Supporting Information

New Delhi Metallo-ß-Lactamase: Structural Insights into ß-Lactam Recognition and Inhibition

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Methods

Protein Expression and Purification

The NDM-1 protein construct includes the mature sequence (M27 to R270) with signal peptide removed and was prepared as previously described.[1] Purified protein was then dialyzed into fresh crystallization buffer (20mM HEPES pH 7.5, 2mM 2-mercaptoethanol, 150 mM NaCl) and concentrated to ~60 mg/ml.

Crystallization, Data Collection and Structure Determination

Benzylpenicillin, oxacillin and methicillin bound product complex NDM-1 crystals were grown using the sitting drop method at 25°C. Drops contained 1μL of (60 mg/mL protein + 40mM methicillin, benzylpenicillin or oxacillin), combined with 1μL of precipitant (0.2M $MgCl_2$, 25% PEG3350, 0.1M bis-tris pH5.5). Drops were then streak seeded with finely crushed ampicillin bound NDM-1 crystals (prepared as previously described).[2] Crystals were soaked in cryoprotectant solution for 30s (precipitant + 25% glycerol), and flash frozen in liquid nitrogen. The benzylpenicillin, oxacillin and methicillin bound crystals diffracted to 1.8, 1.17 and 1.16Å at beamline CMCF-1 of the Canadian light source (CLS).

Meropenem bound product complex crystals were grown using the sitting drop vapor diffusion method at 25°C using 1μL of (55 mg/mL protein + 4mM meropenem), combined with an equal volume of precipitant (1M trisodium cacodylate, 0.1M sodium cacodylate pH 6.5). Crystals were soaked in cryoprotectant solution for 30s (mother liquor + 30% glycerol), and flash frozen in liquid nitrogen. Meropenem bound crystals diffracted to 1.9Å at beamline CMCF-1 of the CLS.

Crystals of ethylene glycol bound NDM-1 grew at 25°C in a condition containing 0.5 μl protein solution mixed with an equal volume of precipitant (10% w/v PEG8K, 8%v/v ethylene glycol, and 0.1M HEPES pH 7.5). L-captopril bound crystals were then attained by soaking ethylene glycol NDM-1 crystals in mother liquor plus 15mM L-captopril for ~30 min. Crystals were soaked in cryoprotectant solution for 30s (precipitant, 25% glycerol, 15mM L-captopril), and flash frozen in liquid nitrogen. Ethylene glycol and L-captopril bound crystals diffracted to 1.47Å and 2.3Å at beam line CMCF-1 at the Canadian Light Source (CLS).

Data were processed using IMOSFILM[3] and CCP4.[4] A total of 5% of reflections were set aside for cross validation. All structures of NDM-1 were solved by molecular replacement using the program Phaser,[5] with chain A of the ampicillin bound NDM-1 structure as a starting model (PDB ID: 3Q6X).[2] Several cycles of manual rebuilding in coot,[6] followed by refinement using REFMAC[7] (CCP4) were carried out. The hydrolyzed methicillin, hydrolyzed oxacillin and ethylene glycol bound structures were refined with anisotropic B-factors. However, hydrolyzed benzylpenicillin, hydrolyzed meropenem and L-captopril bound structures were refined with isotropic B-factors. Zinc, water and the appropriate ligands were added manually by examination of the F_o-F_c and $2F_o$-F_c electron density maps. Coordinates

and structure factors for hydrolyzed methicillin, hydrolyzed oxacillin, hydrolyzed benzylpenicillin, ethylene glycol, L-captopril and hydrolyzed meropenem bound NDM-1 were deposited in the PDB with accession codes (4EY2, 4EYB, 4EYF, 4EXY, 4EXS and 4EYL). Figures 2-4 and S1 were made using PyMol[8] and figures S2-S7 were created using LIGPLOT.[9]

(1) King, D.; Strynadka, N. *Protein Sci.* **2011**. *20*. 1484-1491.
(2) Zhang, H.; Hao, Q. *FASEB J.* **2011**. *25*. 2574-2582.
(3) Leslie, A.G.W. Joint CCP4+ESF-EAMCB Newsletter on Protein Crystallography. No. 26.
(4) Winn, M.D. *Acta Crystallogr*. **2011**. *D67*. 235-242.
(5) McCoy, A.J.; Grosse-Kunstleve, R.W.; Adams, P.D.; Winn, M.D.; Storoni, L.C.; Read, R.J. *J. Appl. Crystallogr*. **2007**. *40*. 658-674.
(6) Emsley, P.; Cowtan, K. *Acta Crystallogr*. **2004**. *D60*. 2126-2132.
(7) Winn, M.; Isupov, M.; Murshudov, G.N. *Acta Crystallogr*. **2000**. *D57*. 122-133.
(8) DeLano, W.L.; (2002) The Pymol Molecular Graphics System, DeLano Scientific, San Carlos, CA, USA. http://www.pymol.org
(9) Laskowski, R.A. *Nucleic Acids Res*. **2009**. *37*. D355-D359.

Table S1. Data Collection and Refinement Statistics.

	Benzyl Pen	OX	METH	MERO	L-CAP	EG
Data Collection[a]	Home Source	CLS CMCF-1	CLS CMCF-1	CLS CMCF-1	Home Source	CLS CMCF-1
Wavelength	1.54 Å	1.00 Å	1.00 Å	1.00 Å	1.54 Å	1.00 Å
Resolution (Å)	29.71-1.8 (1.9 -1.8)	25.42-1.16 (1.23-1.16)	25.43-1.17 (1.23-1.17)	42.19-1.90 (2.00-1.90)	58.71-2.4 (2.59-2.4)	35.74-1.47 (1.55-1.47)
Space group	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$	$P4_12_12$	$P4_12_12$	$P4_12_12$
a(Å)	39.03	39.20	39.12	106.00	107.12	107.22
b(Å)	79.49	79.37	79.36	106.00	107.12	107.22
c(Å)	134.21	134.15	133.80	92.58	92.90	92.85
α(°)	90	90	90	90	90	90
β(°)	90	90	90	90	90	90
γ(°)	90	90	90	90	90	90
R_{merge} (%)[c]	0.103(0.376)	0.079(0.558)	0.061(0.231)	0.114(0.536)	0.142(0.497)	0.060(0.372)
I/σI	7.9(2.8)	9.6 (3.0)	6.9(2.9)	5.9(2.1)	6.9(2.1)	10.6 (3.0)
Completeness	98.7%(97.6%)	99.6%(98.7%)	96.8%(93.9%)	96.1%(92.2%)	99.0%(99.7%)	99.7%(98.4%)
Unique Reflections	39036(5542)	143064(20466)	137386(19417)	40390(5536)	21474(3088)	91755(13015)
Redundancy	3.0(2.9)	2.9(2.6)	2.1(1.9)	3.3(3.0)	3.2(3.2)	4.0(3.7)
Refinement Statistics [d]						
Ligand Occupancy chainA, chainB	1.00, 1.00	1.00, 1.00	1.00, 1.00	0.80, 0.80	1.00, 0.60	1.00,1.00
Average *B* factor (Å²):						
Protein	11.0	14.7	12.6	17.3	18.1	17.4
Zinc	12.5	8.7	6.3	19.2	16.7	13.5
Ligand	11.9	14.5	8.8	43.2	34.1	18.0
Water	17.1	24.8	23.0	20.9	18.5	27.2
Ramachandran statistics:						
Favored	98.1%	98.8%	98.5%	97.7%	96.6%	97.8%
Additionally allowed	0.5%	0.8%	1.3%	1.5%	2.6%	1.9%
Disallowed	0.4%	0.4%	0.2%	0.8%	0.8%	0.4%
R_{work}	16.27%	13.48%	12.98%	18.36%	18.99%	13.99%
R_{free}	19.73%	16.40%	15.87%	22.37%	24.11%	18.20%
r.m.s.[b] bonds	0.012	0.019	0.017	0.013	0.013	0.017
r.m.s. angles	1.29	2.14	2.11	1.97	1.47	2.10

[a] Values in parenthesis represent the highest resolution shell. [b] r.m.s. means root mean square. [c] $R_{merge} = \sum_{hkl}\sum_j[I_{hkl,j} - \langle I_{hkl}\rangle / \sum_{hkl}\sum_j I_{hkl,j}$. [d] 5% of the reflections were excluded from refinement and used to calculate R_{free}. Benzylpenicillin (Benzyl Pen), oxacillin (OX), methicillin (METH), meropenem (MERO), L-captopril (L-Cap), ethylene glycol (EG).

NDM-1 Ligand Complex Crystal Structures

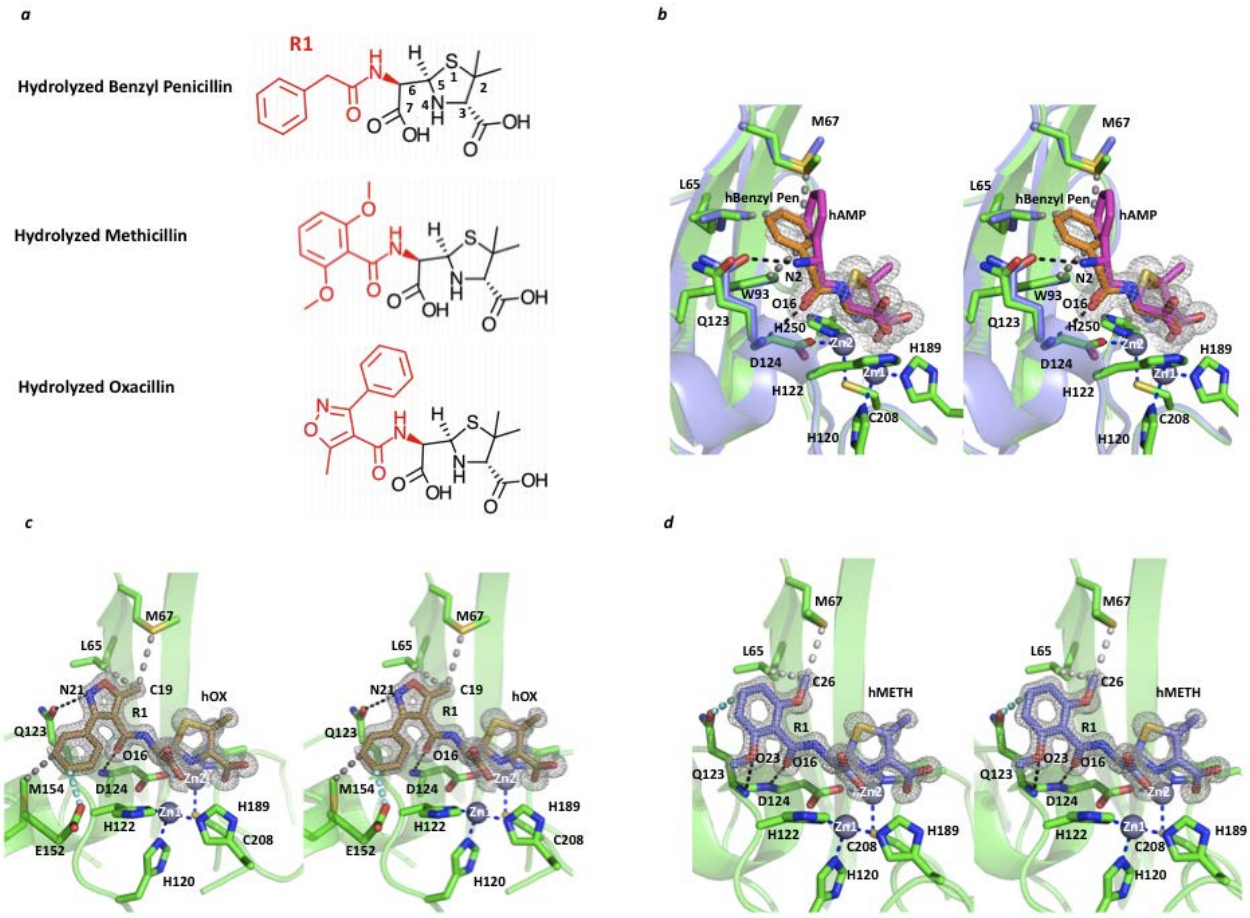

Figure S1. Penicillin product complex crystal structures. (A) Hydrolyzed benzylpenicillin (hBenzyl Pen), hydrolyzed methicillin (hMETH) and hydrolyzed oxacillin (hOX) structures (B) hBenzyl Pen and hydrolyzed ampicillin (hAMP) active site overlay. The hBenzyl Pen and hAMP (PDB ID: 3Q6X)[2] bound NDM-1 cartoons are shown in green and slate with selected active site residues colored by atom type and zinc ions shown as grey spheres. The hBenzyl Pen and hAMP ligands are orange and pink sticks with atoms colored by type. (C) hOX bound NDM-1. The hOX bound NDM-1 cartoon is green with selected active site residues colored by atom and zinc ions displayed as grey spheres. The hOX ligand is brown with atoms colored by type. (D) hMETH bound NDM-1. The hMETH bound NDM-1 cartoon is green with selected active site residues colored by atom and zinc ions colored grey. The hMETH ligand is slate with atoms colored by type. The $2F_o$-F_c maps for hBenzyl Pen, hMETH and hOX are contoured at 1.3, 1.8 and 1.3σ and are represented as grey mesh in B, C and D. Bonds representing zinc coordination, hydrogen bonding, hydrophobic and bond-on amide-π interactions are displayed as thin blue, thin black, thick grey and thick cyan dashes.

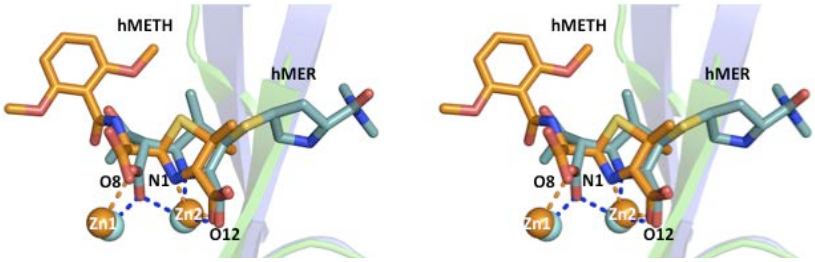

Figure S2. Overlay of hydrolyzed methicillin (hMETH) and hydrolyzed meropenem (hMER) bound NDM-1. The hMETH and hMER and corresponding zinc ions are orange and slate with atoms colored by type. Dashes representing zinc coordination are colored orange and blue for hMETH and hMER.

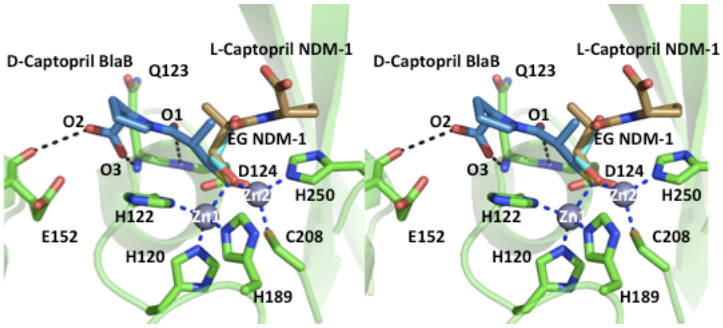

Figure S3. L-captopril, D-captopril and ethylene glycol bound NDM-1 active site overlay. The L-captopril bound NDM-1 protein is shown as green cartoon and selected active site residues are green sticks, which are colored by atom type. L-captopril, D-captopril and ethylene glycol are brown, light blue and teal with atoms colored by type. D-captopril is modeled in the NDM-1 active site using the D-captopril BlaB crystal structure (PDB ID: 1M2X) as a template.[10] Proposed D-captopril hydrogen bonding and zinc coordination interactions are displayed as black and blue dashes.

(10) García-Sáez, I.; Hopkins, J.; Papamicael, C.; Franceschini, N.; Amicosante, G.; Rossoline, G.M.; Galleni, M.; Frère, J.M.; Dideberg, O. *J. Biol. Chem*. **2003**. *278*. 23868-23873.

NDM-1 Substrate Modeling

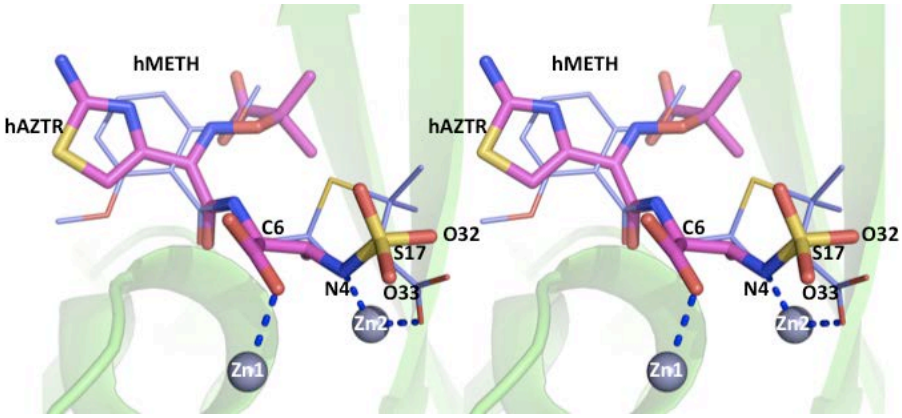

Figure S4. Hydrolyzed Methicillin (hMETH)/modeled hydrolyzed aztreonam (hAZTR) bound NDM-1 active site overlay. The hAZTR C6 carboxylate oxygen and N4 zinc coordinating atoms were fixed in the exact positions as seen in the hMETH bound structure. The hAZTR and hMETH ligands are pink sticks and slate lines with atoms colored by type. NDM-1 is shown in green cartoon representation with zinc ions as grey spheres. The hMETH zinc coordinating interactions are shown as blue dashed lines.

Close NDM-1-Ligand Contacts

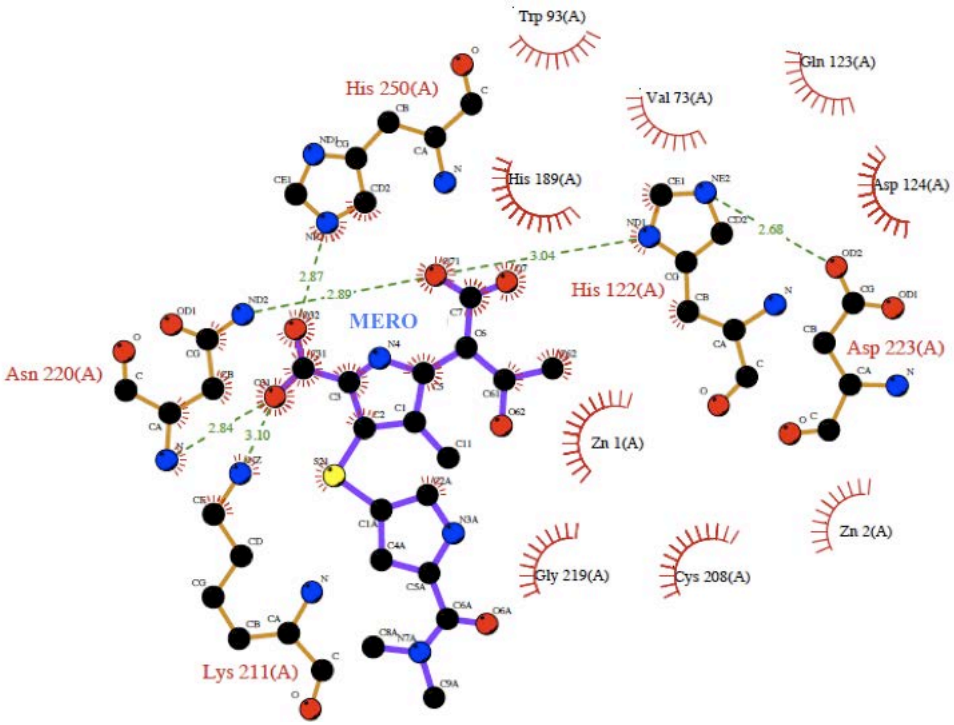

Figure S5. Protein-ligand interactions between NDM-1 and meropenem depicted in monomer A using LIGPLOT.[9] Meropenem and NDM-1 active site residues are shown as purple and orange sticks with atoms colored by type. Hydrogen bonding interactions are shown as dashed green lines. Ligand-protein hydrophobic contacts are shown as curved red combs.

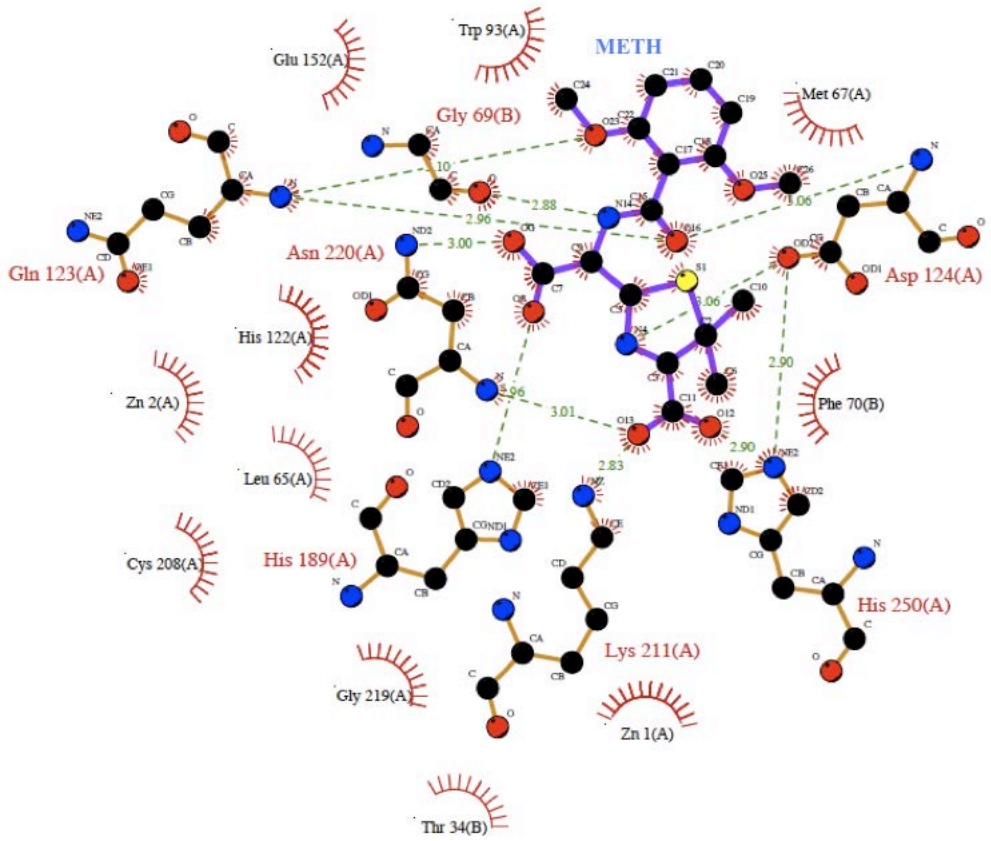

Figure S6. Protein-ligand interactions between NDM-1 and methicillin depicted in monomer A using LIGPLOT.[9] Methicillin and NDM-1 active site residues are shown as purple and orange sticks with atoms colored by type. Hydrogen bonding interactions are shown as dashed green lines. Ligand-protein hydrophobic contacts are shown as curved red combs.

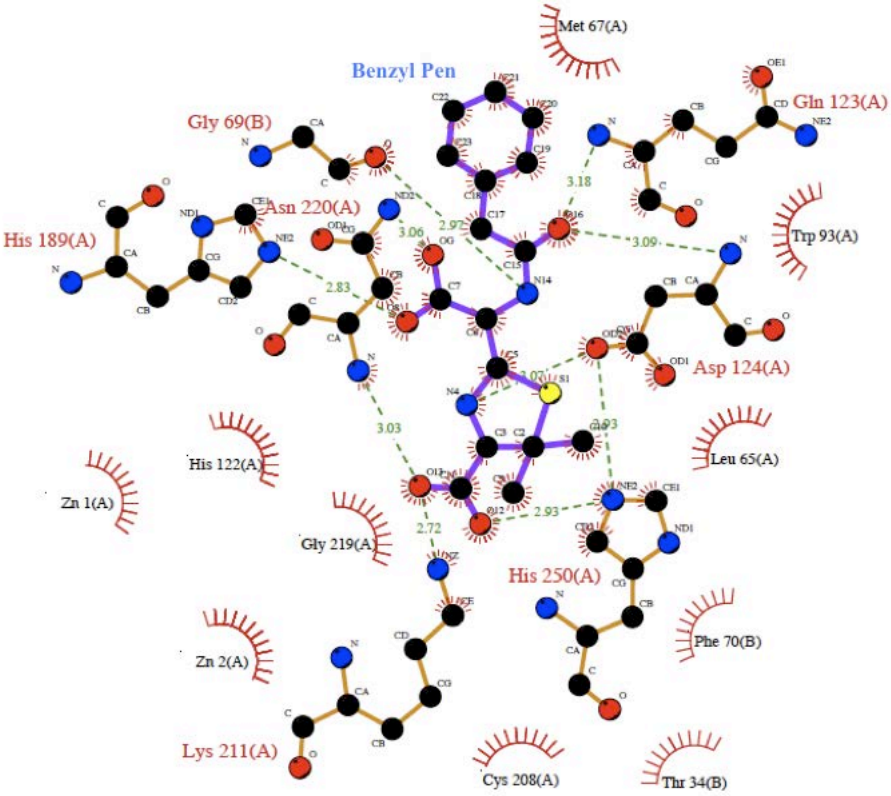

Figure S7. Protein-ligand interactions between NDM-1 and benzylpenicillin depicted in monomer A using LIGPLOT.[9] Benzylpenicillin and NDM-1 active site residues are shown as purple and orange sticks with atoms colored by type. Hydrogen bonding interactions are shown as dashed green lines. Ligand-protein hydrophobic contacts are shown as curved red combs.

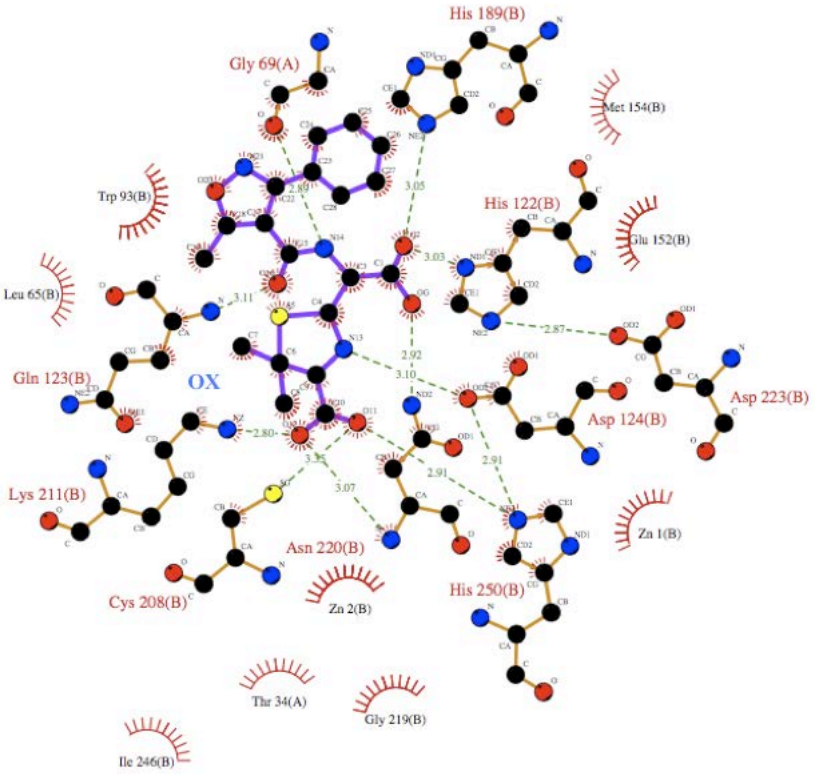

Figure S8. Protein-ligand interactions between NDM-1 and oxacillin depicted in monomer A using LIGPLOT.[9] Oxacillin and NDM-1 active site residues are shown as purple and orange sticks with atoms colored by type. Hydrogen bonding interactions are shown as dashed green lines. Ligand-protein hydrophobic contacts are shown as curved red combs.

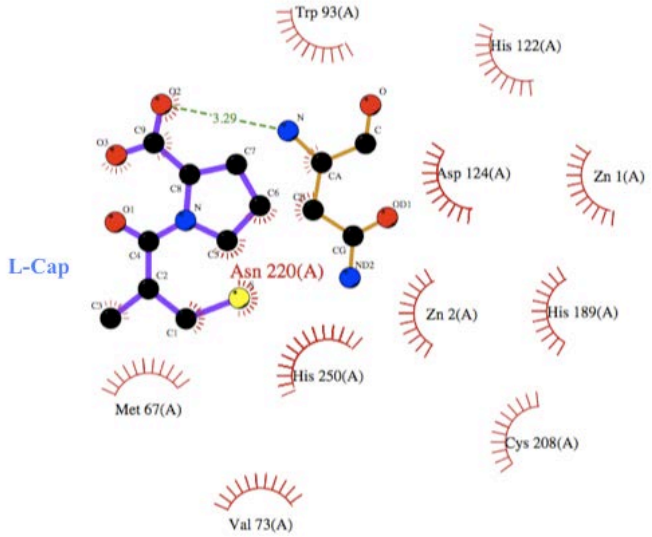

Figure S9. Protein-ligand interactions between NDM-1 and L-captopril depicted in monomer A using LIGPLOT.[9] L-captopril and NDM-1 active site residues are shown as purple and orange sticks with atoms colored by type. Hydrogen bond are shown as dashed green lines. Ligand-protein hydrophobic contacts are shown as curved red combs.

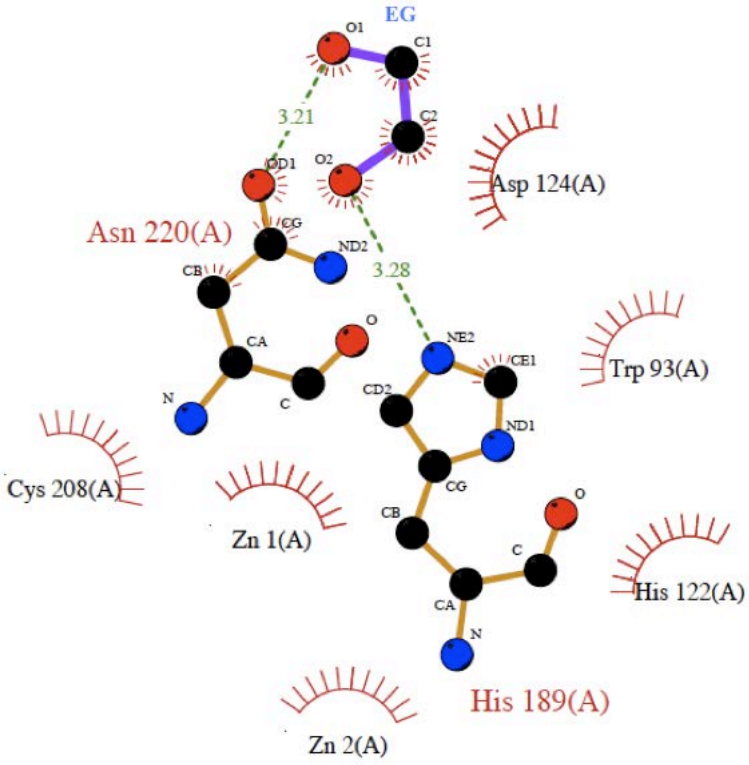

Figure S10. Protein-ligand interactions between NDM-1 and ethylene glycol depicted in monomer A using LIGPLOT.[9] Ethylene Glycol and NDM-1 active site residues are shown as purple and orange sticks with atoms colored by type. Hydrogen bonding interactions are shown as dashed green lines. Ligand-protein hydrophobic contacts are shown as curved red combs.