Supporting Information for

A Reaction Path Study of the Catalysis and Inhibitionof the *Bacillus anthracis* CapD γ-Glutamyl Transpeptidase

Ilja V. Khavrutskii,[1] Patricia M. Legler,[2] Arthur M. Friedlander,[3] and Anders Wallqvist[1]

[1]Biotechnology High Performance Computing Software Applications Institute, Telemedicine and Advanced Technology Research Center, United States Army Medical Research and Materiel Command, Fort Detrick, Maryland 21702, United States

[2]Center of Bio/Molecular Science and Engineering, Naval Research Laboratories, Washington, D.C. 20375, United States

[3]United States Army Medical Research Institute of Infectious Diseases, Fort Detrick, Maryland 21702, United States

Corresponding author's e-mail: ikhavrutskii@bhsai.org

Table of Contents

Methods 3

1. Mechanism of CapD catalysis 3

1.2 CapD Acylation Mechanism by pDGA 3

1.2.1 Formation of tetrahedral CapD intermediate of pDGA (QM1) 3

1.2.2 Formation of acylated CapD intermediate of pDGA (QM2) 6

2. Mechanism of CapD Inhibition with Capsidin 7

2.2 CapD Acylation Mechanism by capsidin 7

2.2.2 Formation of acetylated CapD intermediate of capsidin 7

Table S2. Energies in kcal/mol along computed reaction paths for CapD acylation by capsidin 9

Figure Captions 10

Figure S1. QM regions used in calculations 10

Figure S2. Energy profile for formation of the tetrahedral intermediate between pDGA and CapD using QM1 model 10

Figure S3. Energy profile for collapse of the tetrahedral intermediate of pDGA into γ-glutamylated CapD using QM1 model 10

Figure S4. Energy profile for formation of the tetrahedral intermediate between pDGA and CapD using QM model 10

Figure S5. Energy profile for collapse of the tetrahedral intermediate of capsidin into acetylated CapD using QM model 11

Figure S6. Conformational change coupled to bond stretching 11

Figures 13

Figure S2 14

Figure S3 15

Figure S4 16

Figure S5 17

Figure S6 18

References 19

Methods

For the purposes of the present study the bound complexes of CapD with capsidin and the model of pDGA were prepared as described elsewhere.[1]

1. Mechanism of CapD catalysis

1.2 CapD Acylation Mechanism by pDGA

1.2.1 Formation of tetrahedral CapD intermediate of pDGA (QM1)

The following paragraphs describe formation of the tetrahedral intermediate using QM1 region (see Figure S1). The QM1 reaction paths differ from those for QM2. With QM1, formation of the tetrahedral intermediate requires deprotonated N-terminal NH_2 group of the catalytic Thr352 and proceeds with a barrier of 23.6 kcal/mol (see Table S1). The energy of the tetrahedral intermediate is 23.4 kcal/mol, only 0.2 kcal/mol below the TS. The reaction begins with the Thr352(Oγ)-(C_2)diγDGA and the Thr352(Hγ)-(N)Thr352 distances shrinking. In the transition state, the distances shrink to 1.73 Å and 1.04 Å, respectively. The latter indicates that the proton transfer to the terminal NH_2 group is completed before the transition state is reached. The protonated terminal NH_3^+ group makes stronger hydrogen bond with the second α-carboxyl group as evidenced by reduction in Thr352(HN)-(O_2)diγDGA distance from 2.08 Å at the reactant to 1.56 Å at the transition state. In fact, the Thr352(Hγ) proton transfers from Thr352(Oγ) to Thr352(N) while the Thr352(Oγ)-diγDGA(C) distance changes from 2.5 Å to

1.95 Å. Thus, the proton-coupled Thr352(Oγ)-(C_2)diγDGA bond formation reaction is concerted asynchronous.

The final product is very close to the transition state both in structure and energy. The Thr352(Oγ)-(C_2)diγDGA and the Thr352(Hγ)-(N)Thr352 distances are 1.64 Å and 1.03 Å, respectively. The peptide bond diγDGA(C_2-N_2) that is under the attack elongates from 1.34 Å to 1.45 Å loosing the conjugation with the carbonyl double bond. The carbonyl diγDGA(C_2=O) bond elongates from 1.25 Å to 1.31 Å. Note that the Thr352(Oγ)-(C)diγDGA distance of 1.64 Å is not a typical single bond, which in tetrahedral intermediate is expected to be around 1.54 Å. Therefore, the QM1 selection appears to have stabilized an unusual intermediate that is very close to the transition state. We feel that this finding may be an artifact of the specific choice of the QM region. Therefore, we have revised this section of the reaction path with the larger QM2 region (see Figure S1).

It may seem difficult to explain why this reaction does not immediately revert. One possible explanation is that subsequent proton transfer from the terminal NH_3^+ group to the bulk water may stabilize the system and ensure the reaction does not revert. The distance between the terminal Thr352(NH) and the second α-carboxyl of diγDGA reaches 1.52 Å, while the corresponding Thr352(N-H) bond elongates from 1.03 Å to 1.10 Å. The strong hydrogen bond is suggestive of an imminent proton transfer. Indeed, we find that a small stabilization of 1.3 kcal/mol can be achieved by transferring the proton from the NH_3^+ group to the second α-

carboxyl group of diγDGA. This proton transfer has a barrier of 0.9 kcal/mol. The barrier to revert the transfer is 2.2 kcal/mol.

Although the proton transfer is localized to only three atoms, the structure of the resulting tetrahedral intermediate undergoes noticeable conformational changes, particularly in the donor region of the active site. The conformational changes affect the oxyanion hole and the coordination of the fist α-carboxyl group with the Arg113. The fragment of the substrate in the donor side undergoes a conformational change that is best described as hinging about the first Cα carbon atom. This conformational change alters position of the Phe410 on the surface of CapD, i.e., propagates from the active site to the surface of CapD.

This reaction segment strongly suggests that the protonated N-terminal amino group is not very stable. Furthermore, the second α-carboxyl of the substrate diγDGA is involved in catalysis of its own transpeptidation by CapD. We must note that, our calculations do not incorporate any water molecules and thus explore the ability of the CapD residues to participate in catalysis. We anticipate that including water molecules would result in alternate pathways with potentially lower activation energies. Nevertheless, because of the high degree of difficulties and computational expenses we did not attempt to incorporate water molecules into our calculations at this time.

1.2.2 Formation of acylated CapD intermediate of pDGA (QM2)

Starting from the metastable, tetrahedral intermediate with protonated second α-carboxyl group, the reaction proceeds as follows. First, the nitrogen atom of the γ-peptide bond undergoes an inversion. This inversion increases the energy of the system by 5.8 kcal/mol as seen from Table S1. The barrier for the inversion is 6.5 kcal/mol. The inversion prepares the nitrogen atom for receiving the proton from the carboxyl group in the next step. The proton from the α-carboxyl is hydrogen bonded with the N-terminal α-amino group of Thr352. To break that hydrogen bond and rotate the OH bond of the carboxyl toward the γ-peptide nitrogen takes 10.0 kcal/mol and increases the energy of the system by the same amount.

The system can lower its energy by inverting the NH_2 group of Thr352 to form a hydrogen bond with the second α-carobxyl group. However the stabilization is not substantial (see Table S1). Additional conformational changes can lower the energy of the system down to 25.4 kcal/mol relative to the bound reactant complex. This conformational change perturbs the active site the least. The proton transfer from the carboxyl group to the nitrogen has a relatively small barrier but subsequently achieves significant energy stabilization and greatly perturbs the geometry of the active site. The energy of the glutamylated CapD in kcal/mol is 3.4 kcal/mol above the bound reactant complex using QM2 model. The immediately preceding structure has energy of 25.4 kcal/mol. Both energy and structure depend on the selection of the QM model used in the calculations.

Thus, with a smaller QM1 selection the final step changes energy from 32.3 to -9.4 kcal/mol.

2. Mechanism of CapD Inhibition with Capsidin

2.2 CapD Acylation Mechanism by capsidin

2.2.2 Formation of acetylated CapD intermediate of capsidin

The tetrahedral intermediate may undergo an optional, stabilizing conformational change in the sidechain of Arg113 that propagates to the surface of the enzyme. This conformational change primarily affects the donor region of the binding site. It lowers the energy of the system by 3.3 kcal/mol. The conformational change in the sidechain of Arg113 can be described as crank-shaft motion. The conformational change has a barrier of only 0.2 kcal/mol (see Table S2; Fig. S4, segment S2; and Fig. S5, segment S1). This conformational change is reversed before the reaction can proceed further. The barrier to reverse the conformational change is 3.5 kcal/mol.

A similar crank-shaft conformational change of the Arg113 sidechain has been observed before the rate limiting step (not shown). Such flexibility was not observed in the case with pDGA. Furthermore, the bound capsidin CapD complex experiences a cascade of conformational changes before it arrives in a catalytically competent state. Such cascade was not apparent with pDGA.

Table S1. Energies in kcal/mol along computed reaction paths for CapD acylation by pDGA

R2I QM1 (Fig. S1) S1 (Fig. S2)	**Overall**	**Forward**	**Backward**
R	0.0	0.0	-23.4
TS	23.6	23.6	0.2
P	23.4	23.4	0.0
S2 (Fig. S2)			
R	23.4	0.0	1.3
TS	24.3	0.9	2.2
P	22.1	-1.3	0.0
I2P QM1 (Fig. S1) S1 (Fig. S3)	**Overall**	**Forward**	**Backward**
R	22.0	0.0	-5.8
TS	28.4	6.5	0.7
P	27.7	5.8	0.0
S2 (Fig. S3)			
R	27.8	0.0	-10.0
TS	37.9	10.0	0.0
P	37.8	10.0	0.0
S3 (Fig. S3)			
R	37.8	0.0	5.3
TS	41.4	3.6	8.9
P	32.5	-5.3	0.0
S4			
R	32.3	0.0	41.8
TS	-	-	-
P	-9.4	-41.8	0.0
R2I QM2 (Fig. S1) S1 (Fig. 3)	**Overall**	**Forward**	**Backward**
R (Fig. 4R)	0.0	0.0	-18.0
TS (Fig. 4TS)	22.3	22.3	4.3
P (Fig. 4P)	18.0	18.0	0.0
I2P QM2 (Fig. S1) S4	**Overall**	**Forward**	**Backward**
R	25.4	0.0	22.0
TS	-	-	-
P (Fig. 5P)	3.4	-22.0	0.0

R2I corresponds to the reaction from the bound complex of CapD with pDGA to form the tetrahedral intermediate; **I2P** corresponds to the reaction where the tetrahedral intermediate transforms into the γ-glutamylated CapD. Where available, references are given to matching figures in the main text and supporting information for illustrations.

Table S2. Energies in kcal/mol along computed reaction paths for CapD acylation by capsidin

R2I QM (Fig. S1)	**Overall**	**Forward**	**Backward**
S1 (Fig. S4; Fig. 6)			
R (Fig. 7R)	0.0	0.0	-14.7
TS (Fig. 7TS)	22.9	22.9	8.2
P (Fig. 7P)	14.7	14.7	0.0
S2 (Fig. S4)			
R	14.8	0.0	3.3
TS	15.0	0.2	3.5
P	11.5	-3.3	0.0
I2P QM (Fig. S1)			
S1 (Fig. S5)			
R	11.5	0.0	-3.3
TS	15.0	3.5	0.2
P	14.8	3.3	0.0
S2 (Fig. S5)			
R (Fig. S6R)	14.7	0.0	2.9
TS (Fig. S6TS)	17.1	2.4	5.3
P (Fig. S6P)	11.8	-2.9	0.0
S3 (Fig. S5)			
R	11.9	0.0	13.6
TS	12.0	0.1	13.7
P	-1.7	-13.6	0.0
S4 (iminol to amide)			
R	-1.7	0.0	-2.9
TS	-	-	-
P	1.2	2.9	0.0

R2I corresponds to the reaction from the bound complex of CapD with capsidin to form the tetrahedral intermediate; **I2P** corresponds to the reaction where the tetrahedral intermediate transforms into the acetylated CapD. Where available, references are given to matching figures in the main text and supporting information for illustrations.

Figure Captions

Figure S1. QM regions used in calculations

A and B show QM1 and QM2 selections of CapD with pDGA. C shows the QM selection for CapD with capsidin. Molecules are shown using sticks with balls on the link atoms that represent the boundary between the QM and MM layers.

Figure S2. Energy profile for formation of the tetrahedral intermediate between pDGA and CapD using QM1 model

S1 segment captures formation of a tetrahedral intermediate with unusually long O-C bond and protonated terminal aminogroup. S2 captures transfer of the proton from the amino group to the hydrogen bonding carboxyl group. Note that in QM2 these two segments collaps into one as described in main text.

Figure S3. Energy profile for collapse of the tetrahedral intermediate of pDGA into γ-glutamylated CapD using QM1 model

Segment S1 corresponds to the inversion of the nitrogen of the scissile bond. Segment S2 captures rotation of the OH group of the protonated carboxyl group toward the nitrogen. Segement S3 corresponds to proton transfer from the carboxyl to the nitrogen along with breaking the peptide bond.

Figure S4. Energy profile for formation of the tetrahedral intermediate between pDGA and CapD using QM model

S1 segment corresponds to the formation of a tetrahedral intermediate. S2 captures a conformational change in the side chain of Arg113 that is reminiscent of a “crank-shaft” motion.

Figure S5. ***Energy profile for collapse of the tetrahedral intermediate of capsidin into acetylated CapD using QM model***

Segment S1 corresponds to the conformational change in the side chain of Arg113 that is reminiscent of a "crank-shaft" motion but now in the opposite direction. Segment S2 captures the rotation about the Cα-Cβ bond of the catalytic Thr352 coupled to stretching of the scissile bond of capsidin. Segement S3 corresponds to proton transfer from the N-terminal amino group to the oxygen of the second acetyl group of capsidin that converts into iminol.

Figure S6. Conformational change coupled to bond stretching

The reactant (R), transition state (TS), and product (P) structures along the rotation-coupled stretching of the scissile bond of capsidin that corresponds to segment S2 of Fig. S5. Enzyme carbon atoms are colored green while carbon atoms of capsidin are colored magenta. Hydrogen, nitrogen, oxygen, and sulfur atoms are colored white, blue, red, and yellow, respectively. Hydrogen bonds between the ligands and the enzyme are shown with dashed gray lines. Yellow dashed lines connect the atoms involved in the chemical transformations. These atoms are also enhanced using solid spheres. The residues of the active site that are not directly involved in the reaction are shown in faded, transparent colors. Atoms that link QM region to the rest of the system described by molecular mechanics are depicted with transparent spheres around them. These atoms are substituted for hydrogen atoms and their bond lengths adjusted accordingly

during QM calculations. The red dashed lines connect the atoms of the scissile peptide bond of the inhibitor that has been cleaved.

Figures

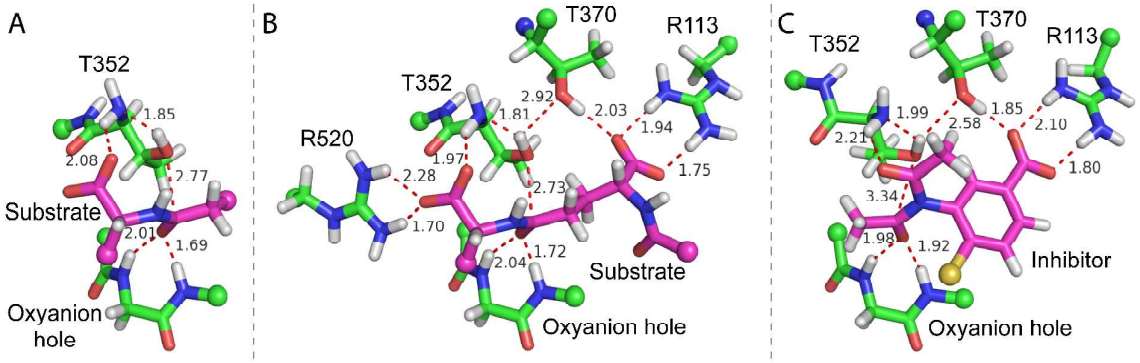

Figure S1

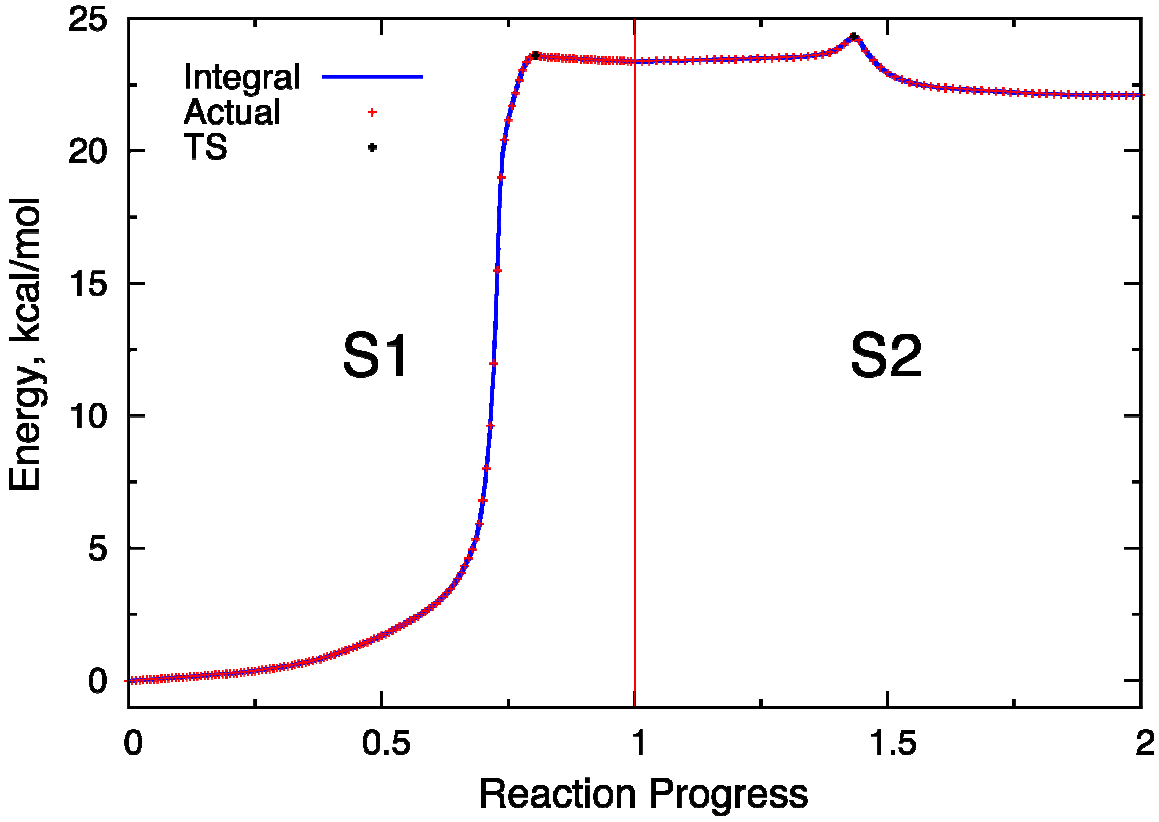
Integral
Actual
TS
S1
S2
Energy, kcal/mol
Reaction Progress
25
20
15
10
5
0
0
0.5
1
1.5
2

Figure S2

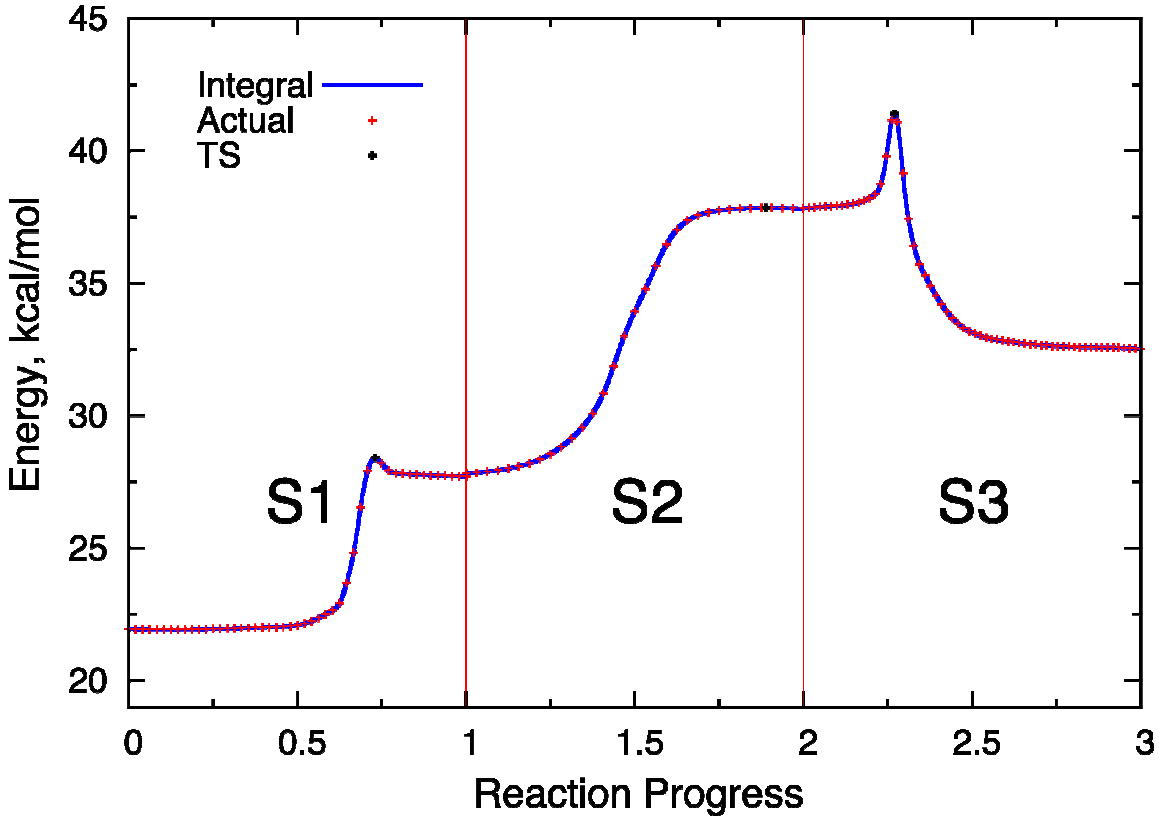
Integral
Actual
TS
S1
S2
S3
Energy, kcal/mol
Reaction Progress
45
40
35
30
25
20
0
0.5
1
1.5
2
2.5
3

Figure S3

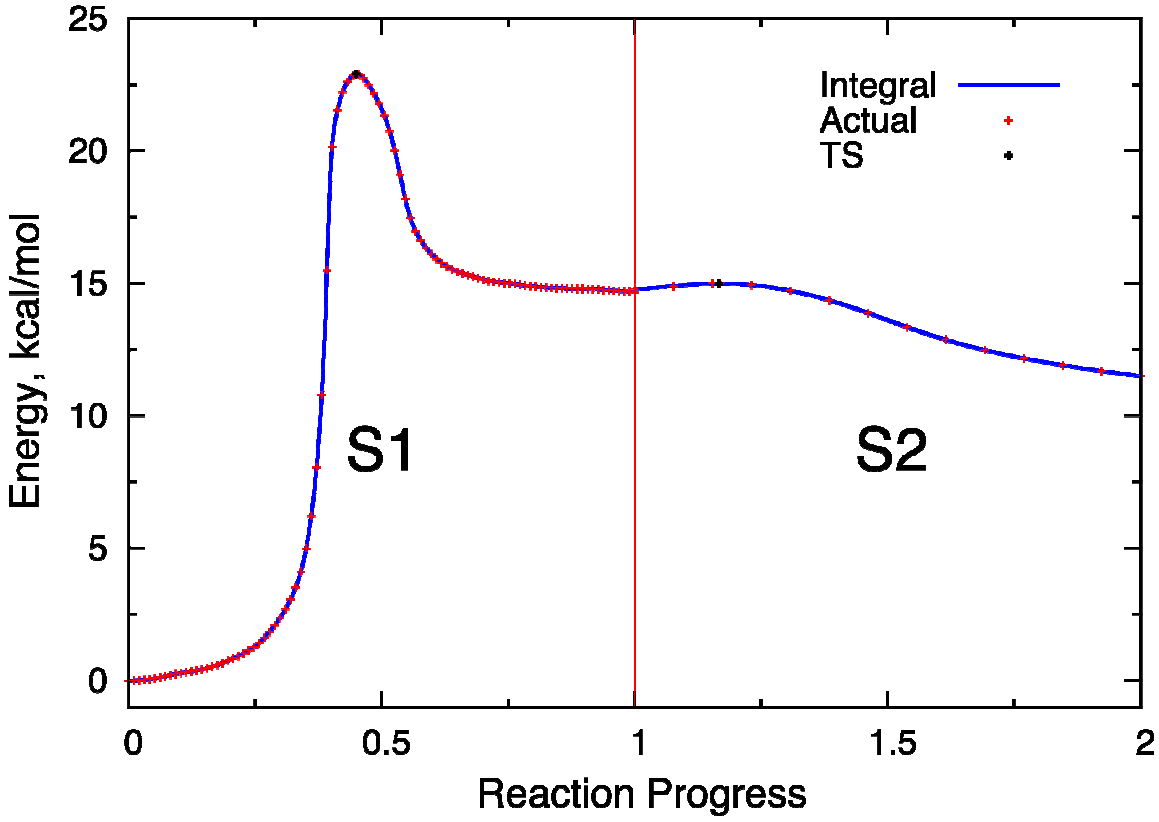
Integral
Actual
TS
S1
S2
Energy, kcal/mol
Reaction Progress
25
20
15
10
5
0
0
0.5
1
1.5
2

Figure S4

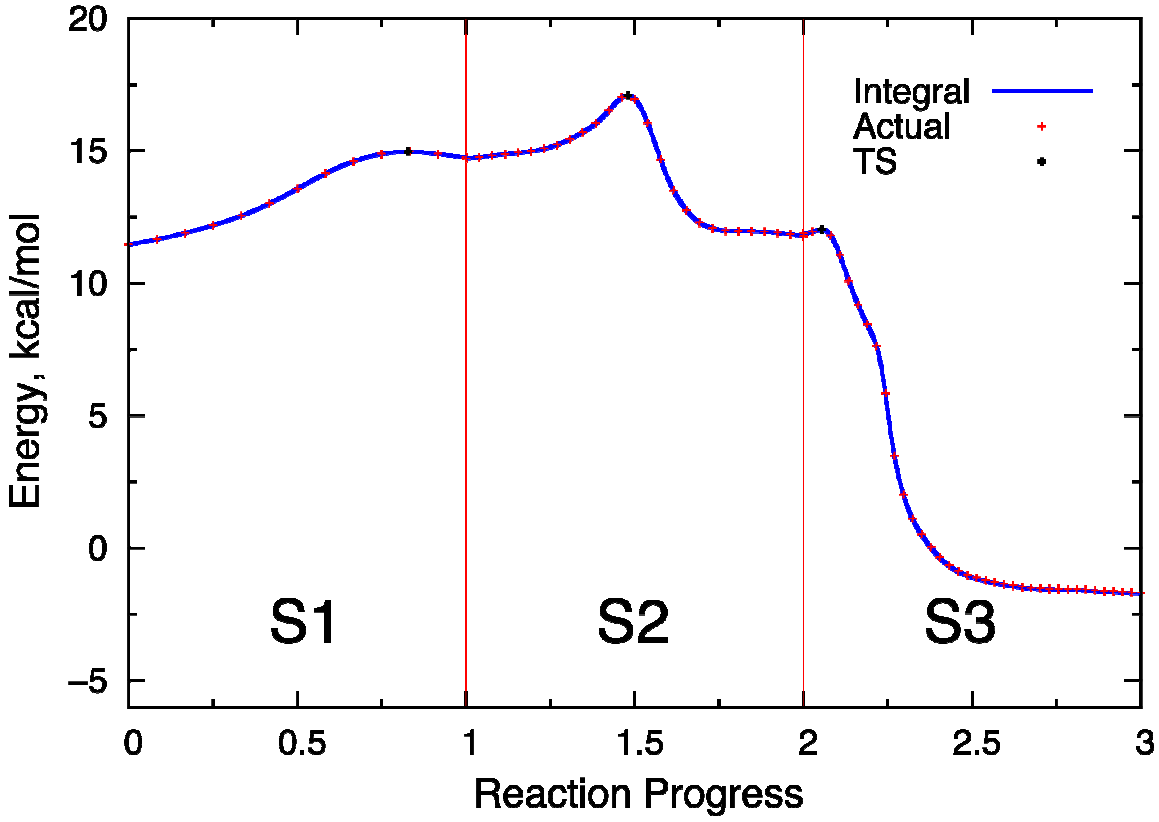
Integral
Actual
TS
S1
S2
S3
Energy, kcal/mol
Reaction Progress
20
15
10
5
0
−5
0
0.5
1
1.5
2
2.5
3

Figure S5

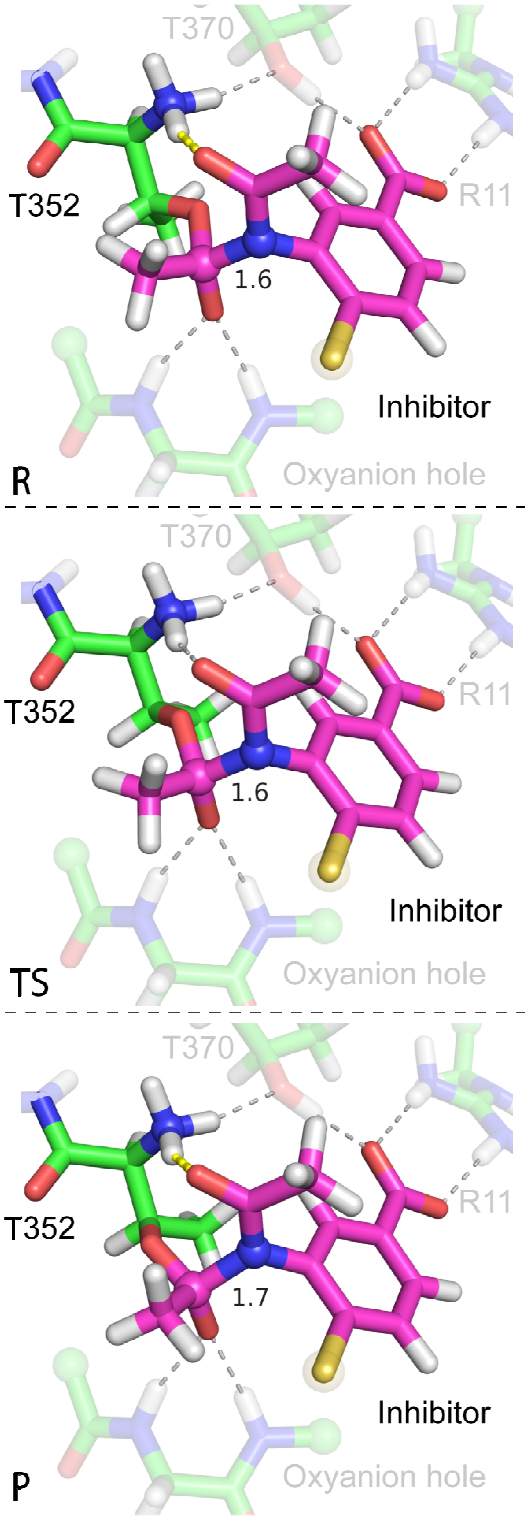
T370
T352
R11
1.6
Inhibitor
Oxyanion hole
R
T370
T352
R11
1.6
Inhibitor
Oxyanion hole
TS
T370
T352
R11
1.7
Inhibitor
Oxyanion hole
P

Figure S6

References

1. Hu, X.; Legler, P. M.; Khavrutskii, I. V.; Scorpio, A.; Compton, J. R.; Robertson, K. L.; Friedlander, A. M.; Wallqvist, A., Probing the Donor and Acceptor Substrate Specificity of the gamma-Glutamyl Transpeptidase. Biochemistry 2012, 51 (6), 1199-1212.