SUPPLEMENTAL MATERIAL

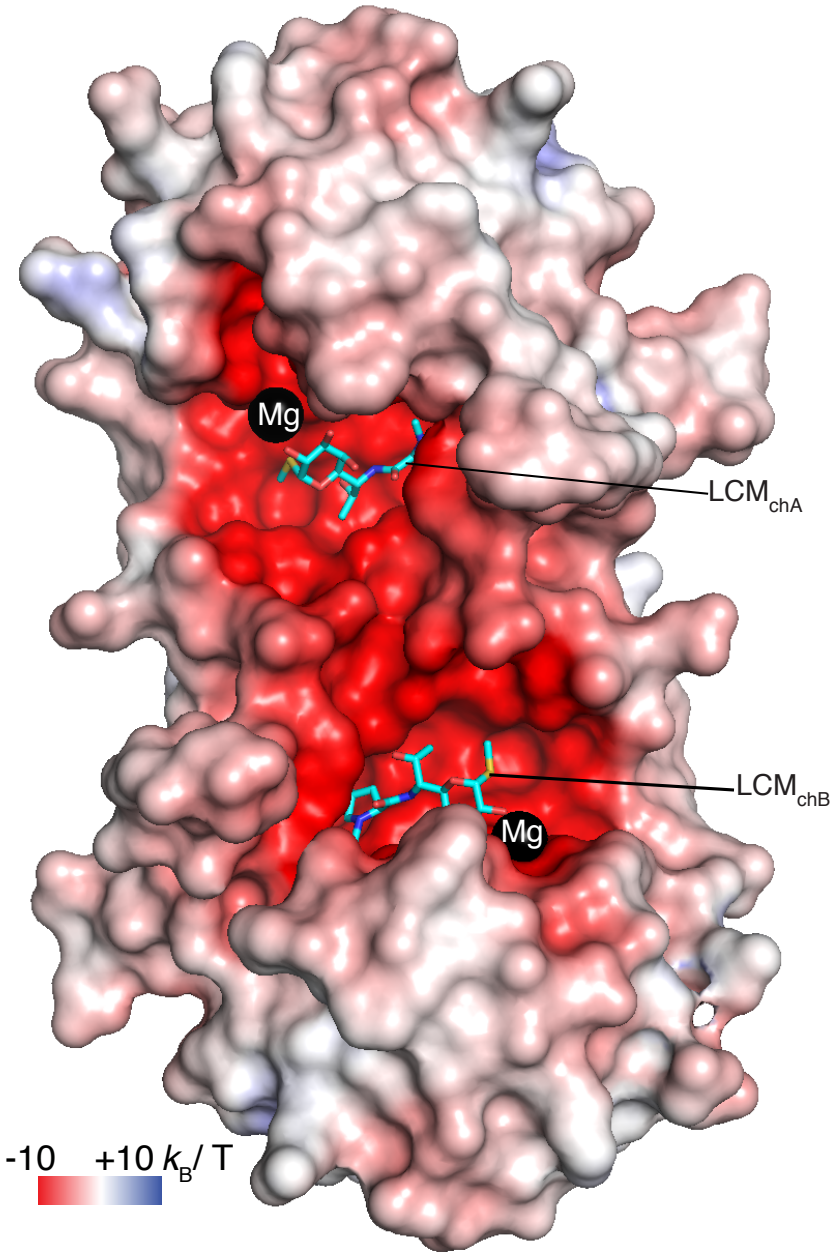
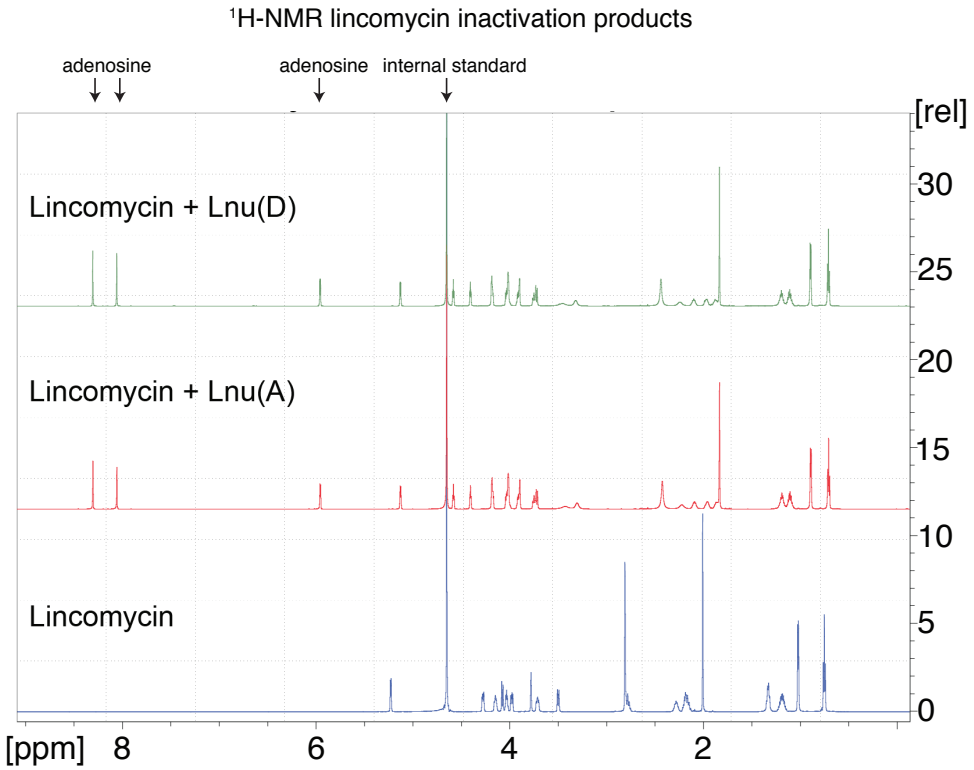


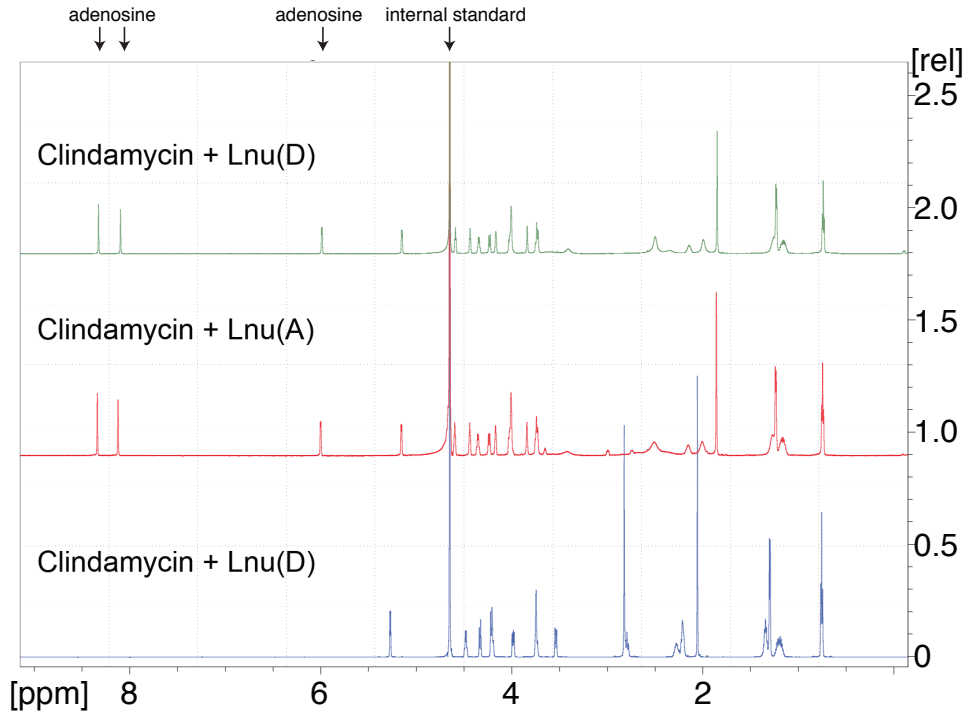
Figure S1. Electrostatic surface representation of Lnu(A) dimer showing the acidic active site cavity.

LCM is shown in sticks and magnesium ions as black spheres. Scale of electrostatic charge is shown at the bottom left; k_B = Bolztmann's constant, T = temperature.

(a)


¹H-NMR lincomycin inactivation products
adenosine
adenosine
internal standard
[rel]
Lincomycin + Lnu(D)
Lincomycin + Lnu(A)
Lincomycin
30
25
20
15
10
5
0
[ppm]
8
6
4
2

(b)


¹H-NMR clindamycin inactivation products
adenosine
adenosine
internal standard
[rel]
Clindamycin + Lnu(D)
Clindamycin + Lnu(A)
Clindamycin + Lnu(D)
2.5
2.0
1.5
1.0
0.5
0
[ppm]
8
6
4
2

(c)

^{1}H chemical shifts

Proton	Clindamycin	3-AMP Clindamycin	Lincomycin	3-AMP Lincomycin
1	5.37	5.25	5.33	5.22
1-SCH_3	2.15	1.97	2.10	1.93
2	4.09	4.12	4.09	4.12
3	3.63	3.84	3.62	3.84
4	3.83	3.90	3.88	3.97
5	4.32	3.83	4.37	4.26
6	4.44	4.33	4.17	4.0
7	4.57	4.44	4.10	4.11
8	1.40	1.34	1.12	0.99
AMP				
2		8.20		8.15
8		8.44		8.40
1’		6.08		6.05
2’		4.69		4.66
3’		4.53		4.49
4’		4.26		4.27
5’		4.10		4.11

^{13}C chemical shifts

Carbon	Clindamycin	3-AMP Clindamycin	Lincomycin	3-AMP Lincomycin
1	87.37	86.5	87.8	87.1
1-SCH_3	12.2	12.0	12.4	12.6
2	67.3	64.1 ($J_{C\text{-}P}$ 7.1 Hz)	67.2	66.1($J_{C\text{-}P}$ 6.9 Hz)
3	70.1	75.1 ($J_{C\text{-}P}$ 6.4Hz)	70.0	75.01($J_{C\text{-}P}$ 5.8Hz)
4	67.6	66.6	68.0	66.9
5	68.7	68.3	53.1	52.3
6	52.7	52.1	68.6	65.8
7	57.2	57.4	65.9	66.2
8	21.2	21.2	15.2	15.0
AMP				
1’		86.7		86.6
2’		74.1		74.0
3’		69.5		69.6
4’		83.0 ($J_{C\text{-}P}$ 9.3Hz)		83.0 ($J_{C\text{-}P}$ 9.8Hz)
5’		64.1 ($J_{C\text{-}P}$ 5.6Hz)		64.2 ($J_{C\text{-}P}$ 6.7Hz)

(d)

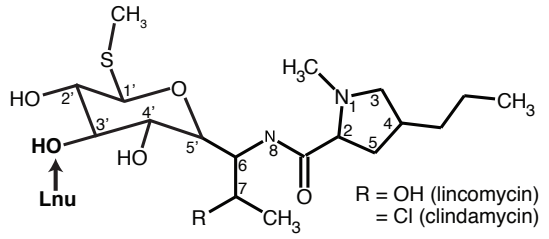


Figure S2. Characterization of inactivation products of Lnu(A) and Lnu(D) by NMR.

(a) ^{1}H-NMR spectra of Lnu(D) plus LCM (top), Lnu(A) plus LCM and LCM alone (bottom).

(b) ^{1}H-NMR spectra of Lnu(D) plus CLI (top), Lnu(A) plus CLI and CLI alone (bottom). For both figures S2a and S2b, positions of adenosine aromatic protons at 8.2 and 8.4 ppm, plus adenosine ribose ring anomeric proton at 6.1 ppm are labelled. Position of internal standard of tetramethylsilane at 4.65 ppm are labelled.

(c) ^{1}H chemical shifts for CLI and LCM and their adenylylated products in D_2O (left), and ^{13}C chemical shifts for CLI and LCM and their adenylylated products in D_2O (right).

(d) Chemical structures of lincomycin and clindamycin.

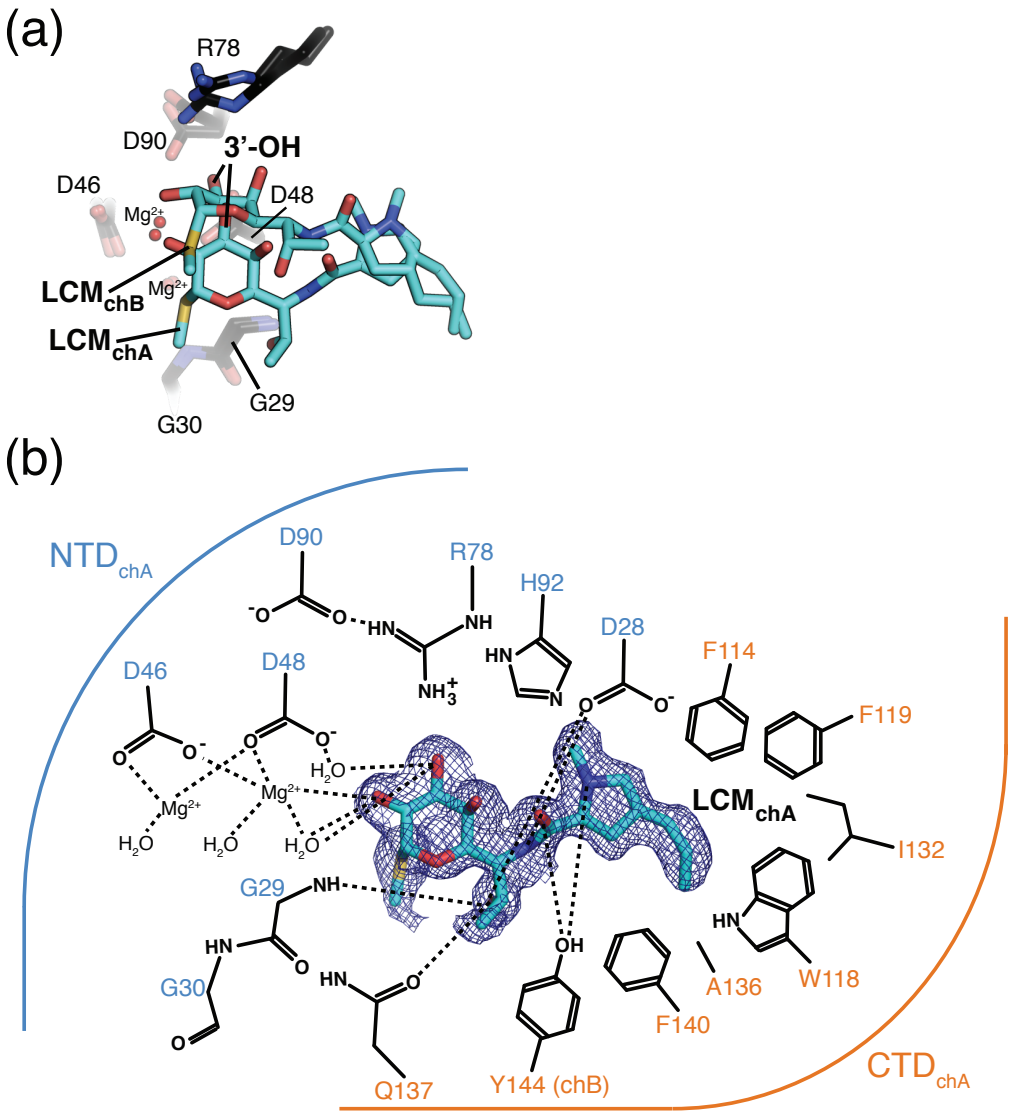


Figure S3. Comparison of two conformations of LCM molecules bound to Lnu(A) dimer.

(a) Relative orientation of two LCM molecules bound in the two Lnu(A) active sites of the dimer. LCM and the representative residues from the NT fold of Lnu(A) are shown in sticks and magnesium ions in red circles.

(b) Details of interactions between Lnu(A) and LCM (chain A complex). Residues shown form hydrogen bonds of hydrophobic interactions with LCM and are coloured according to their presence in the NTD (black) or CTD (orange). Electron density for LCM shown is a F_O-F_C omit map contoured at 2.0 σ. Dashes indicate hydrogen bonds.

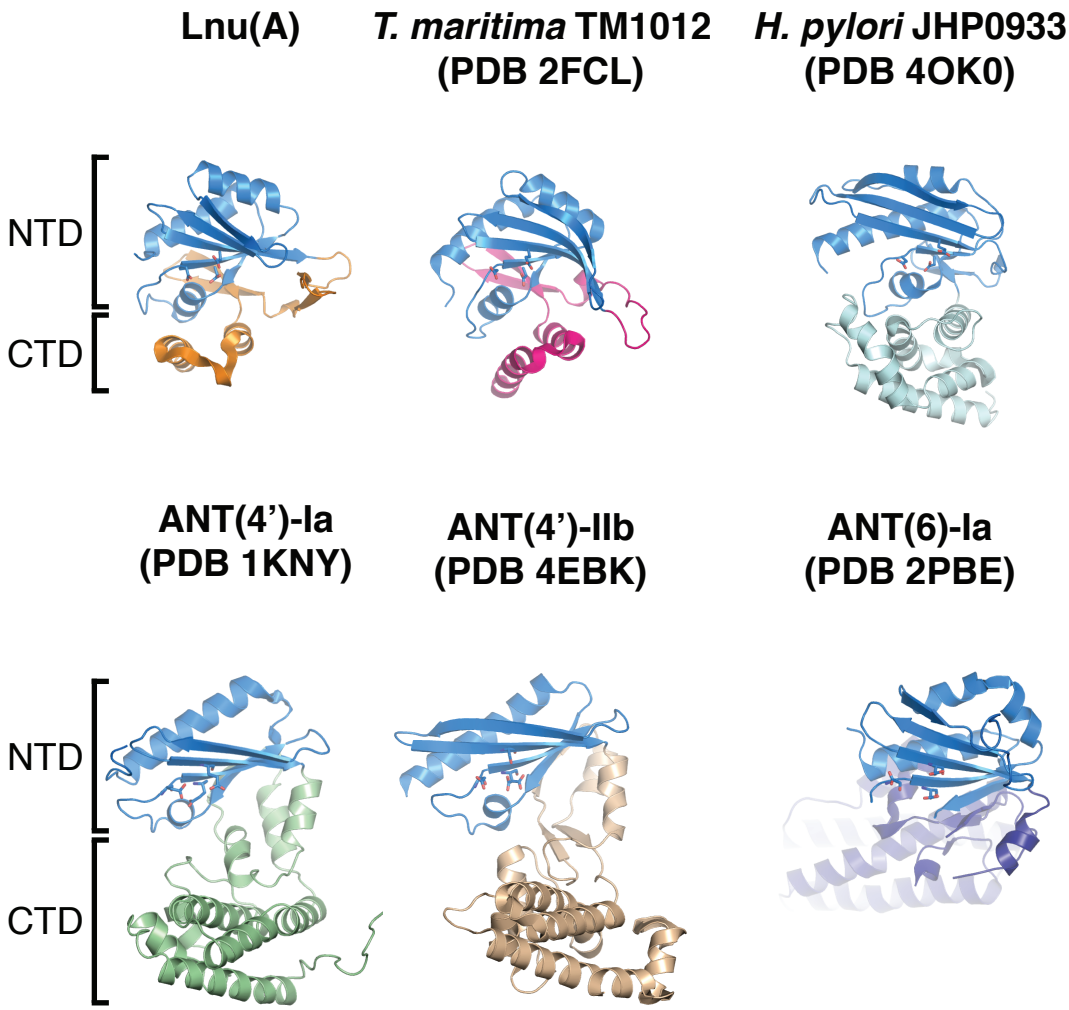


Figure S4. Comparison of the structures of Lnu(A), ANT enzymes and structural homologs as retrieved by search of the PDB.

NTDs are colored light blue, CTDs differently colored. The NTDs are conserved across these enzyme structures but the CTDs are structurally diverse.

Table S1. Characterization of oligomeric state of Lnu(A).

Enzyme	Buffer	Sequence MW (kDa)	Observed MW (kDa)	Oligomer
Lnu(A)	Phosphate[a]	22	20	monomer
Lnu(A)	HEPES[b]	22	84	tetramer
			62	trimer
			39	dimer
Lnu(B)	Phosphate	32	56	dimer
Lnu(D)	Phosphate	21	24	monomer

[a]50 mM sodium phosphate (pH 7.2), 150 mM NaCl.

[b]20 mM HEPES (pH 7.5), 100 mM KCl.