

## Supporting Information

### **Computational studies devoted to the catalytic mechanism of Threonine aldolase – a critical enzyme in the pharmaceutical industry to synthesize $\beta$ -hydroxy- $\alpha$ -amino acids.**

Juliana F. Rocha<sup>(1,2)</sup>, Sérgio F. Sousa<sup>(1,2)</sup> and Nuno M. F. Sousa A. Cerqueira<sup>(1,2)\*</sup>

(1) Associate Laboratory i4HB – Institute for Health and Bioeconomy, Faculty of Medicine, University of Porto, 4200-319 Porto, Portugal

(2) UCIBIO – Applied Molecular Biosciences Unit, BioSIM – Department of Biomedicine, Faculty of Medicine, University of Porto, 4200-319 Porto, Portugal

*\*Corresponding author: [nunoscerqueira@med.up.pt](mailto:nunoscerqueira@med.up.pt)*

Table S1 – All calculated energies for all the complexes for step1, in scenario 2 (Lys mediated) with L-*allo*-threonine as substrate. The results are shown in kcal/mol.

|              | Opt            | Correction to<br>Gibbs Free<br>Energies | Opt            | SP                           | SP                |
|--------------|----------------|-----------------------------------------|----------------|------------------------------|-------------------|
| QM           | B3LYP/6-31G(d) |                                         | B3LYP/6-31G(d) | M06-2x/6-<br>311++G(2df,2pd) | DLPNO-CCSD(T)/CBS |
| MM           | fb15           |                                         | fb15           | fb15                         | fb15              |
| R            | 0.00           | 0.00                                    | 0.00           | 0.00                         | 0.00              |
| TS           | 3.05           | -0.12                                   | 2.93           | 3.38                         | 4.06              |
| P            | 0.80           | 1.92                                    | 2.72           | 6.35                         | 6.02              |
| $\Delta E_a$ | 3.05           | $\Delta G^\ddagger$                     | 2.93           | 3.38                         | 4.06              |
| $\Delta E_r$ | 0.80           | $\Delta G_r^0$                          | 2.72           | 6.35                         | 6.02              |

Table S2 – All calculated energies for all the complexes for step2, in scenario 2 (Lys mediated) with L-*allo*-threonine as substrate. The results are shown in kcal/mol.

|              | Opt            | Correction to<br>Gibbs Free<br>Energies | Opt            | SP                           | SP                |
|--------------|----------------|-----------------------------------------|----------------|------------------------------|-------------------|
| QM           | B3LYP/6-31G(d) |                                         | B3LYP/6-31G(d) | M06-2x/6-<br>311++G(2df,2pd) | DLPNO-CCSD(T)/CBS |
| MM           | fb15           |                                         | fb15           | fb15                         | fb15              |
| R            | 0.80           | 1.92                                    | 2.72           | 6.35                         | 6.02              |
| TS           | 7.09           | 1.29                                    | 8.37           | 15.12                        | 16.67             |
| P            | 6.75           | -0.16                                   | 6.60           | 12.53                        | 14.88             |
| $\Delta E_a$ | 6.29           | $\Delta G^\ddagger$                     | 5.65           | 8.77                         | 10.65             |
| $\Delta E_r$ | 5.95           | $\Delta G_r^0$                          | 3.88           | 6.18                         | 8.86              |

Table S3 – All calculated energies for all the complexes for step3, in scenario 2 (Lys mediated) with L-*allo*-threonine as substrate. The results are shown in kcal/mol.

|              | Opt            | Correction to<br>Gibbs Free<br>Energies | Opt            | SP                           | SP                |
|--------------|----------------|-----------------------------------------|----------------|------------------------------|-------------------|
| QM           | B3LYP/6-31G(d) |                                         | B3LYP/6-31G(d) | M06-2x/6-<br>311++G(2df,2pd) | DLPNO-CCSD(T)/CBS |
| MM           | fb15           |                                         | fb15           | fb15                         | fb15              |
| R            | 0.00           | 0.00                                    | 0.00           | 0.00                         | 0.00              |
| TS           | 5.94           | -1.41                                   | 4.53           | 8.56                         | 7.75              |
| P            | -7.95          | -1.06                                   | -9.01          | -3.21                        | -6.15             |
| $\Delta E_a$ | 5.94           | $\Delta G^\ddagger$                     | 4.53           | 8.56                         | 7.75              |
| $\Delta E_r$ | -7.95          | $\Delta G_r^0$                          | -9.01          | -3.21                        | -6.15             |

Table S4 – All calculated energies for all the complexes for step1, in scenario 3 (phosphate mediated) with L-*allo*-threonine as substrate. The results are shown in kcal/mol.

|              | Opt            | Correction to<br>Gibbs Free<br>Energies | Opt            | SP                           | SP                |
|--------------|----------------|-----------------------------------------|----------------|------------------------------|-------------------|
| QM           | B3LYP/6-31G(d) |                                         | B3LYP/6-31G(d) | M06-2x/6-<br>311++G(2df,2pd) | DLPNO-CCSD(T)/CBS |
| MM           | fb15           |                                         | fb15           | fb15                         | fb15              |
| R            | 0.00           | 0.00                                    | 0.00           | 0.00                         | 0.00              |
| TS           | 9.11           | -5.44                                   | 3.67           | 4.84                         | 7.35              |
| P            | 7.49           | -3.24                                   | 4.25           | 7.17                         | 7.82              |
| $\Delta E_a$ | 9.11           | $\Delta G^\ddagger$                     | 3.67           | 4.84                         | 7.35              |
| $\Delta E_r$ | 7.49           | $\Delta G_r^0$                          | 4.25           | 7.17                         | 7.82              |

Table S5 – All calculated energies for all the complexes for step2, in scenario 3 (phosphate mediated) with L-*allo*-threonine as substrate. The results are shown in kcal/mol.

|              | Opt            | Correction to<br>Gibbs Free<br>Energies | Opt            | SP                           | SP                |
|--------------|----------------|-----------------------------------------|----------------|------------------------------|-------------------|
| QM           | B3LYP/6-31G(d) |                                         | B3LYP/6-31G(d) | M06-2x/6-<br>311++G(2df,2pd) | DLPNO-CCSD(T)/CBS |
| MM           | fb15           |                                         | fb15           | fb15                         | fb15              |
| R            | 7.49           | -3.24                                   | 4.25           | 7.17                         | 7.82              |
| TS           | 13.49          | -3.91                                   | 9.58           | 11.88                        | 14.23             |
| P            | 11.20          | -6.65                                   | 4.56           | 6.85                         | 9.97              |
| $\Delta E_a$ | 6.00           | $\Delta G^\ddagger$                     | 5.33           | 4.71                         | 6.41              |
| $\Delta E_r$ | 3.71           | $\Delta G_r^0$                          | 0.31           | -0.32                        | 2.15              |

Table S6 – All calculated energies for all the complexes for step3, in scenario 3 (phosphate mediated) with L-*allo*-threonine as substrate. The results are shown in kcal/mol.

|              | Opt            | Correction to<br>Gibbs Free<br>Energies | Opt            | SP                           | SP                |
|--------------|----------------|-----------------------------------------|----------------|------------------------------|-------------------|
| QM           | B3LYP/6-31G(d) |                                         | B3LYP/6-31G(d) | M06-2x/6-<br>311++G(2df,2pd) | DLPNO-CCSD(T)/CBS |
| MM           | fb15           |                                         | fb15           | fb15                         | fb15              |
| R            | 0.00           | 0.00                                    | 0.00           | 0.00                         | 0.00              |
| TS           | 6.37           | -1.54                                   | 4.83           | 8.99                         | 8.77              |
| P            | -21.49         | 6.04                                    | -15.45         | -9.97                        | -11.82            |
| $\Delta E_a$ | 6.37           | $\Delta G^\ddagger$                     | 4.83           | 8.99                         | 8.77              |
| $\Delta E_r$ | -21.49         | $\Delta G_r^0$                          | -15.45         | -9.97                        | -11.82            |

Table S7 – All calculated energies for all the complexes for step1, in scenario 2 (Lys mediated) with L-threonine as substrate. The results are shown in kcal/mol.

|              | Opt            | Correction to<br>Gibbs Free<br>Energies | Opt            | SP                           | SP                |
|--------------|----------------|-----------------------------------------|----------------|------------------------------|-------------------|
| QM           | B3LYP/6-31G(d) |                                         | B3LYP/6-31G(d) | M06-2x/6-<br>311++G(2df,2pd) | DLPNO-CCSD(T)/CBS |
| MM           | fb15           |                                         | fb15           | fb15                         | fb15              |
| R            | 0.00           | 0.00                                    | 0.00           | 0.00                         | 0.00              |
| TS           | 3.13           | 0.37                                    | 3.49           | 3.68                         | 4.06              |
| P            | 0.05           | 2.22                                    | 2.27           | 6.22                         | 5.51              |
| $\Delta E_a$ | 3.13           | $\Delta G^\ddagger$                     | 3.49           | 3.68                         | 4.06              |
| $\Delta E_r$ | 0.05           | $\Delta G_r^0$                          | 2.27           | 6.22                         | 5.51              |

Table S8 – All calculated energies for all the complexes for step2, in scenario 2 (Lys mediated) with L-threonine as substrate. The results are shown in kcal/mol.

|              | Opt            | Correction to<br>Gibbs Free<br>Energies | Opt            | SP                           | SP                |
|--------------|----------------|-----------------------------------------|----------------|------------------------------|-------------------|
| QM           | B3LYP/6-31G(d) |                                         | B3LYP/6-31G(d) | M06-2x/6-<br>311++G(2df,2pd) | DLPNO-CCSD(T)/CBS |
| MM           | fb15           |                                         | fb15           | fb15                         | fb15              |
| R            | 0.05           | 2.22                                    | 2.27           | 6.22                         | 5.51              |
| TS           | 6.73           | 0.95                                    | 7.81           | 14.61                        | 15.99             |
| P            | 5.62           | -1.43                                   | 4.19           | 9.99                         | 12.15             |
| $\Delta E_a$ | 6.68           | $\Delta G^\ddagger$                     | 5.42           | 8.39                         | 10.48             |
| $\Delta E_r$ | 5.57           | $\Delta G_r^0$                          | 1.92           | 3.77                         | 6.64              |

Table S9 – All calculated energies for all the complexes for step3, in scenario 2 (Lys mediated) with L-threonine as substrate. The results are shown in kcal/mol.

|    | Opt            | Correction to<br>Gibbs Free<br>Energies | Opt            | SP                           | SP                |
|----|----------------|-----------------------------------------|----------------|------------------------------|-------------------|
| QM | B3LYP/6-31G(d) |                                         | B3LYP/6-31G(d) | M06-2x/6-<br>311++G(2df,2pd) | DLPNO-CCSD(T)/CBS |
| MM | fb15           |                                         | fb15           | fb15                         | fb15              |
| R  | 0.00           | 0.00                                    | 0.00           | 0.00                         | 0.00              |
| TS | 5.94           | -1.41                                   | 4.53           | 8.56                         | 7.75              |
| P  | -7.95          | -1.06                                   | -9.01          | -3.21                        | -6.15             |

  

|              |       |                     |       |       |       |
|--------------|-------|---------------------|-------|-------|-------|
| $\Delta E_a$ | 5.94  | $\Delta G^\ddagger$ | 4.53  | 8.56  | 7.75  |
| $\Delta E_r$ | -7.95 | $\Delta G_r^0$      | -9.01 | -3.21 | -6.15 |

Table S10 – Comparison of the free activation and reaction energies obtained with model containing 124 atoms in HL versus the model with 142 atoms in HL.

|       |                                | 124 atoms<br>HL | 142 atoms<br>HL | Difference |
|-------|--------------------------------|-----------------|-----------------|------------|
| step1 | $\Delta G^\ddagger$ (kcal/mol) | 3.67            | 6.72            | 3.05       |
|       | $\Delta G_r^0$ (kcal/mol)      | 4.25            | 7.89            | 3.64       |
| step2 | $\Delta G^\ddagger$ (kcal/mol) | 5.33            | 5.06            | -0.27      |
|       | $\Delta G_r^0$ (kcal/mol)      | 0.31            | 1.26            | 0.95       |
| step3 | $\Delta G^\ddagger$ (kcal/mol) | 4.83            | 4.13            | -0.70      |
|       | $\Delta G_r^0$ (kcal/mol)      | -15.45          | -16.12          | -0.67      |

\*These energies differ from the ones that were presented in the article, because they are the following theoretical level B3LYP/6-31g(d):fb15.

