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**Supporting information for article:**

# Structure of the N-terminal domain of ClpC1 in complex with the antituberculosis natural product ecumicin reveals unique binding interactions

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**Table S1** RMS and average deviations of the $C_\alpha$ carbons of the 12 ClpC1-NTD molecules within the asymmetric unit of 6PBS for residues 1-144, aligned to chain W (reference).

A total of 66 possible unique combinations are possible. The superposition of chain W against the eleven others was considered to be a reasonable sampling for possible variations in RMS and average deviations.

| Chain | Chain W RMSD (Å) | Chain W Average Deviations (Å) |
|---|---|---|
| A | 0.46 | 0.27 |
| I | 0.29 | 0.24 |
| C | 0.54 | 0.36 |
| Y | 0.44 | 0.28 |
| e | 0.48 | 0.30 |
| B | 0.35 | 0.25 |
| G | 0.30 | 0.25 |
| K | 0.47 | 0.35 |
| M | 0.24 | 0.19 |
| O | 0.48 | 0.31 |
| T | 0.59 | 0.33 |
| Mean | 0.42 ± 0.11 | 0.28 ± 0.15 |

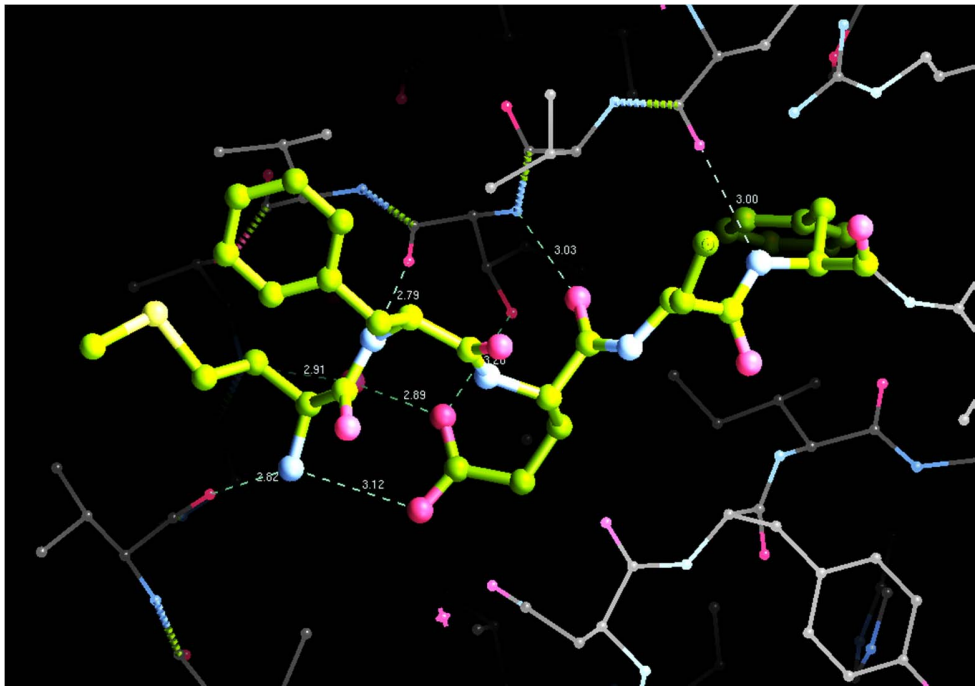


**Figure S1** N-terminal (residues 1-5: Met-Phe-Glu-Arg-Phe, ball and sticks) hydrogen bond interactions between ClpC1-NTD and ECU site 1 (thin bonds) (see Table below).

**Table S2** Distances between the N-terminus (a.a. 1-5) of the ClpC1-NTD-ECU complex (chain W) and the nearby atoms (range 2.7-3.4 Å) of the ECU site 1 (chain N), illustrating the hydrogen bonding network between protein and ligand.

| ClpC1-NTD-Atom 1 | ECU-Atom 2 | Distance (Å) |
|---|---|---|
| Met1-N | Val2-CO | 2.82 |
| Phe2-N | 12(β-hydroxy-Phe)-OXT | 2.79 |
| Glu3-CO | 12(β-hydroxy-Phe)-N | 3.03 |
| Glu3-OE1-Met1-N** | - | 3.12 |
| Glu3-OE2 | 12(β-hydroxy-Phe)-OB | 3.20 |
| Glu3-OE2-W102* | Thr4-N | 2.91 |
| Phe5-N | Trp10-ODJ | 3.0 |

*Water mediated hydrogen bond to ECU.

**Intramolecular hydrogen bond in ClpC1-NTD.

**Table S3** RMS (blue) and average deviations (yellow) of the $C_\alpha$ carbons of ClpC1-NTD (residues 1-145) from various structures.

The most significant movement was seen at the N-terminus when ECU is bound. Therefore, some calculations were repeated omitting residues 1-3 (N-terminus) from the superposition, values in parenthesis.

| | 3wdb | 3wdc | 6cn8 | L92SL96P | 6PBA | 6PBQ | 6PBS-chain W |
|---|---|---|---|---|---|---|---|
| 3wdb | - | 0.81 | 0.83 | 2.06 | 2.00 | 2.07 | 2.45 |
| 3wdc | 0.57 | - | 0.73 | 1.69 | 1.58 | 1.64 | 1.98 |
| 6cn8 | 0.49 | 0.61 | - | 1.91 | 0.73 | 1.98 | 2.18 |
| L92SL96P | 1.81 | 1.52 | 1.74 | - | 1.69 | 0.93 | 1.12 (0.71) |
| 6PBA | 1.65 | 1.39 | 0.61 | 1.52 | - | 0.72 | 1.38 (0.43) |
| 6PBQ | 1.77 | 1.44 | 1.68 | 0.56 | 0.46 | - | 1.36 (0.68) |
| 6PBS-chain W | 1.94 | 1.61 | 1.82 | 0.61 (0.52) | 0.62 (0.37) | 0.69 (0.44) | - |

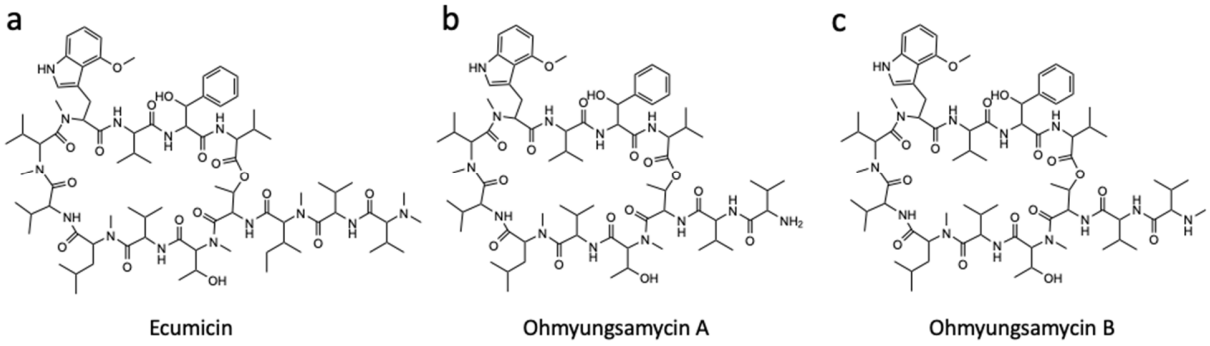


**Figure S2** Two-dimensional drawings of the structurally related ecumicin (a), ohmyungsamycin A (b) and ohmyungsamycin B (c).

**Table S4** Kinetic binding of macrocyclic peptides to ClpC1-FL with various mutations along the amino acid sequence were fit to a 1:1 Langmuir binding equation.

No binding (NB) was detected for ECU and OMS-A binding with the double mutant protein.

| | $K_D$ (nM) | | | $k_a$ ($M^{-1}s^{-1}$) | | | $k_d$ ($s^{-1}$) | | |
|---|---|---|---|---|---|---|---|---|---|
| Mutation | ECU | OMS-A | RUF-I | ECU | OMS-A | RUF-I | ECU | OMS-A | RUF-I |
| V14A | 1030 ± 22 | 1690± 1257 | 654± 9 | $1.42x10^4$ ± $0.93x10^4$ | $8.96x10^3$ ± $1.05x10^3$ | $1.34x10^5$ ± $0.21x10^5$ | $1.46x10^{-2}$ ± $0.12x10^{-2}$ | $2.16x10^{-2}$ ± $0.29x10^{-2}$ | $8.77x10^{-2}$ ± $0.02x10^{-2}$ |
| Q17A | 465 ± 24 | 1260± 280 | 1500 ± 62 | $9.67x10^3$ ± $0.405x10^3$ | $1.79x10^4$ ± $1.29x10^4$ | $7.77x10^4$ ± $0.47x10^4$ | $4.50x10^{-3}$ ± $0.38x10^{-3}$ | $2.03x10^{-2}$ ± $1.35x10^{-2}$ | $1.17x10^{-1}$ ± $0.12x10^{-1}$ |
| K85A | 607 ± 14 | 1720 ± 36 | 433± 10 | $1.61x10^4$ ± $0.16x10^4$ | $8.44x10^3$ ± $2.74x10^3$ | $8.62x10^4$ ± $0.91x10^4$ | $9.69x10^{-3}$ ± $1.16x10^{-3}$ | $1.45x10^{-2}$ ± $0.45x10^{-2}$ | $3.73x10^{-2}$ ± $0.31x10^{-2}$ |
| L92SL96P | NB | NB | 276± 9 | - | - | $2.81x10^5$ ± $1.18x10^5$ | - | - | $7.73x10^{-2}$ ± $3.07x10^{-2}$ |

**Table S5** Kinetic binding of macrocyclic peptides to ClpC1-NTD with various mutations (left most column) in the N-terminus were fit to a 1:1 Langmuir binding equation.

OMS-A was not tested (NT) with MVFER and MVAFER mutant ClpC1-NTD.

| | $K_D$ (nM) | | | $k_a$ ($M^{-1}s^{-1}$) | | | $k_d$ ($s^{-1}$) | | |
|---|---|---|---|---|---|---|---|---|---|
| Mutation | ECU | OMS-A | RUF | ECU | OMS-A | RUF | ECU | OMS-A | RUF |
| MAFER | 3500 ± 90 | 8520± 883 | 848 ± 201 | $2.14x10^3$ ± $0.166x10^3$ | $2.83x10^3$ ± $0.228x10^3$ | $3.90x10^4$ ± 1.15 $x10^4$ | $7.47x10^{-3}$ ± $0.5x10^{-3}$ | $2.40x10^{-2}$ ± $0.08x10^{-2}$ | $3.42x10^{-2}$ ± $1.76x10^{-2}$ |
| MVFER | 3930 ± 72 | NT | 383± 150 | $1.10x10^3$ ± $0.040x10^3$ | - | $2.95x10^4$ ± $0.53x10^4$ | $4.32x10^{-3}$ ± $0.2x10^{-3}$ | - | $1.08x10^{-2}$ ± $0.20x10^{-2}$ |
| MVAFER | 771± 67 | NT | 827± 102 | $1.50x10^4$ ± $0.42x10^4$ | - | $1.75x10^4$ ± $0.39x10^4$ | $1.19x10^{-2}$ ± $0.17x10^{-2}$ | - | $1.71x10^{-2}$ ± $0.08x10^{-2}$ |
| FER | 2960 ± 25 | 4520± 206 | 342 ± 10 | $1.22x10^3$ ± $0.023x10^3$ | $1.36x10^3$ ± $0.066x10^3$ | $1.40x10^5$ ± $0.92x10^5$ | $3.60x10^{-3}$ ± $0.06x10^{-3}$ | $4.40x10^{-3}$ ± $3.31x10^{-3}$ | $3.36x10^{-2}$ ± $2.50x10^{-2}$ |
| AAFER | 3150 ± 107 | 8290 ± 737 | 997 ± 219 | $1.44x10^3$ ± $0.037x10^3$ | $1.07x10^3$ ± $1.33x10^3$ | $8.35x10^4$ ± $1.68x10^4$ | $4.55x10^{-3}$ ± $0.3x10^{-3}$ | $8.92x10^{-3}$ ± $1.65x10^{-3}$ | $8.49x10^{-2}$ ± $3.02x10^{-2}$ |