SUPPLEMENTARY INFORMATION

The Reaction Mechanism of Metallo-β-lactamases is Tuned by the Conformation of an Active Site Mobile Loop

Antonela R. Palacios, María F. Mojica, Estefanía Giannini, Magdalena A. Taracila, Christopher R. Bethel, Pedro M. Alzari, Lisandro H. Otero, Sebastián Klinke, Leticia I. Llarrull, Robert A. Bonomo and Alejandro J. Vila.

Table S1. Zn(II) Dissociation constants of NDM-1 and the L3 variants. K_{d1} and K_{d2} represent the macroscopic dissociation constants of the free enzyme and Zn(II) complex or the mono-metallated enzyme and Zn(II) complex, respectively. K_{d12} is the dissociation constant for the bi-metallated enzyme (L3Pro variant) or K_{d1}*K_{d2} product.

Variant	Zn(II) dissociation constants		
	K_{d1} (nM)	K_{d2} (nM)	$K_{d1,2}$ (nM^2)
NDM-1	6 ± 4	17 ± 6	100 ± 20
L3IMP	2.9 ± 0.7	100 ± 10	290 ± 7
L3VIM	29 ± 6	120 ± 20	3500 ± 100
L3Pro			330 ± 90

Table S2. Data collection and refinement statistics.

	L3IMP	**L3Pro**		
	Native	*Native*	Zn - edge	Ni - edge
PDB code	**6C6I**	**6CAC**	-	-
Data Collection				
Synchrotron source	SOLEIL	SOLEIL	ESRF	ESRF
Beamline	Proxima 1	Proxima 1	ID23	ID23
Number of frames	600	2500	2400	1200
Oscillation step (°)	0.2	0.1	0.15	0.15
Detector distance (mm)	260.5	270.3	148.338	197.604
Wavelength (Å)	0.97857	0.97934	above = 1.27241 below = 1.28348	above = 1.48030 below = 1.48840
Exposure per frame (s)	0.4	0.1	0.037	0.149
Indexing and Scaling				
Cell parameters				
a, b, c (Å)	38.29 39.25 73.87	91.71 91.74 134.33	93.51 93.784 136.70	92.97 92.64 136.08
α, β, γ (°)	90.89 93.10 90.02	90.00 107.16 90.00	90.00 106.90 90.00	90.00 107.10 90.00
Space group	*P*1	*C*2	*C*2	*C*2
Resolution limit (Å)	1.65	1.79	2.00	2.00
Number of total reflections	98791	451488	261904	116319
Number of unique reflections	49940	96133	73935	70934
Average multiplicity [a]	2.0 (2.0)	4.7 (4.2)	6.8 (5.9)	3.3 (3.0)
R_{meas}	0.141 (0.516)	0.074 (0.828)	0.056 (0.121)	0.056 (0.613)
I/σ(I)	5.3 (2.1)	11.9 (1.9)	29.1 (15.4)	18.0 (2.2)
CC(1/2)	0.992 (0.787)	0.998 (0.817)	0.998 (0.992)	0.998 (0.914)
Completeness (%)	96.6 (94.1)	97.0 (80.4)	97.1 (95.5)	95.5 (90.7)
Monomers per A.U.	2	4	4	4
Solvent content (%)	45	54		
B-factors (Wilson plot, $Å^2$)	18	30		
Refinement				
Resolution range (Å)	24.16 - 1.65	45.87 - 1.79		
No. of atoms				
Protein	3396	6883		
Ligands/ions	4	66		
Water	264	915		
R	0.203	0.163		
*R*free	0.229	0.181		
rmsd values from ideal values[b]				
Bond lengths (Å)	0.010	0.010		
Bond angles (°)	1.02	1.01		
B-factors (average, $Å^2$)	20	36		
MolProbity validation[c]				
Clashscore	1.65	0.88		
Poor rotamers (%)	2.03	0.57		
Ramachandran plot (%)				
Favored	98.7	97.6		
Allowed	1.3	2.4		
Disallowed	-	-		

[a] Values in parentheses correspond to the highest-resolution shell: L3IMP: 1.68-1.65; L3Pro: 1.83 - 1.79 Å. [b] (1). [c] (2)

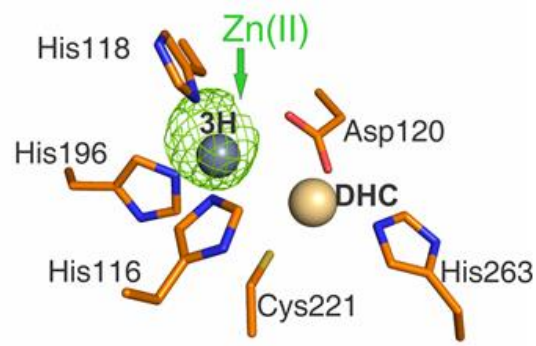

Fig. S1. Anomalous diffraction for Zn(II) in the active site of L3Pro. The presence of Zn(II) in the active site of L3Pro (PDB code: 6CAC) was verified by anomalous diffraction. Peaks of 40-50 rmsd (map in green at level 5) were observed in the 3H site of the four molecules of the asymmetric unit, whereas a signal was not detected at the DCH site.

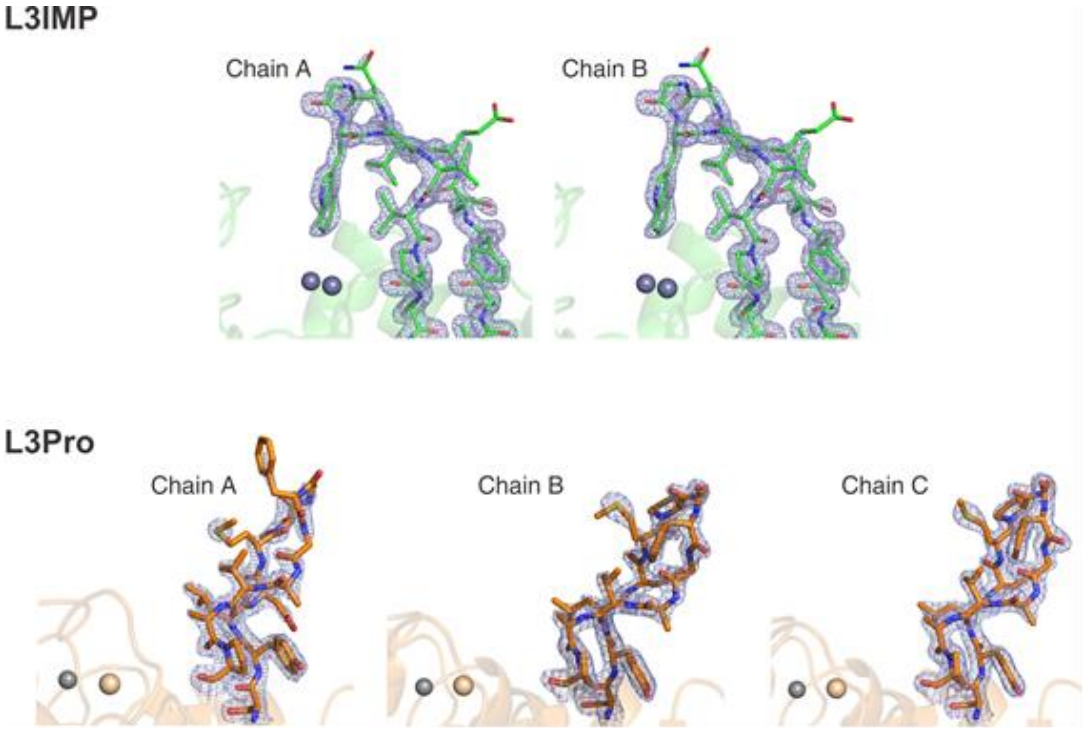

Fig. S2. Electron density of the loop L3 in L3IMP and L3Pro crystal structures. Electron density of loop L3 region of L3IMP (PDB code: 6C6I) and L3Pro (6CAC). Maps, $2F_0$-F_c, are shown at 1.5 rmsd. The loop L3 from chain D in L3Pro was not defined.

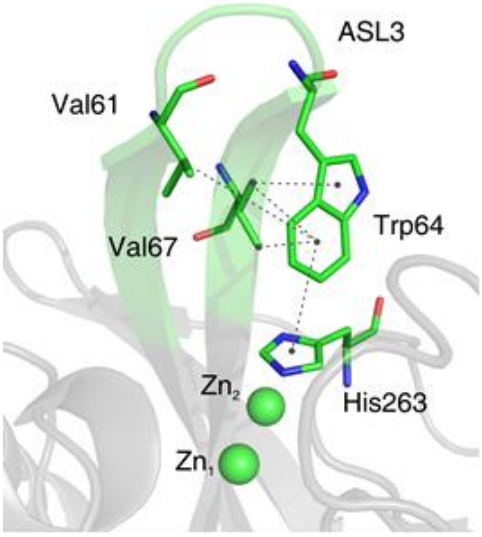

Fig. S3. Hydrophobic interactions that stabilize the Trp64 position in L3IMP. Crystal structure of L3IMP showing hydrophobic interactions of Trp64 with His263 (π-π T shaped interaction), Val67 (π-σ interactions) and Val61 (π-Alkyl interaction). The interactions were identified by Discovery Studio (3).

References

1. Engh RA, Huber R. 1991. Accurate bond and angle parameters for X-ray protein structure refinement. Acta Crystallographica Section A Foundations of Crystallography 47:392-400.
2. Chen VB, Arendall WB, 3rd, Headd JJ, Keedy DA, Immormino RM, Kapral GJ, Murray LW, Richardson JS, Richardson DC. 2010. MolProbity: all-atom structure validation for macromolecular crystallography. Acta Crystallogr D Biol Crystallogr 66:12-21.
3. BIOVIA DS. 2017. Discovery Studio Modeling Environment, San Diego.