Supplementary Information

Simulating the Inhibition Reaction of *Mycobacterium tuberculosis* L,D-Transpeptidase 2 by Carbapenems

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Material and Methods

The system

The enzyme-inhibitor complexes for the starting structures were prepared using the available LDT_{Mt2} crystal structures (3TUR[1] and 3VYP[2] access codes) and theoretical models from our previous computational model of the catalytic mechanism.[3] The bonded form of meropenem in the 3VYP PDB structure was rebuilt and used to build the imipenem-bound structure. Then, the transition state (TS) structure from our previous study was used to construct, *in silico*, both LDT_{Mt2} complexes, considering experimental information from the 3VYP PDB crystal structure.

The Amber12 package[4] with ff99SB[5] (for protein), GAFF[6] (for inhibitors) and TIP3P[7] (for water molecules) parameter sets were used for starting MM simulations. The missing protons of the protein were added using *tleap* Ambertools module. The optimized structures and partial charges for the inhibitors were obtained utilizing the RESP method from HF/6-31G* calculations carried out in the Gaussian09 program.[8] The complexes were solvated in a truncated octahedral cell of TIP3P[7] water molecules, extending 10 Å outside the system on each side. The minimization, heating and equilibration stages used for the complexes were the same applied in the catalytic mechanism[3] (see ref. 3 for details). The SHAKE method[9] was used to restraint MM hydrogen bonds and a cut-off of 8 Å for non-bonded interactions with PME was used for long-range electrostatic interactions.

QM/MM Umbrella Sampling and Potential Mean Force (PMF)

For the hybrid QM/MM MD simulations with enzyme-carbapenem complexes, the atoms of the Cys354, His336, Ser337 and the whole carbapenem were selected for the QM region, which contains 80 atoms for LDT_{Mt2}-meropenem and 66 atoms for LDT_{Mt2}-imipenem. The semi-empirical SCC-DFTB (MIO version) method[10] without any order correction as implemented in Amber12[11], was selected to describe the QM region, while the rest of the system (enzyme and water molecules) was described using the ff99SB[5] and TIP3P[7] classical parameters set, respectively, as described early. To satisfy the valence of the QM fragments in the QM-MM boundary, the link atom method[12] was used. Finally, 100 ps of QM/MM MD simulations were performed for each complex in order to equilibrate the initial structures for the umbrella sampling simulations. PDB files for reactant states are included as ESI.

The umbrella sampling approach was carried out using a linear combination of the distances described in the main manuscript (see Scheme 1). A 2D FES was obtained to explore the first step in the inactivation mechanism of LDT_{Mt2} by carbapanem, while single 1D FES was used to describe the second step. The values of the reaction coordinates during the complete simulations were restrained to their target values with a harmonic potential and a force constant of 250 $kcal \cdot mol^{-1} \cdot Å^{-2}$. The PMFs were unbiased using the variational free energy profile (VFEP) method, which is implemented in the VFEP program written by Lee and coworkers.[13]

1D FES and simulations on the water/enzymatic environments

In order to validate the reliability and convergence of the SCC-DFTB/MM method applied in this study, the 1D FES for the acylation step of enzyme-carbapenem complexes were evaluated in water and enzymatic environments. For the water system, a model containing only the catalytic residues Cys354, His336 and Ser337 main chain and carbapenem was built in a water box. To build this model, we started from the Ldt_{Mt2} crystal structure, cut the bonds linking the Cα atoms to the protein backbone, and then filled the free valence of each Cα with hydrogen atoms. During the MD simulations, a 100 $kcal \cdot mol^{-1} \cdot Å^{-2}$ harmonic restraint on the Cα atom for the Cys354, His336 and Ser337 main chain was applied to their starting positions so as to maintain the same distances as in the enzyme environment. The water molecules were modeled by MM while Cys354-His336-Ser337 residues and carbapanem inhibitor were part of

the QM system (see Scheme 1 in the main text). The same procedure was used in our previous study.[3]

The 1D reaction coordinate used to describe the acylation step was (d(C_7 – N_4) – d(Sγ – C_7)) for both systems (water and enzymatic environments). The harmonic potential applied to restrain the values of the reaction coordinates during the whole simulation was the same used for the 2D FES calculations (force constant of 250 kcal·mol^{-1}·Å^{-2}). Finally, the 1D PMF profile was obtained using VFEP method, as previously described. The results are presented on the Fig. S5.

In order to explore the catalytic nature of enzymes, it is also important to have a perspective about the activation barrier of the reference reaction in solution. The calculated activation energy for reactions in water and in the LDT_{Mt2} involving meropenem and imipenem are shown in Fig. S5. The DFTB/MM activation free energy values correspond to 17.50 and 14.95 kcal·mol^{-1} for the imipenem and meropenem in solution, respectively. While, in the enzymatic environment the $\Delta G^{\ddagger}_{\mathrm{calc}}$ values are 6.15 and 8.00 kcal·mol^{-1}, respectively which suggest that DFTB/MM potential in the enzymatic complex indeed induced a catalytic effect. Note that, to the best of our knowledge, there is no experimental data available for this reaction in solution.

Experimental free energy barriers

The experimental free energy barriers for the inhibitors were obtained by the transition state theory according to follow formulation[14]:

$$\boldsymbol{k_n = \frac{k_B T}{h} e^{-\frac{\Delta G^{\ddagger}}{RT}}}$$

where $\boldsymbol{k_B}$, $\boldsymbol{h}$ and $\boldsymbol{R}$ are the Boltzmann, Planck and gas constants, and **T** is the absolute temperature.

Correction of SCC-DFTB/MM by M06-2X/MM method

In order to fix the possible inaccuracies in the SCC-DFTB semiempirical method and verify its reliability with higher theoretical levels (i.e. DFT), single-point M06-2X-D3/MM calculations were carried out using representative snapshots from R, TS1, INT and TS2 ensembles from PMF QM/MM simulations. A total of 200 representatives' snapshots for each state were used. The corrected SCC-DFTB/MM results based on the M06-2X-D3/MM calculations have led to improved energies at the QM level as well as the MM interactions and its polarization on the QM subsystem,[15] as proposed for the β-

lactam system[16]. The M06-2X-D3/MM//SCC-DFTB/MM corrections were performed using a straightforward one-step free energy perturbation,

$$\Delta G_{M062X-DFTB} = -k\mathrm{T}\ln\langle e^{-\beta(E_{M062X/MM}-E_{DFTB/MM})}\rangle_{DFTB/MM}$$

The M06-2X-D3/MM//SCC-DFTB/MM results are summarized on Table S3.

Supplementary Results

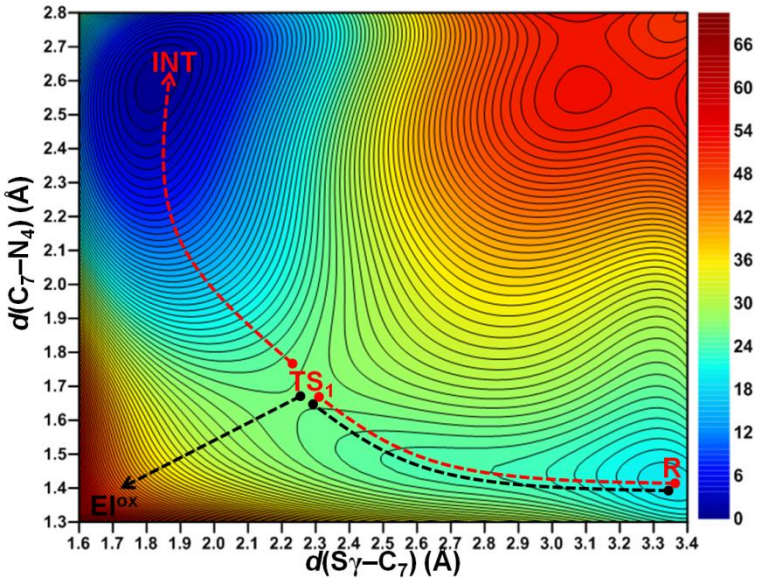

Figure S1. 2D FES for the first step of imipenem in LDT_{Mt2}. R: reactant; TS_1: transition state; INT: intermediate; EI^{ox}: oxyanion state. The energy values are report in kcal mol^{-1}. The red dashed line present the minimum free energy path (MFEP), while the black dashed line present the experimental proposal. The $\Delta G^{\ddagger}_{calc}$ and computed ΔG° values are 6.31 and –20.00 kcal·mol^{-1} for the MFEP, respectively. The computed ΔG° value for the EI^{ox} is 28.00 kcal·mol^{-1}.

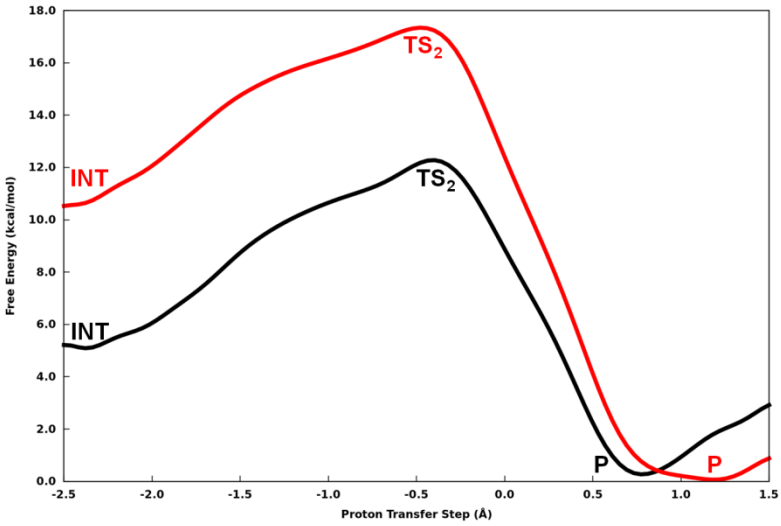

Figure S2. 1D FES for the proton transfer step ($d(N_\varepsilon–H_\varepsilon) – d(N_4–H_\varepsilon)$) of meropenem (black) and imipenem (red) in complex to LDT_{Mt2}. INT: intermediate; TS_2: transition state; P: product (acyl enzyme). The DFTB/MM free energy values are reported in kcal mol^{-1}. The $\Delta G^{\ddagger}_{calc}$ and computed ΔG°values are 8.00 and –5.80 kcal·mol^{-1} for the meropenem, respectively. While these values are 7.10 and –10.50 kcal·mol^{-1} for the imipenem, respectively.

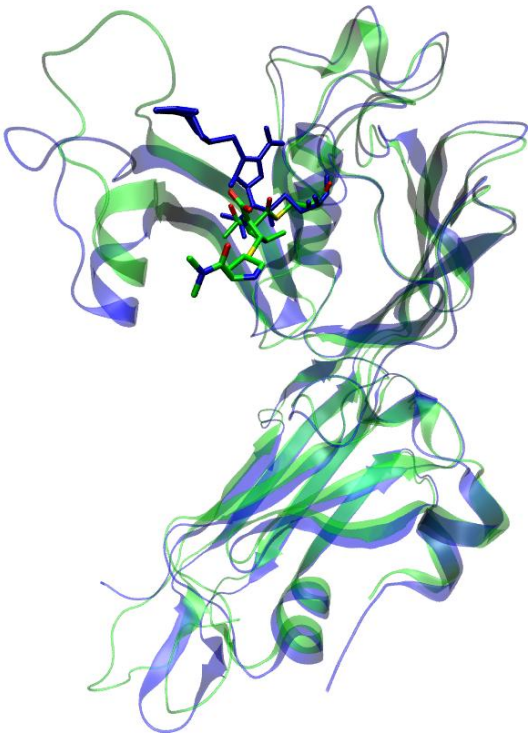

Figure S3. 3D overlap of crystal LDT_{Mt2}-meropem (green) (PDB code: 3VYP[2]) and theoretical model (blue) obtained by QM/MM umbrella sampling simulations. In the crystal structure, the meropenem covalently linked to enzyme is presented in the adduct

state II, while for theoretical model, the adduct state I is shown. The RMSD value for these structures is 1.20 Å.

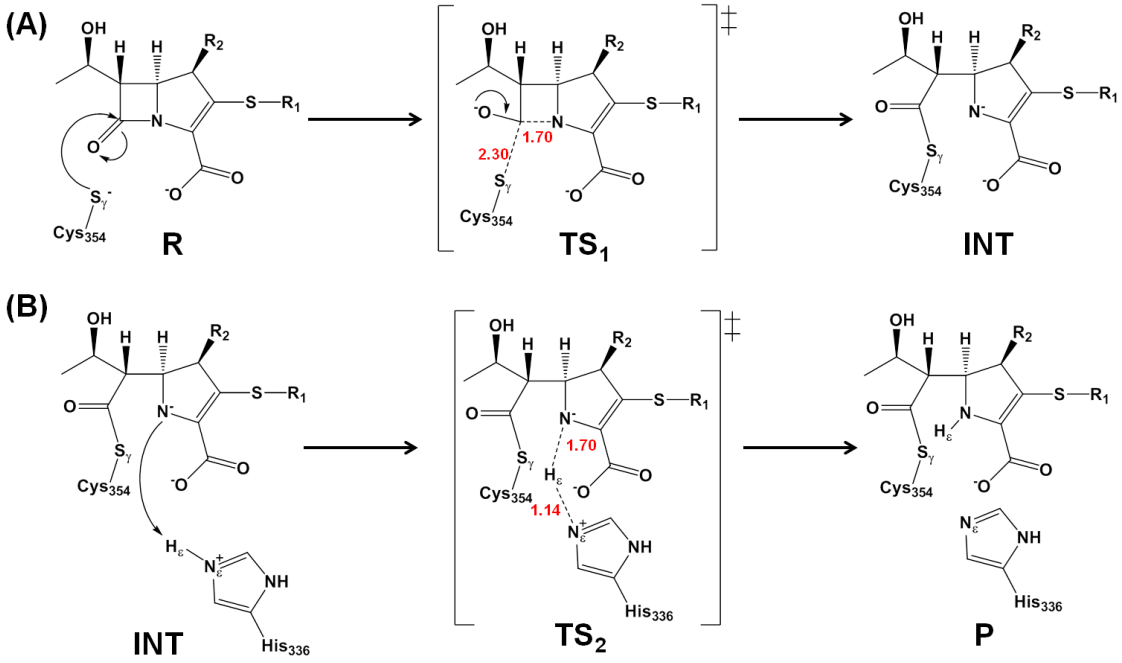

Figure S4. Inactivation mechanism proposal after QM/MM mechanistic study taking into account the MFEP obtained in the FESs. (A) First step (acylation) and (B) second step (proton transfer).

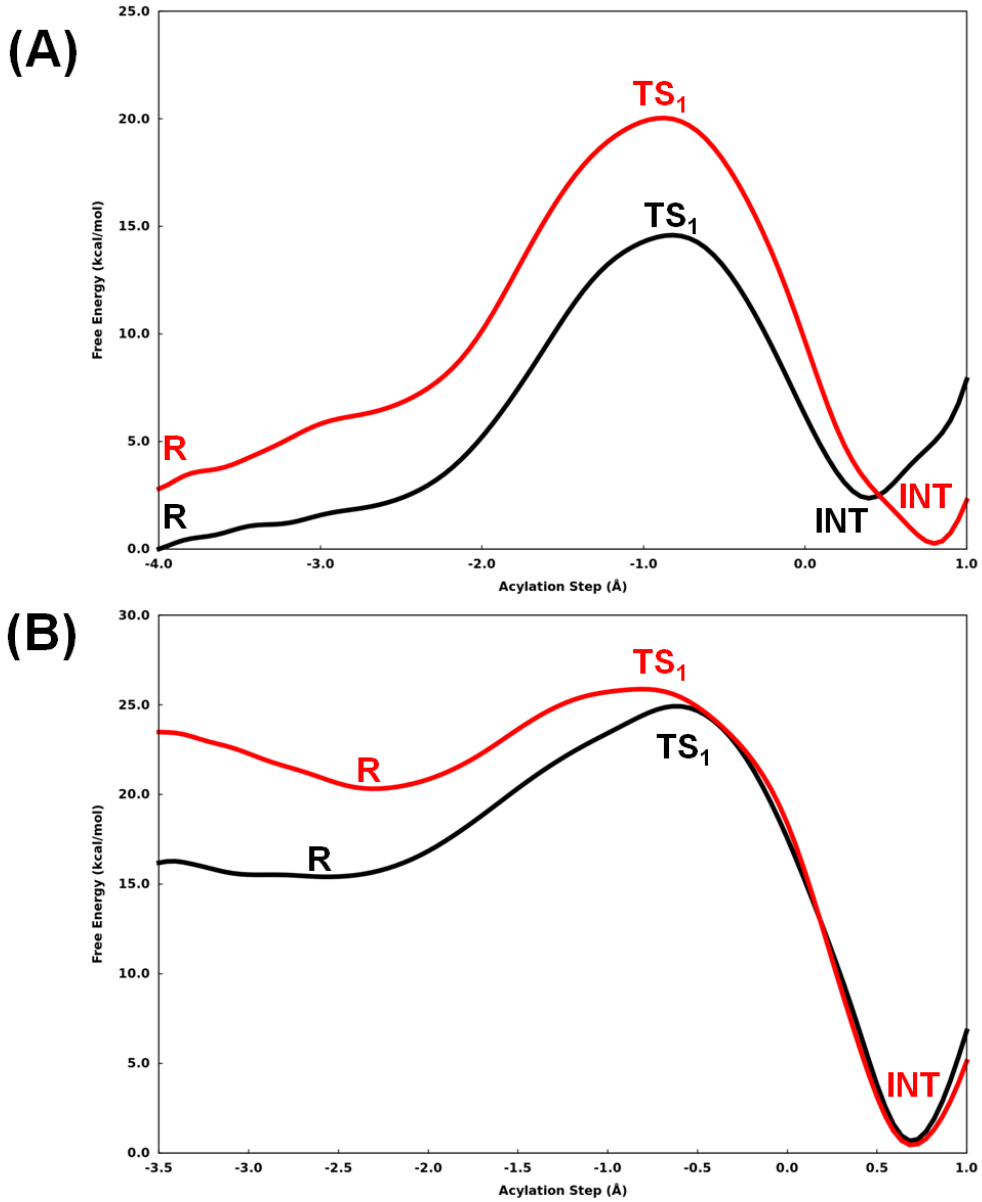

Figure S5. 1D FES for the acylation step ($d(C_7 - N_4) - d(S\gamma - C_7)$) of imipenem (red) and meropenem (black) in water (A) and enzymatic (B) environments. R: reactant; TS_1: transition state; INT: intermediate. The DFTB/MM free energy values are report in kcal mol^{-1}. In the water environment the $\Delta G^{\ddagger}_{calc}$ values are 17.50 and 14.95 kcal·mol^{-1} for the imipenem and meropenem systems, respectively. Whereas, in the enzymatic environment the $\Delta G^{\ddagger}_{calc}$ values are 6.15 and 8.00 kcal·mol^{-1}, respectively.

Table S1. Average values for the relevant distances involved during the QM/MM umbrella sampling simulations for the whole inactivation mechanism. The values are reported in Å.

	LDT_{Mt2}-Meropenem				
	R	**TS_1**	**INT**	**TS_2**	**P**
$d(S_\gamma - C_7)$	3.41±0.03	2.29±0.03	1.81±0.03	1.80±0.03	1.82±0.03
$d(C_7 - N_4)$	1.42±0.02	1.90±0.03	2.50±0.05	2.62±0.06	2.70±0.06
$d(N_\varepsilon - H_\varepsilon)$	1.04±0.03	1.03±0.03	1.02±0.03	1.16±0.03	1.85±0.05
$d(N_4 - H_\varepsilon)$	5.50±0.30	4.24±0.26	5.00±0.05	1.57±0.04	1.05±0.03
	LDT_{Mt2}-Imipenem				
	R	**TS_1**	**INT**	**TS_2**	**P**
$d(S_\gamma - C_7)$	3.40±0.03	2.29±0.04	1.82±0.03	1.81±0.03	1.82±0.03
$d(C_7 - N_4)$	1.42±0.02	1.70±0.04	2.53±0.06	2.66±0.07	2.74±0.07
$d(N_\varepsilon - H_\varepsilon)$	1.08±0.03	1.03±0.03	1.02±0.03	1.13±0.03	3.83±0.04
$d(N_4 - H_\varepsilon)$	5.04±0.24	4.27±0.23	4.43±0.04	1.70±0.04	1.04±0.03

Table S2. Experimental kinetic values[17] and DFTB/MM calculated free energy barriers for the inactivation mechanism of LDT_{Mt2} by carbapanems. *Energy values are reported in kcal·mol^{-1}. The reported values from first step were taken from 2D FES.

	$k_1 \cdot 10^3$ (μM^{-1} min^{-1})	$k_2 \cdot 10^3$ (min^{-1})	$\Delta G^{\ddagger}_{exp}(1)$*	$\Delta G^{\ddagger}_{exp}(2)$*	$\Delta G^{\ddagger}_{calc}(1)$*	$\Delta G^{\ddagger}_{calc}(2)$*
Meropenem	0.67±0.02	660±40	15.74	19.78	7.27	8.00
Imipenem	67±5	11200±700	13.06	18.13	6.31	7.10

Table S3. M06-2X-D3/MM//SCC-DFTB/MM calculated free energy barriers for the inactivation mechanism of LDT_{Mt2} by carbapanems. *Energy values are reported in kcal·mol^{-1}. The reported values from first step were taken from 2D FES.

	$\Delta G^{\ddagger}_{M062X/MM}(1)$*	$\Delta G^{\ddagger}_{M062X/MM}(2)$*
Meropenem	17.41±1.30	20.00±1.05
Imipenem	14.30±1.55	18.47±1.25

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