

**Electronic Supplementary Information**

**for**

**Accounting for the instantaneous disorder in the enzyme-substrate Michaelis complex to calculate the Gibbs free energy barrier of an enzyme reaction**

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We have carried out a test of convergence of the Gibbs free energy barrier and its contributions appearing in equation 5 in the main text for the hydrogen abstraction reactions from arachidonic acid catalyzed by the enzyme rabbit 15-lipoxygenase-1. To this aim, we have divided the 100 ns of the molecular dynamics (MD) simulation into two halves (50 ns each), and we have used the already obtained results to perform a separated analysis of each half. Thus, each half contains a set of  $N = 5000$  selected snapshots uniformly distributed at equal intervals along the MD simulation, the corresponding precatalytic snapshots ( $n$ ), and those of the 25 structures that had been randomly selected as representative of the precatalytic snapshots belonging to each trajectory half of 50 ns. Remember that from those structures the corresponding QM(B3LYP)/AMBER potential energy paths and the potential energy barriers have already been calculated.

Table 1 and Table 2 display the results for the H<sub>7-proS</sub> and the H<sub>13-proS</sub> abstractions, respectively. The results already explained in the main text for the whole simulations are also shown in the last files of those two Tables for the sake of comparison.

**Table 1:** Number of precatalytic snapshots ( $n$ ), total number of selected snapshots ( $N$ ), number of potential energy profiles calculated, first and second terms of equation 5, and  $\Delta G^{\ddagger}$  for the H<sub>7-proS</sub> abstraction from arachidonic acid catalyzed by the enzyme rabbit 15-lipoxygenase-1 for the different fragments of the MD simulation considered.

Fragment of MD considered	Number of precatalytic snapshots ( $n$ )	Total number of selected snapshots ( $N$ )	Number of potential energy profiles calculated	First term (kcal/mol)	Second term (kcal/mol)	$\Delta G^{\ddagger}$ (kcal/mol)
From 0 ns to 50 ns	2	5000	2	4.6	47.4	52
From 50 ns to 100 ns	31	5000	23	3.0	41.2	44.2
From 0 ns to 100 ns	33	10000	25	3.4	41.3	44.7

**Table 2:** Number of precatalytic snapshots ( $n$ ), total number of selected snapshots ( $N$ ), number of potential energy profiles calculated, first and second terms of equation 5, and  $\Delta G^{\ddagger}$  for the H<sub>13-proS</sub> abstraction from arachidonic acid catalyzed by the enzyme rabbit 15-lipoxygenase-1 for the different fragments of the MD simulation considered.

Fragment of MD considered	Number of precatalytic snapshots ( $n$ )	Total number of selected snapshots ( $N$ )	Number of potential energy profiles calculated	First term (kcal/mol)	Second term (kcal/mol)	$\Delta G^{\ddagger}$ (kcal/mol)
From 0 ns to 50 ns	1643	5000	14	0.6	14.2	14.8
From 50 ns to 100 ns	1740	5000	11	0.6	16.3	16.9
From 0 ns to 100 ns	3383	10000	25	0.6	14.6	15.2

It is evident that the longer the MD simulation, the better the results are. We recommend that this test should be carried out to conclude that the Gibbs free energy barrier and its components are well converged. This test is quite fast, because it just consists in the comparison of the entire results with the situation corresponding to the division into two halves. In our case, the results are quite well converged, being the only discordant results the ones corresponding to the simulation from 0 ns to 50 ns for the H<sub>7-proS</sub> abstraction (see Table 1). This is due to the very poor statistic that is obtained when just 2 precatalytic snapshots are found. Indeed, more snapshots (and, therefore, more calculated potential energy barriers) have to exist in a MD simulation to get reliable results. As a matter of fact, in the case of the H<sub>13-proS</sub> abstraction (see Table 2), the most considerable deviation appears in the MD fragment from 50 ns to 100 ns, within which the number of potential energy profiles calculated is somewhat smaller in comparison with the first fragment or the whole MD simulation.

On the other hand, we have also compared our original results (main text) for the 100 ns molecular MD simulation for the hydrogen abstraction reactions from arachidonic acid catalyzed by the enzyme rabbit 15-lipoxygenase-1, with the corresponding results that could have been obtained using a slightly more restrictive filter to identify the precatalytic snapshots. The original conditions were  $d(\text{H}_{7\text{-proS}}\text{-OH}) \leq 4.0 \text{ \AA}$  and  $d(\text{H}_{7\text{-proS}}\text{-OH}) < d(\text{C}_7\text{-OH})$  for the H<sub>7-proS</sub> abstraction, and  $d(\text{H}_{13\text{-proS}}\text{-OH}) \leq 3.0 \text{ \AA}$  and  $d(\text{H}_{13\text{-proS}}\text{-OH}) < d(\text{C}_{13}\text{-OH})$  for the H<sub>13-proS</sub> abstraction. The results in Table 3 show that the slightly more severe restriction clearly diminishes, as expected, the number of precatalytic

snapshots, and alters the values of the two terms of equation 5 (it specially increases the contribution of the first term), although the change of  $\Delta G^{\ddagger}$  is not very significant. However, the use of a quite restrictive filter could reduce the number of precatalytic snapshots enough to lose the statistic required to get reliable results.

**Table 3:** Number of precatalytic snapshots ( $n$ ), total number of selected snapshots ( $N$ ), number of potential energy profiles calculated, first and second terms of equation 5, and  $\Delta G^{\ddagger}$  for the H<sub>7-proS</sub> and H<sub>13-proS</sub> abstractions from arachidonic acid catalyzed by the enzyme rabbit 15-lipoxygenase-1 for the whole MD simulation applying a more restricted filter to search precatalytic snapshots.

Precatalytic snapshot filter	Number of precatalytic snapshots ( $n$ )	Total number of selected snapshots ( $N$ )	Number of potential energy profiles calculated	First term (kcal/mol)	Second term (kcal/mol)	$\Delta G^{\ddagger}$ (kcal/mol)
$d(\text{H}_{7\text{-proS}}\text{-OH}) \leq 3.8 \text{ \AA}$ and $d(\text{H}_{7\text{-proS}}\text{-OH}) < d(\text{C}_7\text{-OH})$	12	10000	7	4.0	40.5	44.5
$d(\text{H}_{13\text{-proS}}\text{-OH}) \leq 2.7 \text{ \AA}$ and $d(\text{H}_{13\text{-proS}}\text{-OH}) < d(\text{C}_{13}\text{-OH})$	1457	10000	9	1.1	14.9	16.0