On the catalytic activity of the engineered coiled-coil heptamer mimicking the hydrolase enzymes: insights from a computational study

Mario Prejanò, Isabella Romeo, Nino Russo*, Tiziana Marino*

Dipartimento di Chimica e Tecnologie Chimiche, Università della Calabria, 87036 Rende, CS, Italy.

correspondence should be addressed to: tiziana.marino65@unical.it; nrusso@unical.it

SUPPORTING INFORMATION

Table of contents:

Fig. S1 The initial structure adopted in the present study (PDB: 5ECZ)..................................................................................................................................................S4

Fig. S2 Superposition between 10 representative structures generated by cpptraj, after 200 ns of the apo-form protein MDs with Nδ protonation state in A) top and B) front view. Each structure is represented in ribbon form.
..................................................................................................................................................................S4

Fig. S3 Representation of the unnatural substrate pNPA binding mode into the channel of the de novo protein for each obtained representative structure. 10 poses are generated by using AutoDock Vina. The protein and the relative pNPA binding poses are shown in light grey cartoon and coloured carbon sticks, respectively.
.............................................................................................................................................................S5
**Fig. S4** The pair correlation function for the sulphur of cysteine and the carbon of pNPA...

**Fig. S5** Plot of the distance calculated between the centroids of all the seven Gly, Cys and Arg residues of the protein and the corresponding Ca of Gly, Cys and Arg of each chain as a measure of the diameter of the channel at bottom (blue line), middle and top (green and red lines), respectively, relative to the initial structure. The time of simulations (ns) and the values of the Ca distance (Å) are reported, respectively, on the abscissa and on the ordinate axes for apo-form (Nδ protonation state) and pNPA-bound protein for A) Conformation 1, B) Conformation 2 and C) Conformation 3...

**Fig. S6** Hydrogen bond network for water molecules explicitly considered in the QM/MM model for the pNPA-bound protein are represented as black lines for A) Conformation 1, B) Conformation 2. Three water molecules are indicated as red balls. The substrate is shown in green sticks, meanwhile, Cys, His and Glu are indicates as yellow, blue and red sticks, respectively...

**Fig. S7** PES for the nucleophilic attack of the neutral thiol form of Cys-SH and deprotonated one Cys-S(-) to the ester carbonyl for Conformation 1 and Conformation 2...

**Fig. S8** Optimized geometries of TS species for A) conformation 1, B) conformation 2, intercepted along the PES of the hydrolytic process. For clarity, only the amino acid residues taking part in the reaction are shown, in which Cys18-His22-Glu25 residues and pNPA are represented in ball and sticks...
**Fig. S9** Optimized geometries of TS1 species for A) conformation 1, B) conformation 2, intercepted along the PES of the hydrolytic process. For clarity, only the amino acid residues taking part in the reaction are shown, in which Cys18-His22-Glu25 residues and pNPA are represented in ball and sticks.

**Fig. S10** Optimized geometries of I2A species for A) conformation 1, B) conformation 2, intercepted along the PES of the hydrolytic process. For clarity, only the amino acid residues taking part in the reaction are shown, in which Cys18-His22-Glu25 residues and pNPA are represented in ball and sticks.

**Fig. S11** Free Energy Surfaces for proposed hydrolysis catalyzed by de novo protein obtained at B3LYP-D3/6-311+(2d,2p):ff14SB/B3LYP/6-31+G(d,p):ff14SB level of theory, according to the B mechanism for the two adopted conformation.

**Fig. S12** Optimized geometries of TS3B species for A) conformation 1, B) conformation 2, intercepted along the PES of the hydrolytic process. For clarity, only the amino acid residues taking part in the reaction are shown, in which Cys18-His22-Glu25 residues and pNPA are represented in ball and sticks.

**Table S1.** Calculated pKa for ionizable residues of CC-Hept-CHE. Residues fully protonated (positively charged) or deprotonated (negatively charged) are highlighted in blue and red, respectively. Residues belonging to the catalytic triad are underlined.

**Table S2.** Best pNPA docked pose for each representative structure and adopted geometrical parameters in order to predict binding mode into de novo
Table S3. Calculated parameters for pNPA.

Table S4. Adopted geometrical filters on each representative structure of pNPA-bound protein for the two analyzed conformations in order to select the starting point for the QM/MM investigation.

Table S5. NBO charges (|e|) of selected atoms in all the species found on the PES.

Table S6. Energy contributions, calculated at ONIOM[B3LYP-D3/6-311+G(2d,2p):ff14SB] level of theory, extracted to obtain Free Energy Surfaces, for each stationary point. Imaginary frequencies calculated for transition states are also reported (cm⁻¹).
Figure S1. The initial structure adopted in the present study (PDB: 5ECZ).

Fig. S2 Superposition between 10 representative structures generated by cpptraj, after 200 ns of the apo-form protein MDs with Nδ protonation state in A) top and B) front view. Each structure is represented in ribbon form.
Fig. S3 Representation of the unnatural substrate pNPA binding mode into the channel of the *de novo* protein for each obtained representative structure. 10 poses are generated by using AutoDock Vina. The protein and the relative pNPA binding poses are shown in light grey cartoon and coloured carbon sticks, respectively.
**Fig. S4.** The pair correlation function for the sulphur of cysteine and the carbon of pNPA.
**Fig. S5** Plot of the distance calculated between the centroids of all the seven Gly\textsubscript{1}, Cys\textsubscript{18} and Arg\textsubscript{30} residues of the protein and the corresponding C\textalpha{} of Gly, Cys and Arg of each chain as a measure of the diameter of the channel at bottom (blue line), middle and top (green and red lines), respectively, relative to the initial structure. The time of simulations (ns) and the values of the C\textalpha{} distance (Å) are reported, respectively, on the abscissa and on the ordinate axes for apo-form (N\textdelta{} protonation state) and pNPA-bound protein for A) Conformation 1, B) Conformation 2 and C) Conformation 3.

---

**Fig. S6.** Hydrogen bond network for water molecules explicitly considered in the QM/MM model for the pNPA-bound protein are represented as black lines for A) Conformation 1, B) Conformation 2. Three water molecules are indicated as red balls. The substrate is shown in green sticks, meanwhile, Cys, His and Glu are indicates as yellow, blue and red sticks, respectively.
**Fig. S7** PES for the nucleophilic attack of the neutral thiol form of Cys-SH and deprotonated one Cys-S⁻ to the ester carbonyl for **A)** Conformation 1, **B)** Conformation 2.

**Fig. S8** Optimized geometries of TS species for **A)** conformation 1, **B)** conformation 2, intercepted along the PES of the hydrolytic process. For clarity, only the amino acid residues taking part in the reaction are shown, in which Cys^{18}-His^{22}-Glu^{25} residues and pNPA are represented in ball and sticks.
Fig. S9. Optimized geometries of TS1 species for A) conformation 1, B) conformation 2, intercepted along the PES of the hydrolytic process. For clarity, only the amino acid residues taking part in the reaction are shown, in which Cys18-His22-Glu25 residues and pNPA are represented in ball and sticks.

Fig. S10. Optimized geometries of I2A species for A) conformation 1, B) conformation 2, intercepted along the PES of the hydrolytic process. For clarity, only the amino acid residues taking part in the reaction are shown, in which Cys18-His22-Glu25 residues and pNPA are represented in ball and sticks.
In the channel B the hydrolysis of thioester involves the w1 that donates the proton to the sulfur atom ($O_{w1}$--H 1.61 Å and H--S 1.78 Å) producing the $O_{w1}$--C bond formation (1.60 Å for conformation 2) and the S--C bond cleavage (1.98 Å for conformation 2) in concerted way. The corresponding transition state ($TS2B$) has in all cases one imaginary frequency of 750i cm$^{-1}$ and a Gibbs activation energy of 31.8 and 22.5 kcal/mol (Figure S12) for conformation 1 and 2. The I2B resulting species are almost isoenergetic with I1 for the conformation 1 and 2. The next step accounts for restoring of the catalytic center through a concerted proton cascade involving w2, His, Glu and pNPA (see $TS3B$ in Figure S13). The imaginary frequencies of about 1000i cm$^{-1}$ for the two paths confirm the main role played by water in this rearrangement. The final reaction Gibbs energy results to be exothermic for configuration 1 and 2 (Figure S12).

**Fig. S11** Free Energy Surfaces for proposed hydrolysis catalyzed by de novo protein obtained at B3LYP-D3/6-311+(2d,2p):ff14SB//B3LYP/6-31+G(d,p):ff14SB level of theory, according to the B mechanism for the two adopted conformations.
**Fig. S12** Optimized geometries of TS3B species for A) conformation 1, B) conformation 2, intercepted along the PES of the hydrolytic process. For clarity, only the amino acid residues taking part in the reaction are shown, in which Cys18-His22-Glu25 residues and pNPA are represented in ball and sticks.

**Table S1.** Calculated pKa for ionizable residues of CC-Hept-CHE. Residues fully protonated (positively charged) or deprotonated (negatively charged) are highlighted in blue and red, respectively. Residues belonging to the catalytic triad are underlined.

|     | A   | B   | C   | D   | E   | F   | G   |
|-----|-----|-----|-----|-----|-----|-----|-----|
| Glu3| 1.5 | 1.3 | 1.5 | 1.3 | 1.4 | 1.2 | 1.8 |
| Lys6| 10.0| 10.1| 9.9 | 8.8 | 10.1| 10.0| 10.1|
| Arg9| ≈12.0| ≈12.0| ≈12.0| ≈12.0| ≈12.0| ≈12.0| ≈12.0|
| Glu10| 1.9 | 0.8 | 0.5 | 1.3 | 2.3 | 0.5 | 2.1 |
| Lys13| 10.1| 10.1| 10.1| 10.1| 10.0| 10.0| 10.0|
| Arg16| ≈12.0| ≈12.0| ≈12.0| ≈12.0| ≈12.0| ≈12.0| 12.0|
|      | Glu17 | Cys18* | His22* | Arg23 | Glu24 | Glu25* | Lys27 | Arg30 |
|------|-------|--------|--------|-------|-------|--------|-------|-------|
|      | 1.6   | 1.6    | 1.6    | 1.6   | 1.6   | 1.6    | 1.6   | 1.6   |
|      | 2.1   | 2.1    | 2.1    | 2.1   | 2.1   | 2.1    | 2.1   | 2.1   |
|      | 2.2   | 2.2    | 2.2    | 2.2   | 2.2   | 2.2    | 2.2   | 2.2   |
|      | 1.7   | 1.7    | 1.7    | 1.7   | 1.7   | 1.7    | 1.7   | 1.7   |
|      | 2.0   | 2.0    | 2.0    | 2.0   | 2.0   | 2.0    | 2.0   | 2.0   |
|      | 1.5   | 1.5    | 1.5    | 1.5   | 1.5   | 1.5    | 1.5   | 1.5   |

|      | ≈12.0 | ≈12.0  | ≈12.0  | ≈12.0 | ≈12.0 | ≈12.0  | ≈12.0 | ≈12.0 |
|      | ≈12.0 | ≈12.0  | ≈12.0  | ≈12.0 | ≈12.0 | ≈12.0  | ≈12.0 | ≈12.0 |
|      | 5.9   | 4.4    | 5.5    | 6.1   | 5.5   | 5.4    | 4.4   | 4.4   |
|      | 4.4   | 4.4    | 4.4    | 4.4   | 4.4   | 4.4    | 4.4   | 4.4   |

|      | ≈12.0 | ≈12.0  | ≈12.0  | ≈12.0 | ≈12.0 | ≈12.0  | ≈12.0 | ≈12.0 |
|      | ≈12.0 | ≈12.0  | ≈12.0  | ≈12.0 | ≈12.0 | ≈12.0  | ≈12.0 | ≈12.0 |
|      | 6.4   | 7.0    | 8.3    | 6.8   | 5.7   | 5.4    | 8.0   | 8.0   |
|      | 7.0   | 7.0    | 7.0    | 7.0   | 7.0   | 7.0    | 7.0   | 7.0   |
|      | 8.3   | 8.3    | 8.3    | 8.3   | 8.3   | 8.3    | 8.3   | 8.3   |
|      | 6.8   | 6.8    | 6.8    | 6.8   | 6.8   | 6.8    | 6.8   | 6.8   |
|      | 5.7   | 5.7    | 5.7    | 5.7   | 5.7   | 5.7    | 5.7   | 5.7   |
|      | 5.4   | 5.4    | 5.4    | 5.4   | 5.4   | 5.4    | 5.4   | 5.4   |

|      | 3.4   | 2.8    | 3.0    | 2.8   | 3.0   | 2.8    | 3.0   | 2.8   |
| Glu25* | 2.8   | 2.8    | 2.8    | 2.8   | 2.8   | 2.8    | 2.8   | 2.8   |
|      | 3.0   | 3.0    | 3.0    | 3.0   | 3.0   | 3.0    | 3.0   | 3.0   |
|      | 0.2   | 0.2    | 0.2    | 0.2   | 0.2   | 0.2    | 0.2   | 0.2   |
|      | 3.8   | 3.8    | 3.8    | 3.8   | 3.8   | 3.8    | 3.8   | 3.8   |
|      | 2.9   | 2.9    | 2.9    | 2.9   | 2.9   | 2.9    | 2.9   | 2.9   |
|      | 3.1   | 3.1    | 3.1    | 3.1   | 3.1   | 3.1    | 3.1   | 3.1   |

|      | 10.9  | 10.9   | 10.9   | 11.5  | 11.0  | 11.2   | 10.8  | 10.8  |
| Lys27 | 10.8  | 10.8   | 10.8   | 11.5  | 11.0  | 11.2   | 10.8  | 10.8  |
|      | 10.7  | 10.7   | 10.7   | 11.5  | 11.0  | 11.2   | 10.8  | 10.8  |
|      | 11.5  | 11.5   | 11.5   | 11.5  | 11.5  | 11.5   | 11.5  | 11.5  |
|      | 11.0  | 11.0   | 11.0   | 11.0  | 11.0  | 11.0   | 11.0  | 11.0  |
|      | 11.2  | 11.2   | 11.2   | 11.2  | 11.2  | 11.2   | 11.2  | 11.2  |
|      | 10.8  | 10.8   | 10.8   | 10.8  | 10.8  | 10.8   | 10.8  | 10.8  |
|      | ≈12.0 | ≈12.0  | ≈12.0  | ≈12.0 | ≈12.0 | ≈12.0  | ≈12.0 | ≈12.0 |
|      | ≈12.0 | ≈12.0  | ≈12.0  | ≈12.0 | ≈12.0 | ≈12.0  | ≈12.0 | ≈12.0 |

**Table S2.** Best pNPA docked pose for each representative structure and adopted geometrical parameters in order to predict binding mode into de novo protein.
| Cluster | Vina binding scores (kcal/mol) | $S_{CN}$-C$_{PTRA}$ atomic distance (Å) | $S_{CN}$-N$_{dila}$ atomic distance (Å) | N$_{dila}$-O$_{dila}$ Atomic distance (Å) |
|---------|---------------------------------|--------------------------------------|--------------------------------------|--------------------------------------|
| Cluster 1 | -5.4                            | 13.697                               | 3.472                                | 8.584                                |
| Cluster 2 | -5.5                            | 4.355                                | 3.795                                | 2.815                                |
| Cluster 3 | -5.7                            | 13.487                               | 5.732                                | 12.683                               |
| Cluster 4 | -6.3                            | 5.796                                | 6.306                                | 2.855                                |
| Cluster 5 | -5.8                            | 11.351                               | 3.357                                | 9.517                                |
| Cluster 6 | -5.0                            | 11.200                               | 6.098                                | 6.066                                |
| Cluster 7 | -5.3                            | 13.027                               | 5.273                                | 11.417                               |
| Cluster 8 | -5.3                            | 4.566                                | 5.356                                | 9.883                                |
| Cluster 9 | -5.2                            | 11.066                               | 3.553                                | 8.030                                |
| Cluster 10 | -4.9                           | 7.732                                | 7.963                                | 5.774                                |
**Table S3.** Calculated parameters for pNPA.

| Atomtype | Charge |
|----------|--------|
| Ca       | -0.010 |
| Ha       | 0.193  |
| No       | 0.813  |
| O        | -0.473 |
| Os       | -0.524 |
| C        | 0.938  |
| c3       | -0.558 |
| Hc       | 0.162  |

| Bond | $K_r$/ Kcal mol$^{-1}$ Å$^{-2}$ | $l$/Å |
|------|---------------------------------|-------|
| ca-ca | 461.10                           | 1.398 |
| ca-no | 321.70                           | 1.469 |
| ca-ha | 345.80                           | 1.086 |
| no-o  | 741.80                           | 1.226 |
| ca-os | 376.60                           | 1.370 |
| os-c  | 390.80                           | 1.358 |
| c-c3  | 313.00                           | 1.524 |
| c-o   | 637.70                           | 1.218 |
| c3-hc | 330.60                           | 1.097 |

| Dihedral | $V_d$/ Kcal mol$^{-1}$ rad$^2$ | $\gamma$/deg | n |
|-----------|---------------------------------|---------------|---|
| ca-ca-ca-ha | 1                               | 3.625         | 180.000 | 2.000 |
| ca-ca-ca-ca | 1                               | 3.625         | 180.000 | 2.000 |
| ca-ca-no-o | 1                               | 0.600         | 180.000 | 2.000 |
| ca-ca-ca-os | 1                               | 3.625         | 180.000 | 2.000 |
| ca-ca-ca-no | 1                               | 3.625         | 180.000 | 2.000 |
| ca-ca-os-c | 1                               | 0.900         | 180.000 | 2.000 |
| ha-ca-ca-ha | 1                               | 3.625         | 180.000 | 2.000 |
| ha-ca-ca-os | 1                               | 3.625         | 180.000 | 2.000 |
| ha-ca-ca-no | 1                               | 3.625         | 180.000 | 2.000 |
| ca-os-c-c3 | 1                               | 2.700         | 180.000 | 2.000 |
| ca-os-c-o | 1                               | 2.700         | 180.000 | 2.000 |
| os-c-c3-hc | 1                               | 0.000         | 180.000 | 2.000 |
| hc-c3-c-o | 1                               | 0.800         | 0.000   | -1.000 |
| hc-c3-c-o | 1                               | 0.000         | 0.000   | -2.000 |

| Angle   | $K_{\theta}$/ Kcal mol$^{-1}$ rad$^2$ | $\theta$/deg |
|---------|--------------------------------------|--------------|
| ca-cac  | 66.620                               | 120.020      |
| ca-ca-ha | 48.180                                | 119.880      |
| ca-no-o | 68.700                                | 117.760      |

S15
Table S4. Adopted geometrical filters on each representative structure of pNPA-bound protein for the two analyzed conformations in order to select the starting point for the QM/MM investigation.

|                  | VdW     | ℇ/Å    | ℇ/ kJ mol⁻¹ |
|------------------|---------|--------|-------------|
| Improper         |         |        |             |
| ca-ca-ca-no      | 1.1     | 180.0  | 2.0         |
| ca-ca-ca-ha      | 1.1     | 180.0  | 2.0         |
| ca-o-no-o        | 1.1     | 180.0  | 2.0         |
| ca-ca-ca-os      | 1.1     | 180.0  | 2.0         |
| c3-o-c-os        | 10.5    | 180.0  | 2.0         |
|                  |         |        |             |
| Cluster 1        | 6.44    | 9.80   | 8.11        |
|                  | 4.08    | 7.24   | 4.697       |
|                  | 3.98    | 6.68   | 5.78        |
| Cluster 2        | 5.46    | 5.66   | 2.79        |
|                  | 4.28    | 7.48   | 4.73        |
|                  | 3.54    | 5.32   | 10.62       |
| Cluster 3        | 3.70    | 6.57   | 7.78        |
|                  | 5.02    | 7.57   | 5.05        |
|                  | 4.48    | 5.29   | 14.58       |
| Cluster 4        | 7.09    | 3.82   | 6.66        |
|                  | 5.77    | 5.35   | 2.89        |
|                  | 10.43   | 4.65   | 11.46       |
| Cluster 5        | 4.69    | 5.76   | 8.13        |
|                  | 5.71    | 5.59   | 6.80        |
|                  | 4.89    | 8.64   | 11.61       |
| Cluster 6        | 4.89    | 7.65   | 3.13        |
|                  | 5.29    | 8.18   | 4.87        |
|                  | 5.54    | 4.28   | 2.71        |
| Cluster 7        | 7.28    | 6.82   | 5.70        |
|                  | 4.59    | 6.98   | 4.90        |
|                  | 6.93    | 7.72   | 4.70        |
| Cluster 8        | 3.99    | 6.36   | 7.74        |
|                  | 4.09    | 7.58   | 4.91        |
|                  | 6.35    | 5.01   | 15.46       |
| Cluster 9        | 5.71    | 4.62   | 8.24        |
|                  | 5.84    | 3.69   | 9.69        |
|                  | 4.98    | 4.31   | 6.63        |
Table S5. NBO charges (|e|) of selected atoms in all the species found on the PES.

|        | ES     | TS1   | I1     | TS2   | I2     |
|--------|--------|-------|--------|-------|--------|
| S Cys  | -0.138 | -0.166| -0.652 | -0.239| 0.174  |
| C NPA  | 0.837  | 0.841 | 0.820  | 0.616 | 0.401  |
| Ow1    | -1.013 | -1.101| -1.090 | -1.066| -1.041 |
| O1NPA  | -0.593 | -0.599| -0.603 | -0.667| -0.577 |
| O2NPA  | -0.559 | -0.563| -0.535 | -0.604| -0.809 |

Table S6. Energy contributions, calculated at ONIOM[B3LYP-D3/6-311+G(2d,2p):ff14SB] level of theory, extracted to obtain Free Energy Surfaces, for each stationary point. Imaginary frequencies calculated for transition states are also reported (cm⁻¹).

| Species | Conformation | E_{B3LYP} (a.u.) | E_{EP} (a.u.) | E_{D3} (a.u.) | \(-T\Delta S\) (kcal/mol) | E_{total} (a.u.) | \(\nu\) (cm⁻¹) |
|---------|--------------|------------------|--------------|--------------|-----------------|-----------------|---------------|
| ES      |              |                  |              |              |                 |                 |               |
| 1       | -6065.269687 | 29.113458        | -0.179546    | 0.0          | -6036.335829    |                 |               |
| 2       | -6065.276575 | 29.114817        | -0.177247    | 0.0          | -6036.339006    |                 |               |
| TS1     |              |                  |              |              |                 |                 |               |
| 1       | -6065.226492 | 29.103458        | -0.179946    | 1.2          | -6036.30498     | 800i            |               |
| 2       | -6065.250377 | 29.104580        | -0.177202    | -0.9         | -6036.322998    | 892i            |               |
| I1      |              |                  |              |              |                 |                 |               |
| 1       | -6065.299978 | 29.115458        | -0.180146    | -0.4         | -6036.325544    |                 |               |
|  | Z   | E   | X   | Y   | W   |
|---|-----|-----|-----|-----|-----|
| TS2 | 1   | -6065.25711 | 29.116487 | -0.179815 | 1.7 | -6036.320438 | 119i |
|    | 2   | -6065.256521 | 29.115570 | -0.174065 | -1.0 | -6036.315019 | 153i |
| I2  | 1   | -6065.301561 | 29.118462 | -0.175890 | 1.1 | -6036.362859 |
|    | 2   | -6065.304601 | 29.119100 | -0.171312 | 0.1 | -6036.356814 |
| TS3A | 1 | -6065.296611 | 29.165740 | -0.177796 | -1.1 | -6036.308667 | 680i |
|    | 2   | -6065.270381 | 29.117517 | -0.174539 | -0.2 | -6036.327403 | 752i |
| TS3B | 1 | -6065.319528 | 29.13874 | -0.173698 | -0.3 | -6036.334486 | 600i |
|    | 2   | -6065.266646 | 29.112971 | -0.172782 | 0.1 | -6036.326458 | 559i |
| I3A | 1   | -6065.312226 | 29.115701 | -0.169985 | -1.6 | -6036.366510 |
|    | 2   | -6065.300780 | 29.115517 | -0.170976 | 1.3 | -6036.356239 |
| I3B | 1   | -6065.277880 | 29.117852 | -0.168562 | 0.9 | -6036.32859 |
|    | 2   | -6065.273341 | 29.116018 | -0.170753 | 0.1 | -6036.328076 |
| TS4A | 1 | -6065.294370 | 29.109483 | -0.169589 | -0.5 | -6036.354476 | 998i |
|    | 2   | -6065.284532 | 29.107267 | -0.168867 | -0.3 | -6036.346132 | 1002i |
| TS4B | 1 | -6065.254587 | 29.115754 | -0.170123 | -0.7 | -6036.308956 | 81i |
|    | 2   | -6065.270212 | 29.115973 | -0.172060 | 0.2 | -6036.326298 | 79i |
| I4B | 1   | -6065.285765 | 29.116895 | -0.171456 | 0.7 | -6036.340326 |
|    | 2   | -6065.279843 | 29.116563 | -0.172460 | 0.1 | -6036.335741 |
|       | E1        | E2        | T         | B         | E1        | E2        | T         | B         |
|-------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|
| TS5B  | -6065.275598 | 29.104528 | -0.166555 | 0.9       | -6036.337625 | 860i     |
|       | -6065.271466 | 29.106182 | -0.163046 | -0.3      | -6036.328330 | 882i     |
| EP    | -6065.299092 | 29.111256 | -0.167222 | 0.5       | -6036.355058 |
|       | -6065.298616 | 29.114800 | -0.169381 | -0.4      | -6036.353198 |
| pNP   | -512.141645  | 0.107308  | -0.010257 |           | -512.042105 |
| CH3COOH | -229.172132 | 0.061779  | -0.003000 |           | -229.111027 |