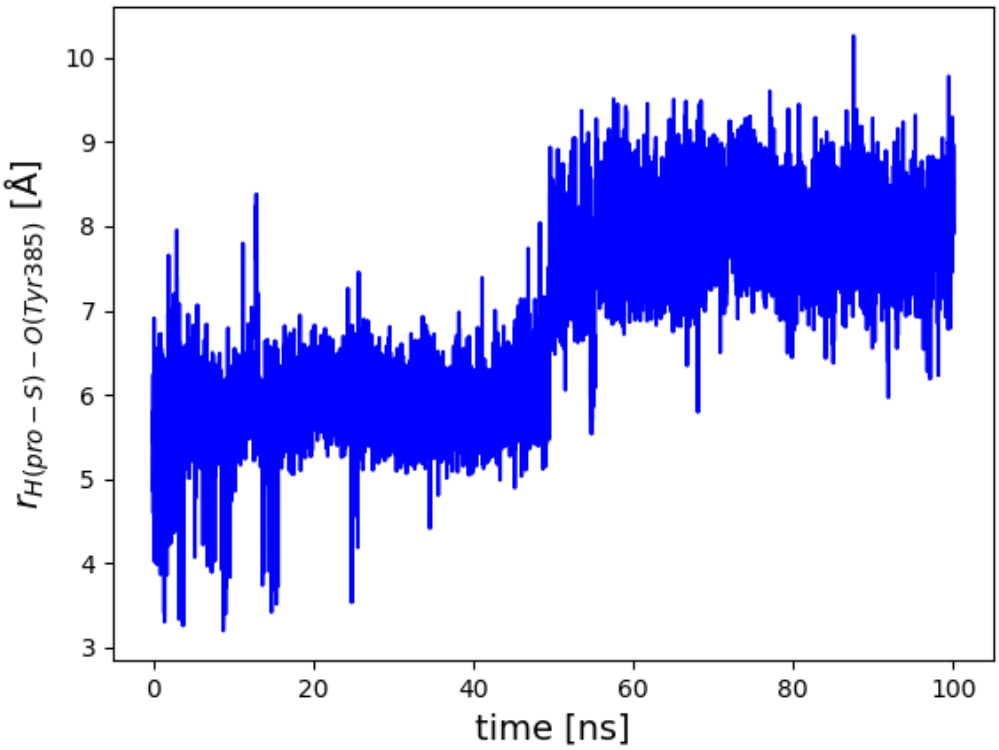

Figure S2. Evolution of the C_{13} pro-*S* hydrogen – O(Tyr385) distance along the 100 ns of the first MD simulation of the Gly526Ser COX-2/AA Michaelis complex.

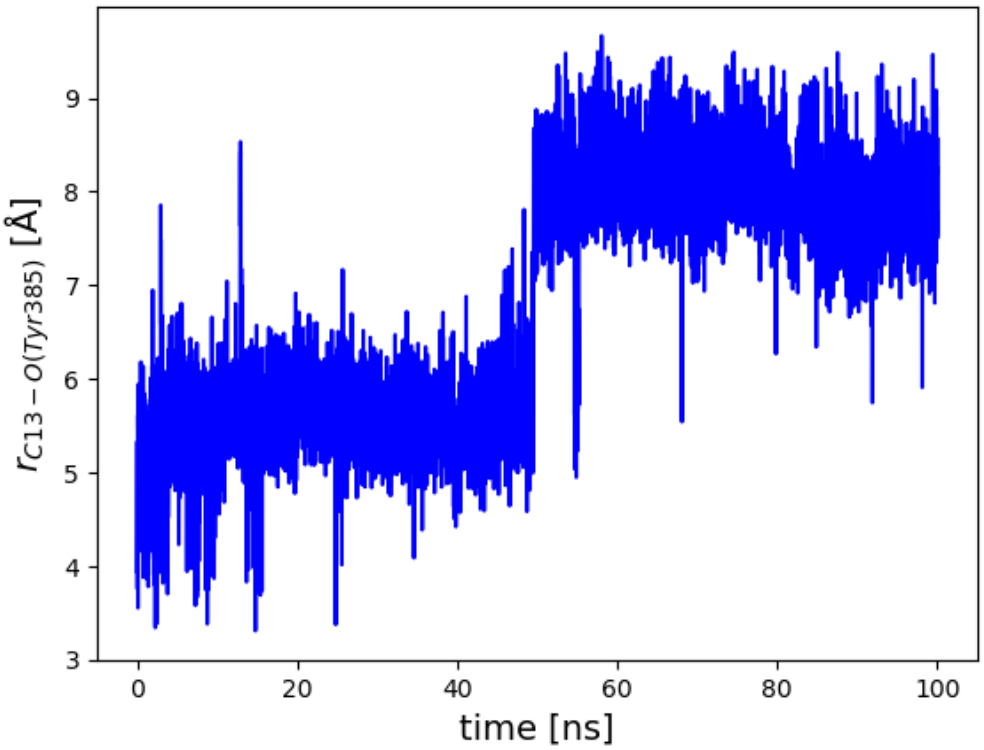

Figure S3. Evolution of the C_{13} – O(Tyr385) distance along the 100 ns of the first MD simulation of the Gly526Ser COX-2/AA Michaelis complex.

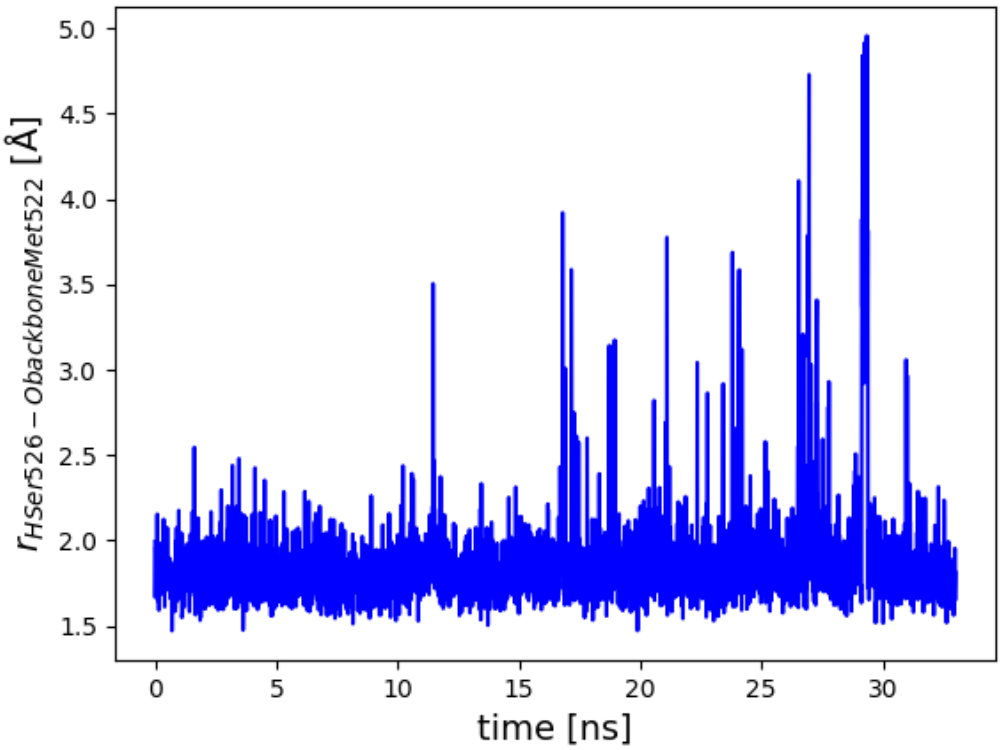

Figure S4. Distance between the hydrogen of the Ser526 OH group and the oxygen atom of the Met522 backbone along the 10 ns (equilibration) + 20 ns (production) MD simulation of the Gly526Ser COX-2 mutant enzyme without AA.

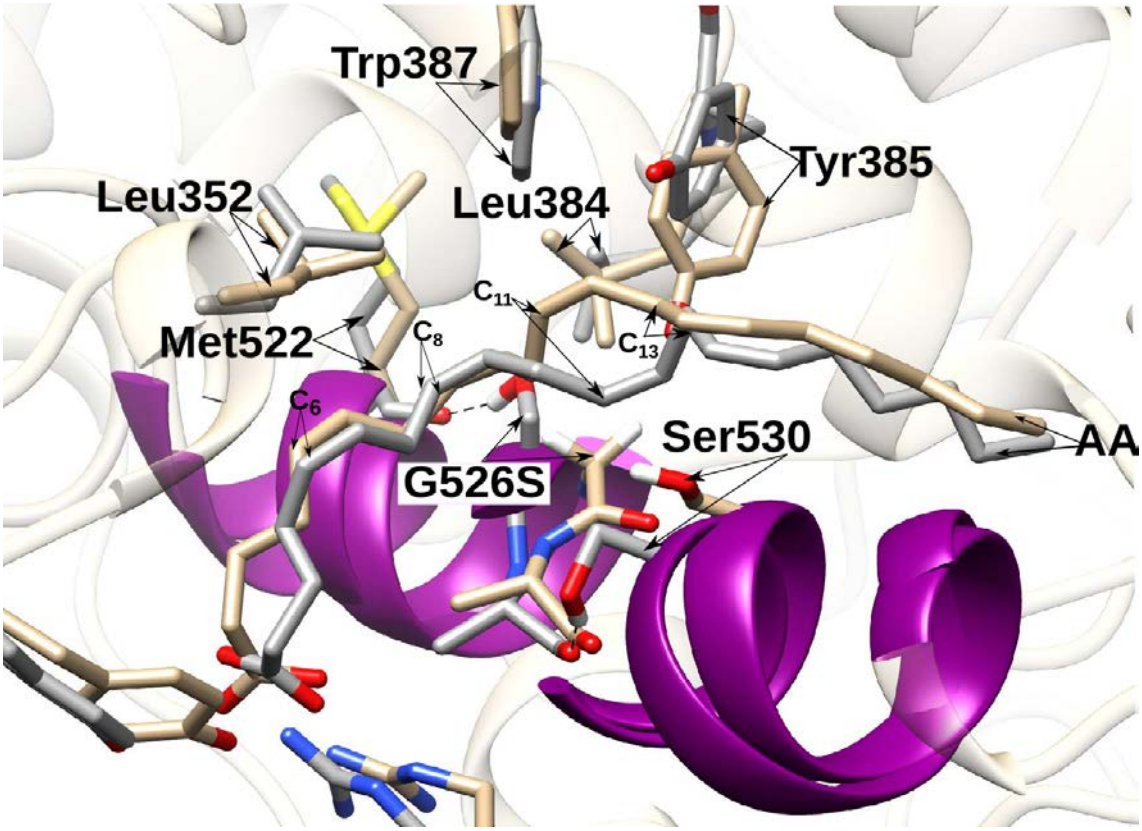

Figure S5. Comparison between the structure (in tan) corresponding to snapshot 1 extracted from the MD simulation of the COX-2/AA Michaelis complex (see reference 13) and the centroid snapshot (in grey) of the most populated cluster of the last MD simulation of the Gly526Ser COX-2/AA Michaelis complex shown in Figure 4. G526S stands for Gly or Ser for COX-2/AA or Gly526Ser COX-2/AA, respectively.

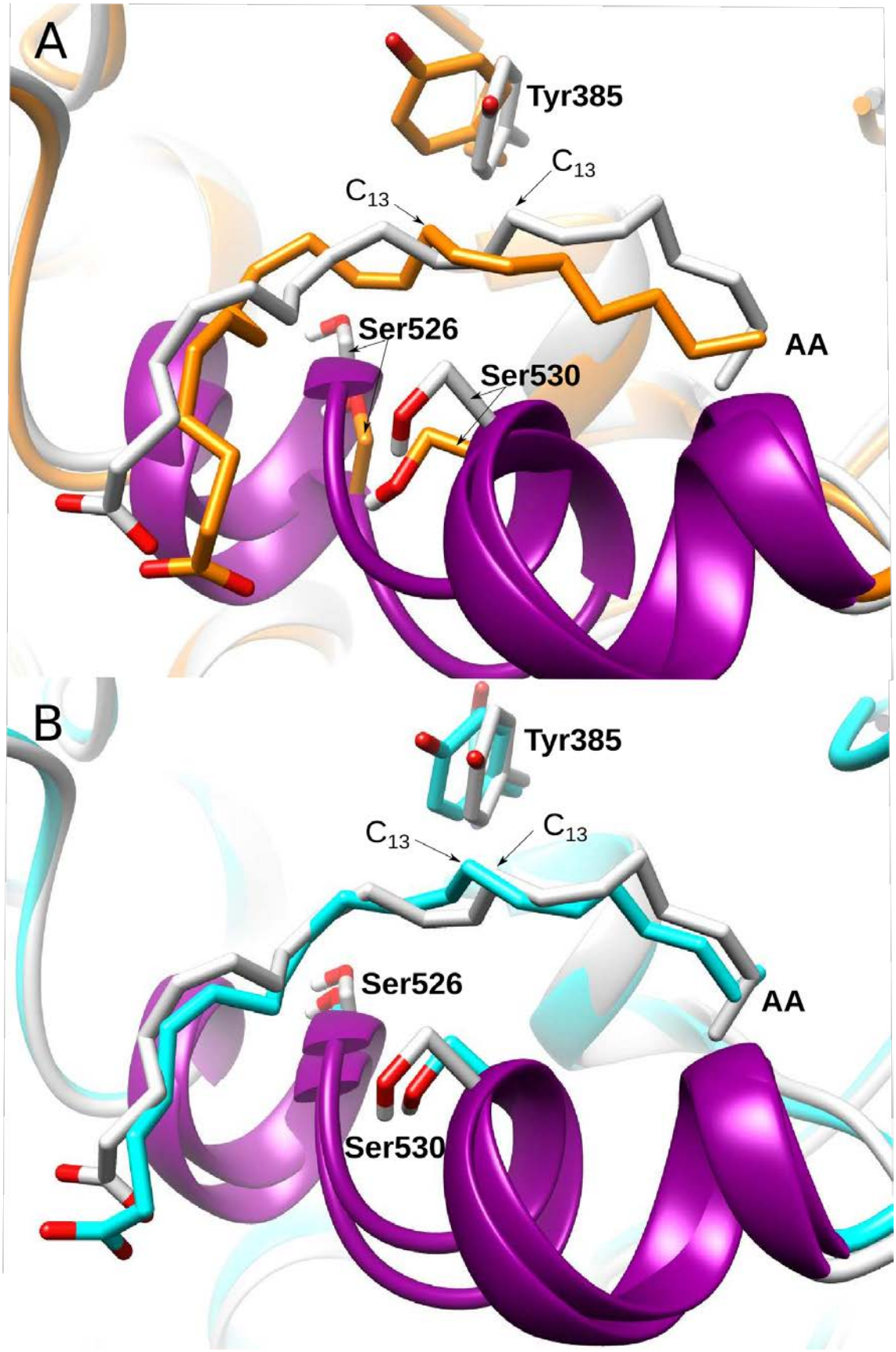
A
Tyr385
C13
C13
Ser526
Ser530
AA
B
Tyr385
C13
C13
Ser526
AA
Ser530

Figure S6. Comparison between the centroid of the most populated cluster (in grey) and A) the centroid of the second most populated cluster (in orange); B) the centroid of the third most populated cluster (in cyan).

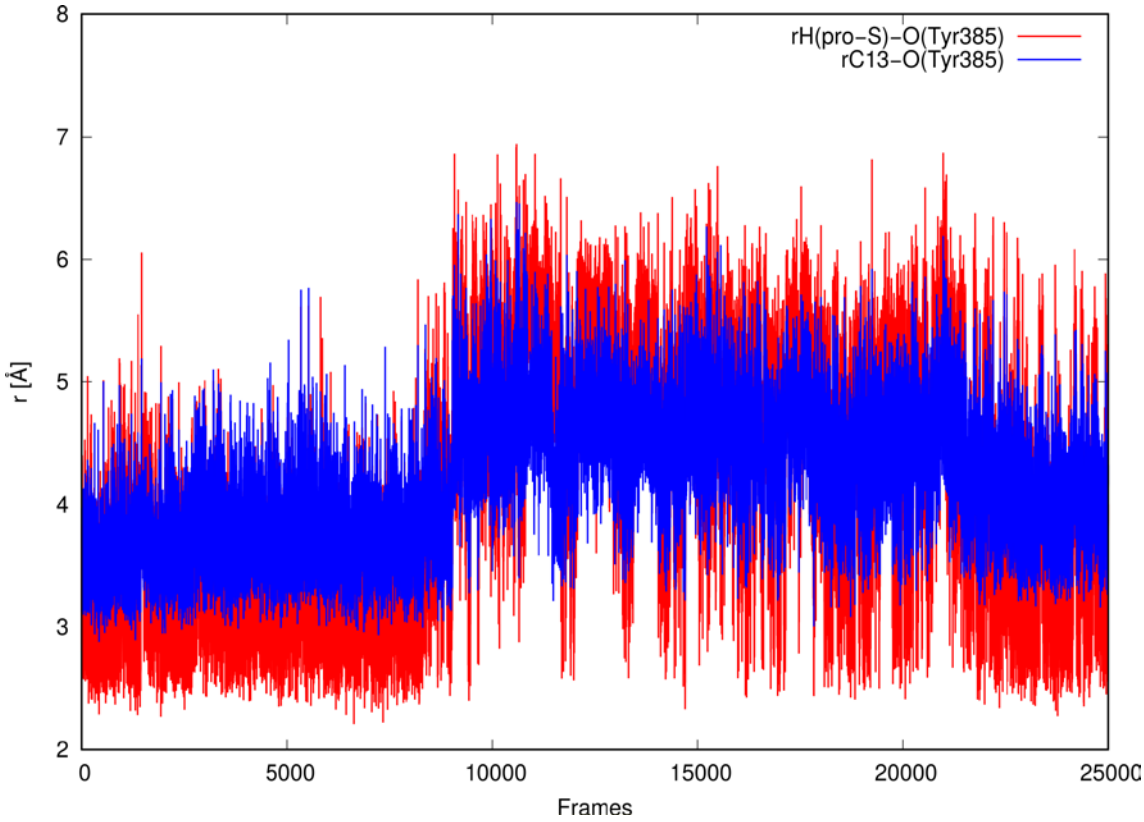

Figure S7. Evolution of the distances C_{13} pro-*S* hydrogen – O(Tyr385) (red line) and C_{13} – O(Tyr385) (blue line) along 250 ns of the last MD simulation of the Gly526Ser COX-2/AA Michaelis complex. The first 100 ns have already been pictured in Figure 3. A frame has been taken each 10 ps.

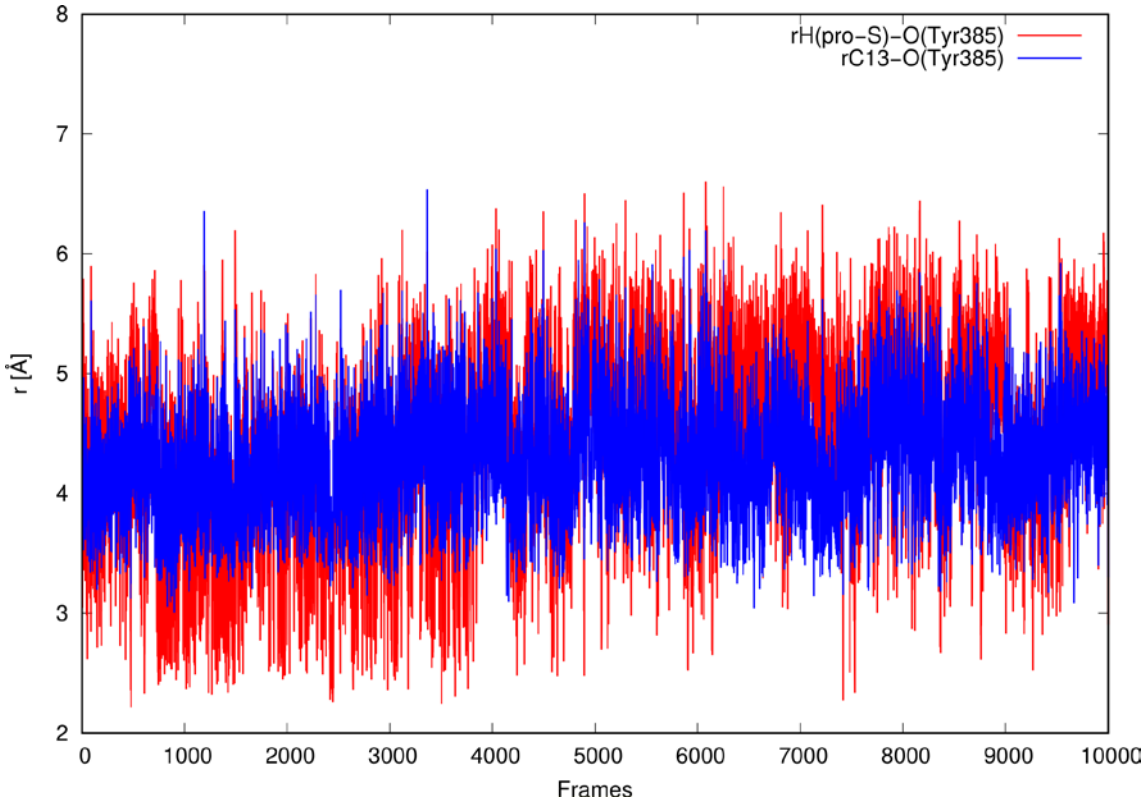

Figure S8. Evolution of the distances C_{13} pro-*S* hydrogen – O(Tyr385) (red line) and C_{13} – O(Tyr385) (blue line) along 100 ns MD simulation of the Gly526Ser COX-2/AA Michaelis complex. To carry out this MD simulation, we have selected at random one of the structures of the 20 ns MD simulation of the Gly526Ser COX-2 mutated enzyme, and after re-introducing AA in the cavity of the mutant as explained in section 3.1 of the main text, we have run the simulation. This new MD simulation is equivalent to the one shown in Figure 3, but starting from a different structure and different initial velocities. A frame has been taken each 10 ps.

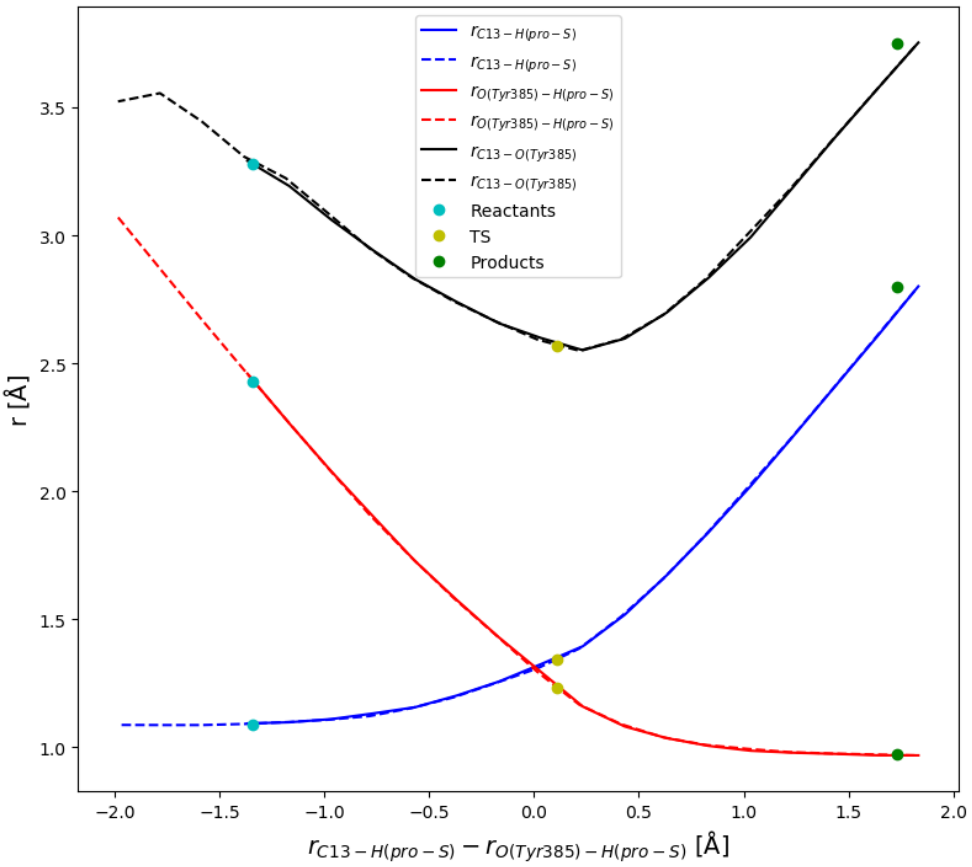

Figure S9. Evolution of the main distances along the C_{13} pro-*S* hydrogen abstraction by the tyrosyl radical. The points indicate the location of the stationary points. The continuous and dashed lines correspond to the forward and backward potential energy profiles, respectively.

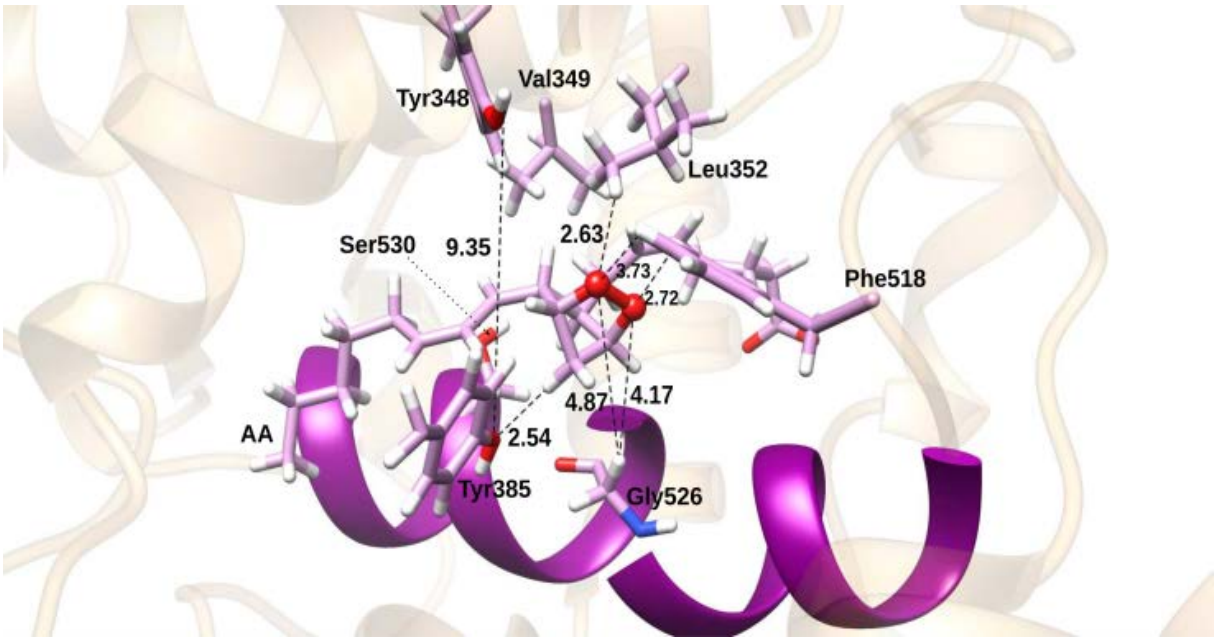

Figure S10. Structure obtained after the 8,12-cyclization to give a bicyclo endoperoxide that occurs in wild type COX-2.

Table S1. Distances (in Å) between side chain carbon atoms of selected residues of snapshot 1 extracted from the MD simulation of the COX-2/AA Michaelis complex (see reference 13) or the centroid snapshot of the most populated cluster of the last MD simulation of the Gly526Ser COX-2/AA Michaelis complex shown in Figure 4, and selected carbon atoms of arachidonic acid bound in the corresponding active site. G526S stands for Gly or Ser for COX-2/AA or Gly526Ser COX-2/AA, respectively.

Residue	C-atom	COX-2/AA				Gly526Ser COX-2/AA			
		C_6	C_8	C_{11}	C_{13}	C_6	C_8	C_{11}	C_{13}
G526S	C_A or C_B	5.9	3.8	5.5	6.1	6.0	3.6	4.2	6.4
Ser530	C_B	7.7	6.4	5.1	4.1	5.4	5.5	3.6	5.0
Tyr385	C_Z [a]	7.8	5.4	4.3	3.7	8.7	7.0	5.2	3.7
Met522	C_G [b]	7.6	6.4	9.3	10.8	10.2	8.3	10.5	12.3
Trp387	CH_2 [c]	7.2	5.8	4.5	5.6	7.3	5.2	5.6	5.7
	CZ_2	7.1	5.5	5.0	6.2	7.8	5.5	6.1	6.5
Leu352	CD_1 [d]	4.9	5.9	5.9	8.3	4.1	4.8	6.1	6.9
	CD_2	4.3	4.7	3.6	5.9	5.3	5.8	8.2	8.9
Leu384	CD_1 [d]	7.5	5.2	6.8	7.4	10.6	8.2	8.5	9.8
	CD_2	7.6	5.4	6.8	7.7	9.8	7.3	7.4	8.2

[a] C_Z is the 4'-carbon of the benzyl ring of Tyr.
[b] C_G is the CH_2 carbon bonded to sulfur atom.
[c] CH_2 and CZ_2 are carbons 5' and 4' of the indole side chain of Trp.
[d] CD_1 and CD_2 are the terminal methyl carbons of the leucine side chain.

Table S2. Distances (Å) corresponding to the three atoms that directly participate in the breaking/forming bonds for the reactant, transition state structure and product [a] corresponding to the C_{13} pro-*S* hydrogen abstraction by the tyrosyl radical.

$d(C\text{-}H)_R$	$d(H\text{-}O)_R$	$d(C\text{-}O)_R$	$d(C\text{-}H)_{TS}$	$d(H\text{-}O)_{TS}$	$d(C\text{-}O)_{TS}$	$d(C\text{-}H)_P$	$d(H\text{-}O)_P$	$d(C\text{-}O)_P$
1.09	2.43	3.28	1.34	1.23	2.57	2.78	0.97	3.74

[a] C, H and O stand, respectively, for C_{13}, $H_{pro\text{-}S,}$ and O of the Tyr385 radical