Common Mechanistic Features among Metallo-β-Lactamases: a Computational Study of *Aeromonas hydrophila* CphA Enzyme

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Running head: Common role of zinc in metallo β-lactamases.

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Supporting information: MD Simulations of the intermediate of ES1-based reaction.

SI: METHODS

Two classical MD simulations were carried out, **IntA** and **IntB**. In both models, the force field parameters of the protein (except for the protein active site, Fig. 2), water, counterions were treated with the AMBER PARM99, as in ref. [1]. Active site and **Int** charges, in both models, were calculated with the RESP method [2, 3]. In both simulations, **Int** parameters were constructed using the gaff force field [4].

The simulations differed for the parametrization of ligand-Zn bonds in the active site. In **IntA**, a non-bonded approach was used, in which **Int** were not explicitly bound to the metal by any atom.

In **IntB**, the atom donors N(1)@**Int** and O(2)@**Int**, formed a covalent bond with the Zn(II) ion, following the parametrization of Merz and coworkers [5, 6, 7]

5 ns MD calculations were carried out in the NPT ensemble using the NAMD program [8, 9], an integration time step of 1.5 fs was used, with the SHAKE algorithm [10] to constrain bonds involving hydrogen atoms. Electrostatic interactions were computed using the Particle Mesh Ewald (PME) [11]. A cut-off of 12 Å was used for van der Waals and for short range component of electrostatic interactions. The systems were initially relaxed with 5,000 steps of optimization of the solvent, while the protein was held fix, followed by 5,000 steps of optimization of the whole system. The systems were slowly heated to 300 K performing runs at constant volume and constant temperature using Langevin thermostat [8, 9]. Constant temperature and pressure (1 atm, 300 K) MD runs were finally performed [12]. The pressure coupling was accomplished with Langevin piston [13], and the temperature with the Nosé-Hoover method [14, 15].

All the structural analysis of the trajectories have been performed with the VMD visualization software [16].

The RMSD was calculated over the backbone atoms (hydrogen atoms not included) [17]. Hydrogen bonds were assumed to be present if donor and acceptor were located within 3.4 Å and the angle between donor, hydrogen and acceptor was comprised between 150 and 210 degrees.

SI: RESULTS

During the 5 ns of dynamics, the overall folds of **IntA** and **IntB** were fully maintained (RMSD = 0.9 ± 0.1 Å), pointing to a good stability of the MD structures, as observed in all our MD simulations of this system [18] and B1 enzymes [5, 6, 7, 19, 20, 21, 22]

The substrate formed the following non-bonded interactions: Lys224-O(2)@**Bia**, Asn223@NH-O(2)@**Bia** along with two weak H-bonds between His118@NεH-O(2)@**Bia** and between Asn223@NH_2-O(1)@**Bia** (Tab. 4).

The **Bia**@N(1)-Zn distance and **Bia**@O(2)-ZN one (Tab. 4) turned out to increase in **IntA**, whilst kept the same values as the initial ones in **IntB** (Tab. 4). These different results are to be ascribed to the different parametrizations at the active site.

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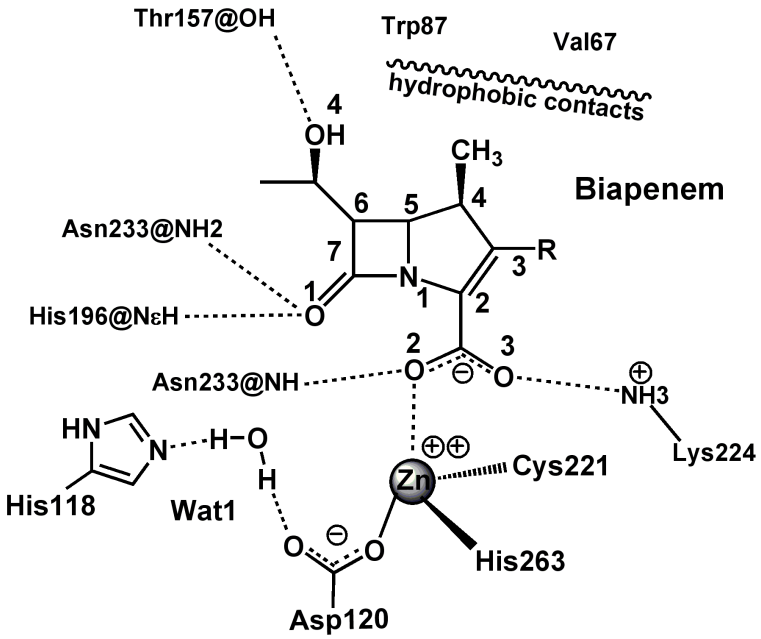
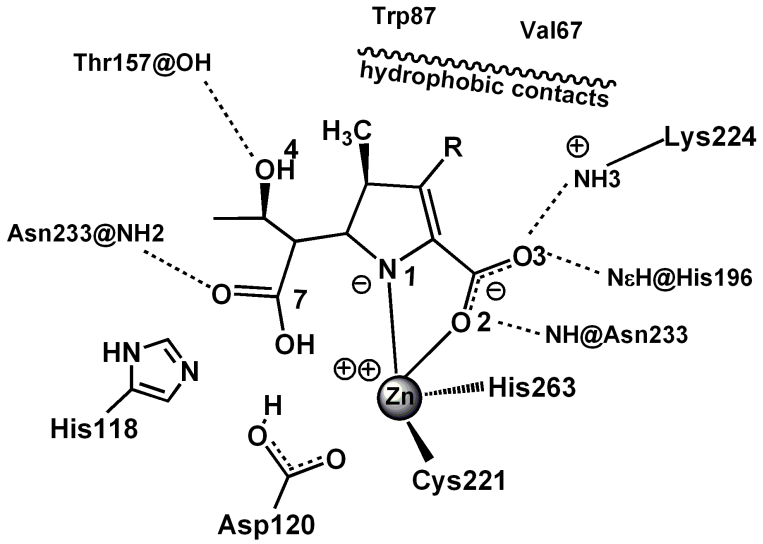


RC	Asp120@O-Zn	His263@Nε-Zn	**Bia**@O(2)-Zn	Cys221@S-Zn	Lys224@NH_3-O(3)@**Bia**	**Bia**@C(7)-N(1)@**Bia**	**Wat1**@O-Zn	Asn233@NH-O(3)@**Bia**	Asn233@NH_2-O(1)@**Bia**	Thr157@OH-O(4)@**Bia**
Initial	2.0	2.2	2.4	2.3	2.6	1.9	1.4	2.1	1.9	1.9
Free	2.1 ± 0.1	2.1 ± 0.1	3.8 ± 0.2	2.3 ± 0.1	2.0 ± 0.1	1.3 ± 0.1	2.1 ± 0.1	1.9 ± 0.1	2.0 ± 0.1	1.9 ± 0.1
3.1	2.1 ± 0.1	2.1 ± 0.1	3.8 ± 0.3	2.3 ± 0.1	2.0± 0.1	1.3 ± 0.1	2.12 ± 0.1	2.0 ± 0.1	1.9 ± 0.1	2.0 ± 0.1
2.9	2.1 ± 0.1	2.1 ± 0.1	3.8 ± 0.2	2.3 ± 0.1	2.0 ± 0.1	1.3 ± 0.1	2.1 ± 0.1	1.9 ± 0.1	1.9 ± 0.1	1.9 ± 0.2
2.7	2.1 ± 0.1	2.1 ± 0.1	3.9 ± 0.3	2.3 ± 0.1	2.0 ± 0.2	1.4 ± 0.1	2.14 ± 0.1	1.9 ± 0.1	2.0 ± 0.1	2.0 ± 0.1
2.5	2.1 ± 0.1	2.1 ± 0.1	3.7 ± 0.2	2.3 ± 0.1	1.9 ± 0.2	1.4 ± 0.1	2.16± 0.1	1.9 ± 0.1	1.9 ± 0.1	1.9 ± 0.1
2.3	2.1 ± 0.1	2.1 ± 0.1	3.5 ± 0.2	2.3 ± 0.1	1.9 ± 0.1	1.4 ± 0.1	2.19 ±0.1	2.0 ± 0.1	2.0 ± 0.1	2.0 ± 0.1
2.1	2.1 ± 0.1	2.1 ± 0.1	3.2 ± 0.2	2.3 ± 0.1	1.8 ± 0.2	1.4 ± 0.1	2.2 ± 0.1	1.9 ± 0.1	1.9 ± 0.1	1.9 ± 0.2
1.9	2.1 ± 0.1	2.1 ± 0.1	3.2 ± 0.2	2.3 ± 0.1	1.8 ± 0.1	1.5 ± 0.1	2.3 ± 0.1	2.1 ± 0.1	2.0 ± 0.1	1.9 ± 0.1
1.8	2.0 ± 0.1	2.1 ± 0.1	3.2 ± 0.5	2.3 ± 0.1	1.8 ± 0.1	1.5 ± 0.2	3.0 ± 0.2	2.0 ± 0.1	2.0 ± 0.1	2.0 ± 0.1
1.7	2.4 ± 0.4	2.1 ± 0.2	2.4 ± 0.3	2.3 ± 0.1	1.8 ± 0.1	1.6 ± 0.2	2.8 ± 0.2	1.9 ± 0.1	1.9 ± 0.1	1.9 ± 0.1
RC	**Bia**@N(1)-Zn	His263@$N_{epsilon}$-Zn	**Bia**@O(2)-Zn	Cys221@S-Zn	**Wat1**@O-Zn					
1.6	2.0 ± 0.1	2.2 ± 0.2	2.2 ± 0.1	2.3 ± 0.1	3.1 ± 0.2	2.0 ± 0.1	2.2 ± 0.2	2.0 ± 0.1	1.9 ± 0.1	2.0 ± 0.1
1.5	2.0 ± 0.1	2.2 ± 0.2	2.2 ± 0.1	2.3 ± 0.1	3.4 ± 0.2	1.9 ± 0.2	2.5 ± 0.2	1.9 ± 0.1	1.9 ± 0.1	1.9 ± 0.1
1.4	2.0 ± 0.1	2.2 ± 0.2	2.2 ± 0.1	2.3 ± 0.1	3.7 ± 0.2	1.9 ± 0.1	2.6 ± 0.2	2.1 ± 0.3	1.9 ± 0.1	2.0 ± 0.1
Free	2.0± 0.1	2.2 ± 0.1	2.2 ± 0.1	2.3 ± 0.1	3.8 ± 0.2	1.9 ± 0.1	2.6 ± 0.1	2.1 ± 0.3	1.9 ± 0.1	2.0 ± 0.1

RC	Asp120@O-Zn-NεHis263	Cys221@S-Zn-Nε@His263	Asp120@O-Zn-O@**Wat1**	Asp120@O-Zn-S@Cys221	His263@Nε-Zn-O@**Wat1**	Cys221@S-Zn-O@**Wat1**
Initial	95	112	94	108	125	130
Free	93 ± 4	110 ± 5	92 ± 5	110 ± 6	123 ± 9	125 ± 8
3.1	92 ± 4	109 ± 6	92 ± 6	110 ± 7	123 ± 9	123 ± 9
2.9	93 ± 4	110 ± 7	95 ± 5	110 ± 6	119 ± 7	121 ± 7
2.7	92 ± 4	111 ± 5	94 ± 4	109 ± 5	122 ± 8	118 ± 9
2.5	92 ± 4	109 ± 6	93 ± 4	105 ± 6	122 ± 6	122 ± 6
2.3	92 ± 4	110 ± 5	93 ± 4	105 ± 6	123 ± 7	110 ± 5
2.1	92 ± 3	110 ± 4	92 ± 3	103 ± 4	128 ± 5	117 ± 5
1.9	92 ± 3	109 ± 4	90 ± 3	101 ± 4	129 ± 5	118 ± 5
1.8	99 ± 8	117 ± 9	84 ± 5	111 ± 11	126 ± 15	105 ± 8
RC	Asp120@O-Zn-Nε@His263	Cys221@S-Zn-NεHis263	Asp120@O-Zn-O(2)@**Bia**	Asp120@O-Zn-S@Cys221	His263@ε-Zn-O(2)@**Bia**	Cys221@S-Zn-O(2)@**Bia**
1.7	86 ± 6	107 ± 6	164 ± 7	89 ± 9	90 ± 9	102 ± 6
RC	His263@Nε-Zn-N(1)@**Bia**	Cys221@S-Zn-Nε@His263	**Bia**@N(1)-Zn-O(2)@**Bia**	Cys221@S-Zn-N(1)@**Bia**	His263@Nε-Zn-O(2)@**Bia**	Cys221@S-Zn-O(2)@**Bia**
1.6	103 ± 5	151 ± 6	80 ± 2	143 ± 9	96 ± 5	106 ± 4
1.5	103 ± 6	101 ± 6	79 ± 3	149 ± 6	95 ± 7	110 ± 5
1.4	104 ± 7	100 ± 5	80 ± 3	149 ± 8	96 ± 6	109 ± 6
Free	103 ± 6	101 ± 6	83 ± 2	150 ± 7	96 ± 6	110 ± 5

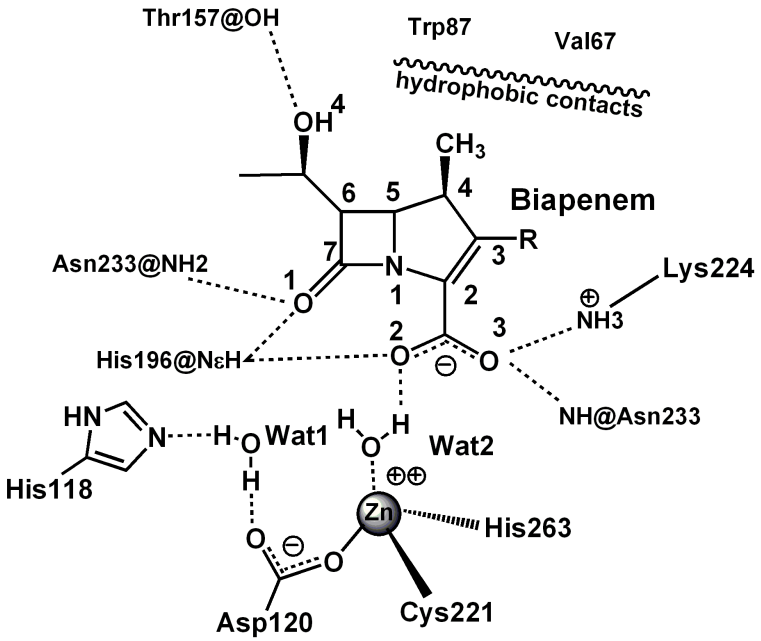
Table 1: QM/MM calculations based on **ES1**-based reaction (first step: the nucleophilic attack to the β-lactam ring). We report here selected ligand/enzyme distances, metal coordination distances (in Å), and bond angles (in degrees), during the constrained simulations along the reaction coordinates. For this step we defined RC as the distance between O@**Wat1** and C(7)@**Bia**.



RC	**Bia**@N(1)-Zn	**Bia**@O(2)-Zn	His263@Nε-Zn	Cys221@S-Zn	**Bia**@C(7)-N(1)@**Bia**	His196@NεH-O(2)@**Bia**	Lys224NH_3-O(3)@**Bia**	Asn233@NH-O(2)@**Bia**	Asn233@NH_2-O(1)@**Bia**	Thr157@OH-O(4)@**Bia**
3.1	2.0 ± 0.1	2.2 ± 0.1	2.2 ± 0.1	2.3 ± 0.1	2.7 ± 0.1	2.6 ± 0.6	1.9 ± 0.1	2.0 ± 0.1	1.9 ± 0.1	1.9 ± 0.1
2.9	2.0 ± 0.1	2.1 ± 0.1	2.2 ± 0.1	2.3 ± 0.1	2.7 ± 0.1	2.2 ± 0.2	1.9 ± 0.1	2.1 ± 0.2	1.8 ± 0.1	1.9 ± 0.1
2.7	2.0 ± 0.1	2.1 ± 0.1	2.2 ± 0.1	2.3 ± 0.1	2.7 ± 0.1	2.2 ± 0.2	1.8 ± 0.1	2.0 ± 0.1	1.8 ± 0.1	2.0 ± 0.1
2.5	2.0 ± 0.1	2.1 ± 0.2	2.2 ± 0.1	2.3 ± 0.1	2.7 ± 0.1	2.1 ± 0.2	1.9 ± 0.7	1.9 ± 0.1	1.8 ± 1.9	2.1 ± 0.1
2.3	2.0 ± 0.1	2.1 ± 0.2	2.2 ± 0.1	2.3 ± 0.1	2.7 ± 0.1	2.1 ± 0.1	1.8 ± 0.1	2.1 ± 0.2	1.8 ± 0.1	2.0 ± 0.1
2.1	2.1 ± 0.1	2.1 ± 0.1	2.1 ± 0.1	2.3 ± 0.1	2.7 ± 0.1	2.1 ± 0.1	1.8 ± 0.1	2.1 ± 0.2	1.8 ± 0.1	1.9 ± 0.1
1.9	2.1 ± 0.1	2.1 ± 0.1	2.1 ± 0.1	2.3 ± 0.1	2.8 ± 0.1	2.0 ± 0.2	1.9 ± 0.1	2.1 ± 0.2	1.8 ± 0.1	2.0 ± 0.1
1.7	2.2 ± 0.1	2.0 ± 0.1	2.1 ± 0.1	2.3 ± 0.1	2.7 ± 0.1	1.9 ± 0.1	1.9 ± 0.1	2.2 ± 0.2	1.8 ± 0.1	2.1 ± 0.1
1.6	2.2 ± 0.1	2.0 ± 0.1	2.1 ± 0.1	2.3 ± 0.1	2.8 ± 0.1	1.9 ± 0.1	1.8 ± 0.1	2.2 ± 0.2	1.9 ± 0.1	2.0 ± 0.1
1.5	2.4 ± 0.3	2.0 ± 0.1	2.1 ± 0.1	2.3 ± 0.1	2.8 ± 0.1	1.9 ± 0.1	1.9 ± 0.1	2.2 ± 0.2	1.8 ± 0.1	2.1 ± 0.1
RC	Asp120@O-Zn	**Bia**@O(2)-Zn	His263@Nε-Zn	Cys221@S-Zn						
1.4	2.2 ± 0.2	2.0 ± 0.1	2.1 ± 0.1	2.3 ± 0.1	2.7 ± 0.1	2.4 ± 0.4	1.8 ± 0.1	2.3 ± 0.2	1.9 ± 0.1	2.0 ± 0.1

RC	Cys221@S-Zn-O(2)@**Bia**	Cys221@S-Zn-Nε@His263	His263@Nε-Zn-O(2)@**Bia**	His263@Nε-Zn-N(1)@**Bia**	**Bia**@O(2)-Zn-N(1)@**Bia**	Cys221@S-Zn-N(1)@**Bia**
3.1	112 ± 5	103 ± 6	95 ± 6	103 ± 6	81 ± 3	150 ± 7
2.9	111 ± 3	101 ± 7	97 ± 7	103 ± 5	82 ± 2	149 ± 9
2.7	108 ± 5	102 ± 5	98 ± 5	103 ± 4	83 ± 2	147 ± 7
2.5	106 ± 5	104 ± 5	95 ± 4	102 ± 3	83 ± 3	149 ± 5
2.3	105 ± 5	104 ± 4	98 ± 5	104 ± 5	84 ± 2	146 ± 5
2.1	106 ± 4	104 ± 6	99 ± 5	106 ± 6	84 ± 2	144 ± 7
1.9	107 ± 5	105 ± 7	100 ± 6	104 ± 5	84 ± 2	144 ± 8
1.7	108 ± 4	108 ± 6	99 ± 4	100 ± 6	83 ± 2	144 ± 4
1.6	108 ± 2	112 ± 4	99 ± 3	100 ± 2	83 ± 1	141 ± 3
1.5	112 ± 4	112 ± 5	97 ± 5	96 ± 4	78 ± 6	145 ± 5
RC	Cys221@S-Zn-O(2)@**Bia**	Cys221@S-Zn-Nε@His263	His263@Nε-Zn-O(2)@**Bia**	His263@Nε-Zn-O@Asp120	**Bia**@O(2)-Zn-O@Asp120	Cys221@S-Zn-O@Asp120
1.4	121 ± 4	115 ± 6	93 ± 4	96 ± 7	119 ± 10	115 ± 7

Table 2: QM/MM calculations of the **ES1**-based reaction (second step: the protonation of N(1)@**Bia**). Selected ligand/enzyme distances, metal coordination distances (in Å), and bond angles (in degrees) at different value of RC, defined here as distance between Asp120@OH and N(1)@**Bia**.



RC	Cys221@S-Zn	His263@Nε-Zn	Asp120@O-Zn	O@**Wat2**-Zn	Thr157@OH-O(4)@**Bia**	NH_2@Asn233-O(1)@**Bia**	Asn233@NH-O(3)@**Bia**	Lys224@NH_3-O(2)@**Bia**	His196@Nε-O(1)@**Bia**	His196@Nε-O(2)@**Bia**
Initial	2.3	2.0	1.9	2.2	2.2	1.9	2.1	1.6	2.2	2.4
free	2.2 ± 0.1	2.1 ± 0.1	2.1 ± 0.1	2.2 ± 0.1	2.4 ± 0.3	1.9 ± 0.1	2.0 ± 0.1	2.0 ± 0.1	2.4 ± 0.1	2.2 ± 0.1
3.1	2.3 ± 0.1	2.2 ± 0.1	2.1 ± 0.1	2.3 ± 0.1	2.5 ± 0.6	1.9 ± 0.2	2.0 ± 0.1	2.0 ± 0.1	2.6 ± 0.2	2.2 ± 0.2
2.9	2.3 ± 0.1	2.1 ± 0.1	2.1 ± 0.1	2.2 ± 0.2	2.5 ± 0.5	1.9 ± 0.1	2.0 ± 0.1	2.2 ± 0.1	2.4 ± 0.2	2.3 ± 0.3
2.7	2.3 ± 0.1	2.1 ± 0.15	2.1 ± 0.1	2.2 ± 0.2	2.0 ± 0.4	1.9 ± 0.1	2.0 ± 0.1	2.3 ± 0.1	2.3 ± 0.3	2.6 ± 0.2
2.5	2.4 ± 0.1	2.1 ± 0.1	2.1 ± 0.1	2.2 ± 0.2	1.9 ± 0.2	1.9 ± 0.1	2.0 ± 0.1	2.1 ± 0.1	2.3 ± 0.3	2.4 ± 0.3
2.3	2.3 ± 0.1	2.1 ± 0.1	2.0 ± 0.1	2.1 ± 0.1	2.1 ± 0.4	1.9 ± 0.1	1.9 ± 0.1	2.2 ± 0.1	2.4 ± 0.2	2.3 ± 0.2
2.1	2.3 ± 0.1	2.1 ± 0.1	2.0 ± 0.1	2.1 ± 0.1	2.0 ± 0.3	1.9 ± 0.1	1.8 ± 0.1	2.1 ± 0.1	2.6 ± 0.3	2.3 ± 0.3
1.9	2.3 ± 0.1	2.1 ± 0.1	2.0 ± 0.1	2.1 ± 0.1	1.9 ± 0.2	2.0 ± 0.1	2.0 ± 0.1	2.0 ± 0.1	2.3 ± 0.3	2.4 ± 0.3
1.8	2.3 ± 0.1	2.0 ± 0.1	2.1 ± 0.2	2.1 ± 0.1	1.9 ± 0.3	1.9 ± 0.1	2.0 ± 0.1	2.1 ± 0.1	2.4 ± 0.2	2.5 ± 0.3
1.7	2.3 ± 0.1	2.0 ± 0.1	2.1 ± 0.2	2.1 ± 0.1	1.9 ± 0.2	1.9 ± 0.1	2.0 ± 0.1	2.0 ± 0.1	2.3 ± 0.2	2.4 ± 0.3
free	2.3 ± 0.1	2.0 ± 0.1	2.1 ± 0.2	2.1 ± 0.1	1.9 ± 0.2	1.9 ± 0.1	1.9 ± 0.1	2.1 ± 0.1	2.4 ± 0.1	2.5 ± 0.2

RC	Cys221@S-Zn-Nε@His263	Cys221@S-Zn-O@**Wat2**	Cys221@S-Zn-O@Asp120	His263@Nε-Zn-O@**Wat2**	Asp120@O-Zn-Nε@His263	Asp120@O-Zn-O@**Wat2**
Initial	130	108	110	96	90	125
free	134 ± 7	105 ± 6	107 ± 6	100 ± 6	93 ± 6	143 ± 7
3.1	130 ± 6	103 ± 6	109 ± 6	103 ± 6	95 ± 6	140 ± 10
2.9	118 ± 7	103 ± 6	109 ± 8	102 ± 6	92 ± 7	138 ± 7
2.7	120 ± 8	96 ± 7	108 ± 7	95 ± 4	91 ± 4	140 ± 15
2.5	121 ± 6	93 ± 6	106 ± 9	92 ± 8	90 ± 6	150 ± 20
2.3	121 ± 8	99 ± 7	110 ± 5	98 ± 7	93 ± 5	145 ± 10
2.1	122 ± 7	102 ± 9	117 ± 7	101 ± 9	96 ± 6	114 ± 11
1.9	125 ± 4	97 ± 5	111 ± 4	101 ± 4	94 ± 3	128 ± 10
1.8	124 ± 5	99 ± 5	115 ± 4	102 ± 5	95 ± 3	124 ± 10
1.7	121 ± 8	101 ± 8	120 ± 7	106 ± 11	96 ± 6	107 ± 14
free	120 ± 7	105 ± 6	123 ± 7	108 ± 12	97 ± 7	108 ± 12

Table 3: QM/MM calculations of **ES2**-based reaction mechanism. Metal coordination distances and ligand/enzyme bond lenghts (in Å) and angles (in degrees) during QM/MM constrained simulations during hydrolysis, along different RC values, defined here as the distance between O@**Wat1** and C(7)@**Bia**.

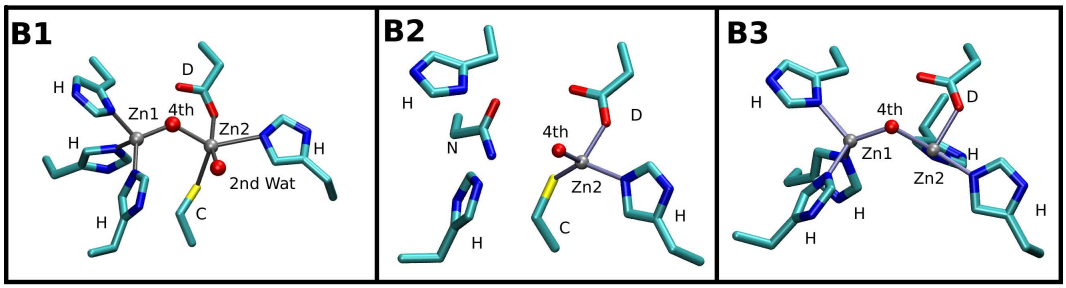


Figure 1: The protein provides three metal ligands in each site: the 'Zn2' site is composed by Asp120, Cys221 and His263 in the B1 and B2 subclasses, and the Asp120, His121 and His263 triad in B3 subclasses. His116, His118 and His196 form the 'Zn1' site both in B1 and B3 subclasses. In B2 subclasses the residue in position 116 is is replaced by Asn [23]. This mutation is most likely responsible for the experimentally observed low Zn(II) binding affinity of this site [24]. The Zn(II) coordination spheres are completed by solvent molecules.

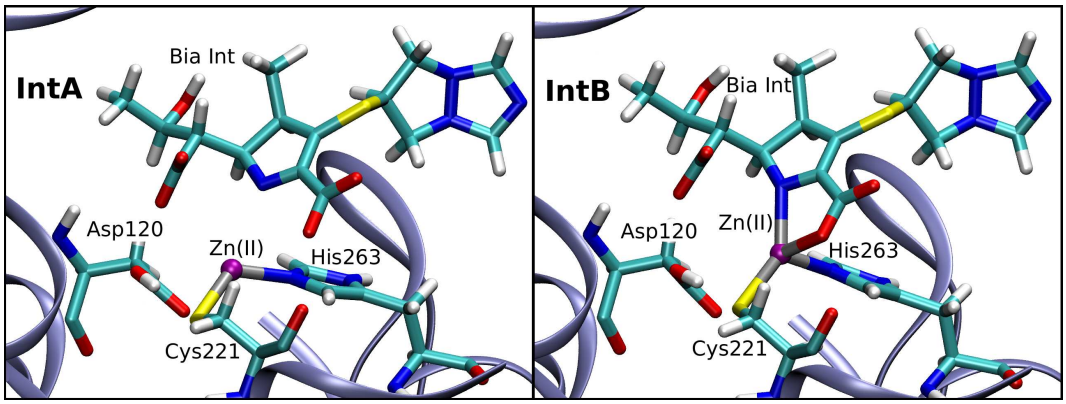
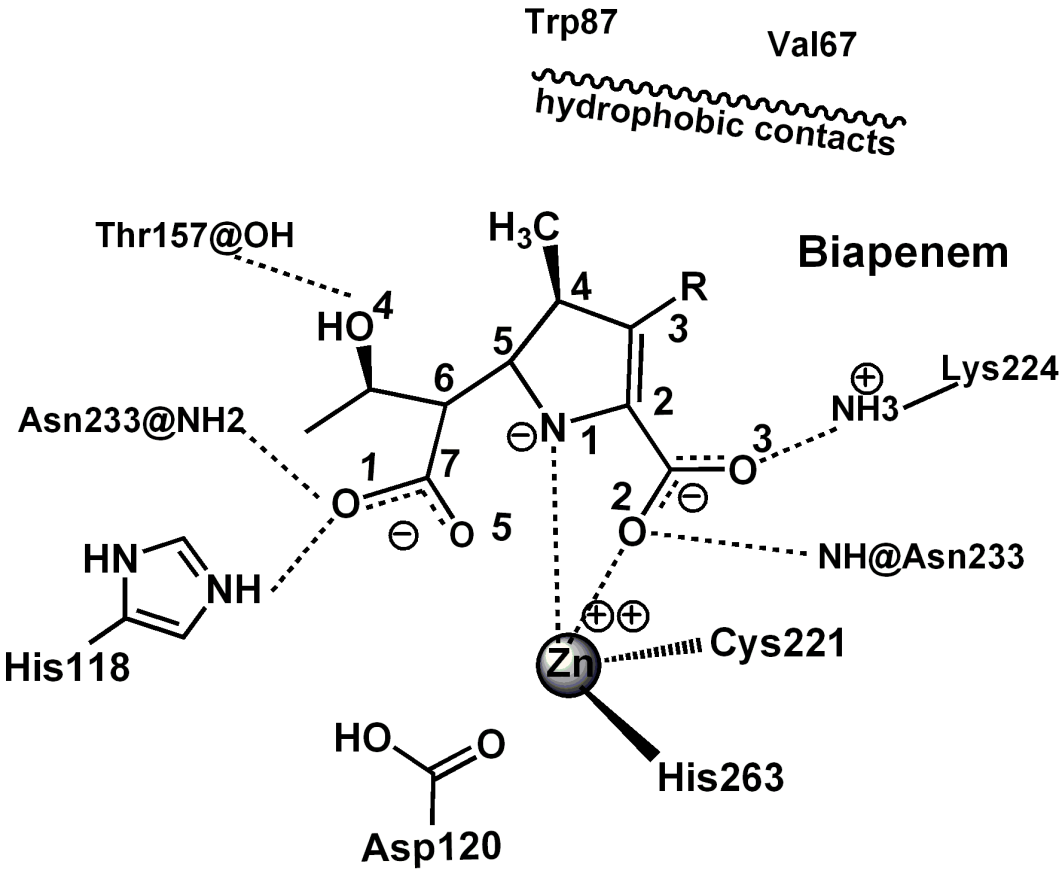


Figure 2: **IntA** and **IntB** active sites with two different parametrizations of Zn environment: in the first N(1)@**Bia** and C(7)@**Bia** are not directly bound to the metal, while in the second model, they are explicitly coordinated with it.



IntA	Initial	Average	Final	**IntB**	Initial	Average	Final
Bia@N(1)-Zn	2.0	const.	const.	**Bia**@N(1)-Zn	2.0	3.1 ± 0.3	2.8
Bia@O(2)-Zn	2.0	const.	const.	**Bia**@O(2)-Zn	2.0	4.0 ± 0.7	4.7
Lys224@NH_3-O(2)@**Bia**	2.7	2.7 ± 0.2	2.7	Lys224@NH_3-O(2)@**Bia**	2.7	2.8 ± 0.1	2.7
Lys224@NH_3-O(3)@**Bia**	2.8	2.8 ± 0.1	2.8	Lys224@NH_3-O(3)@**Bia**	2.8	3.1 ± 0.3	3.3
Thr157@OH-O(4)@**Bia**	2.2	2.3 ± 0.2	2.3	Thr157@OH-O(4)@**Bia**	2.2	2.3 ± 0.3	2.4
His118@NεH-O(2)@**Bia**	1.9	2.2 ± 0.3	2.2	His118@NεH-O(2)@**Bia**	1.9	2.5 ± 0.4	2.1
Asn223@NH-O(2)@**Bia**	2.6	2.6 ± 0.2	2.6	Asn223@NH-O(2)@**Bia**	2.6	2.6 ± 0.3	2.5
Asn223@NH_2-O(1)@**Bia**	2.0	2.0 ± 0.2	2.6	Asn223@NH_2-O(1)@**Bia**	2.0	1.9 ± 0.1	1.8

Table 4: **IntA** and **IntB** ligand/enzyme interaction distances (in Å): initial, averaged over 5 ns of MD, and final values.

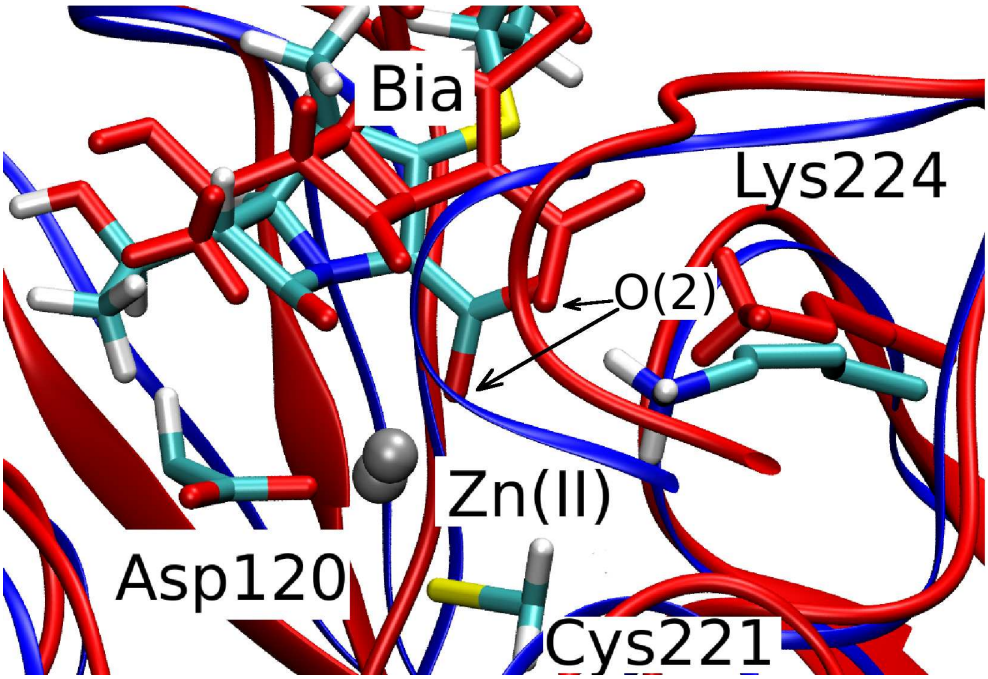


Figure 3: QM/MM calculations of **ES1**. Superimposition of initial (backbone trace colored in blue and atoms colored using different color for each atom type) and final QM/MM structures (colored in red). Whilst **Bia** keeps its salt bridge with the protein, it readily loses its interaction with the metal ion.

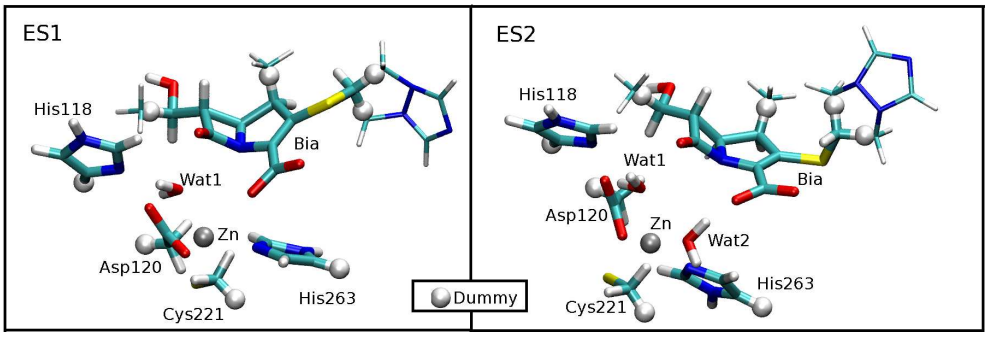


Figure 4: QM region in the QM/MM calculations performed to characterize the reaction mechanism of **ES1** and **ES2**: QM atoms are showed in bold licorice, while the sidechain atoms of **Bia** and the remaining methyl groups, described as MM atoms, are depicted in thin licorice. Dummy hydrogens are shown as balls.

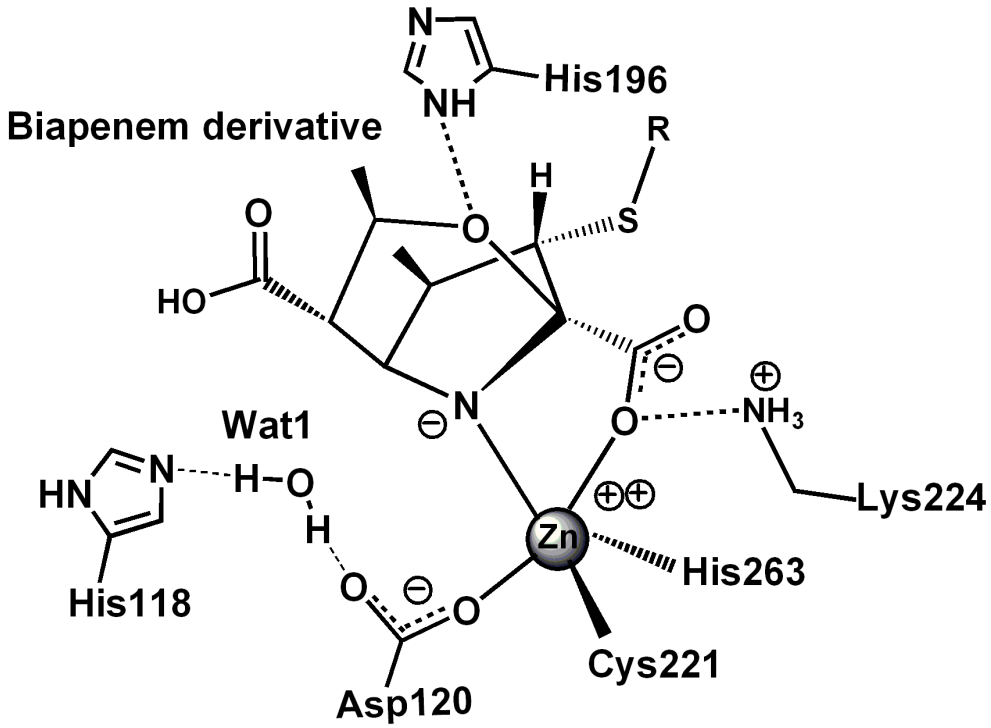


Figure 5: Schematic representation of X-ray structure of CphA in complex with the antibiotic derivative (PDB entry 1X8I).

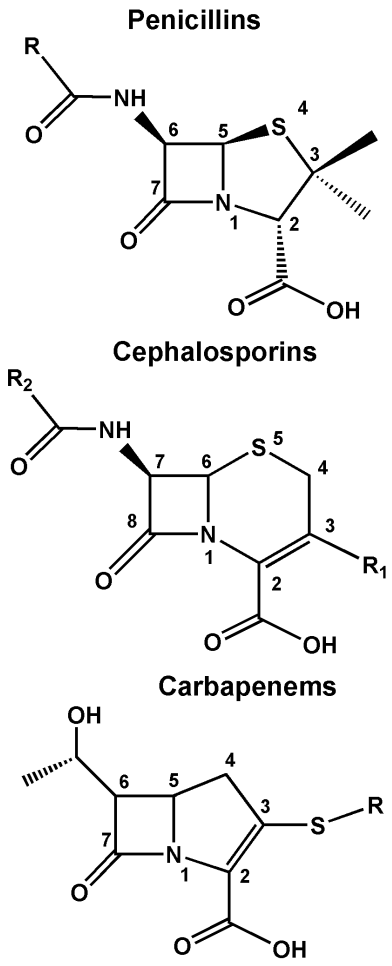


Figure 6: Schematic representation of the three major β-lactam antibiotics used in the clinical settings [25]. For **Bia**, R is a bicyclotriazolium group. Carbapenems are similar to penicillins with the exception of a carbon atom in position 4 and the presence of an unsaturated bond between C(2) and C(3) in the backbone [26, 27]. They have the broadest spectrum within the β-lactam class, and exhibit bactericidal activity against numerous pathogens, and finally, they are stable to almost all β-lactamases [26]. This is mainly due to the *trans* orientation of the hydroxyethyl substituent in position C(6), which is present in carbapenems only, whereas cephalosporins and penicillins always possess a *cis* orientation. The spreading among pathogenic bacteria of genetically encoded carbapenemases [28, 29, 30] further increases the need of developing novel more powerful inhibitors against MβLs.