The structure of ribosome-lankacidin complex reveals ribosomal sites for synergistic antibiotics

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Crystallographic analysis revealed that the 17-member polyketide antibiotic lankacidin produced by Streptomyces rochei binds at the peptidyl transferase center of the eubacterial large ribosomal subunit. Biochemical and functional studies verified this finding and showed interference with peptide bond formation. Chemical probing indicated that the macrolide lankamycin, a second antibiotic produced by the same species, binds at a neighboring site, at the ribosome exit tunnel. These two antibiotics can bind to the ribosome simultaneously and display synergy in inhibiting bacterial growth. The binding site of lankacidin and lankamycin partially overlap with the binding site of another pair of synergistic antibiotics, the streptogramins. Thus, at least two pairs of structurally dissimilar compounds have been selected in the course of evolution to act synergistically by targeting neighboring sites in the ribosome. These results underscore the importance of the corresponding ribosomal sites for development of clinically relevant synergistic antibiotics and demonstrate the utility of structural analysis for providing new directions for drug discovery.

lankamycin | ribosomes | synergism | resistance | rRNA

Biochemical, genetic and functional evidence indicate that a great variety of antibiotics inhibit protein synthesis by binding to ribosomal functional regions. Crystallographic studies performed over the last decade revealed the exact binding sites of a variety of such drugs (see refs.1 and 2 for review). Many natural antibiotics, as well as their clinically relevant semisynthetic derivatives, bind at the peptidyl transferase center (PTC) in the large ribosomal subunit (3–14). Most of these compounds inhibit cell growth by interfering with peptide bond formation (15). The second major antibiotic binding site in the large ribosomal subunit is located at the upper segment of the nascent peptide exit tunnel (NPET), adjacent to the PTC, and is used by macrolides and type B streptogramins (3, 7, 16–21). Binding to this site impedes progression of the nascent proteins toward the tunnel exit. Thus, compounds binding to the PTC and NPET inhibit successive steps in protein synthesis: formation of the nascent chains and their export from the ribosome.

Simultaneous inhibition of successive steps of a specific biochemical pathway often results in a synergistic action of the inhibitors (22). Nature has not ignored this opportunity when evolving ribosomal antibiotics. For example, streptogramin antibiotics, produced by several Streptomyces species, are secreted as a combination of two structurally distinct compounds that inhibit cell growth by acting upon the PTC and NPET (23, 24). Streptogramin A (S_A) compounds are cyclic poly-unsaturated macrolactones that bind in the PTC, whereas type B streptogramins (S_B) compounds are cyclic depsipeptides that bind in the NPET (6, 7, 25). Each of the individual streptogramin components is a fairly weak antibiotic on its own, but in combination they exhibit strong inhibitory action. The synergistic antibiotic effect of streptogramins is medically relevant—the semisynthetic formulation Synercid composed of dalfopristin (the S_A derivative) and quinupristin (the S_B derivative) is widely used for treatment of complicated Gram-positive infections (26).

Lankacidin C (LC) and lankamycin (LM) are two inhibitory compounds produced by Streptomyces rochei 7434 AN4 (27, 28). The structures of LC and LM are chemically distinct and rather different from those of streptogramins (Fig. 1). LC is a macrocyclic compound composed of a 17-membered carbocyclic ring, bridged by a 6-membered lactone. LM is a macrolide whose 14-member lactone ring is decorated with 4-acetyl-L-arcanose and D-chalcose sugars (29, 30) resembling erythromycin (ERY) (Fig. 1). LC, which is used in veterinary medicine, inhibits growth of Gram-positive bacteria by interfering with protein synthesis but has little effect on eukaryotic cell-free translation (31–34). LC and its derivatives also exhibit antitumor activity, although it is unclear whether this phenomenon is related to the drug’s effect upon protein synthesis (35). Lankamycin exhibits a weak antibiotic activity against several Gram-positive bacteria and low toxicity in animal models (36, 37).

LC competition with chloramphenicol for binding to the ribosome reveals the large ribosomal subunit as a likely target of its action, in accord with its classification as a protein synthesis inhibitor (32, 38). Though little data is available about activity of LM, the similarity of its structure to that of ERY indicates that it may act as a typical macrolide. Furthermore, similar to ERY and other macrolides, desacetyl-LM was reported to be capable of activating expression of inducible macrolide-resistance genes (37). Coregulation of production of LC and LM (39, 40) suggests that these drugs have been evolutionary optimized to work together. Nevertheless, although LC and LM are coproduced by the Streptomyces strain, there has been no information about their sites of action, nor any evidence of functional interaction between these two antibiotic compounds.

Here, we investigated the sites of binding and the modes of action of LC and LM by crystallographic and biochemical analyses. We show that these two compounds bind at neighboring sites in the large ribosomal subunit, which partially overlap with the binding sites of two components of streptogramin antibiotics. We present evidence that LC can bind simultaneously with LM, and that the two drugs inhibit bacterial growth synergistically, suggesting that the structures of LC and LM have been optimized in the course of evolution to allow for their simultaneous cooperative action. Based on our structural results we also suggest means for enhancing their synergistic inhibitory effect. This study...
presents the cases in which crystallographic analysis provided functional insights that, in turn, stimulated advanced biochemical and genetic studies, which yielded further clinical implications.

**Results**

**Lankacidin Binding Site.** The 3.5 Å resolution (Table S1) difference electron density map calculated between the structure amplitudes of the large ribosomal subunits from *Deinococcus radiodurans* (D50S) in complex with LC (D50S-LC) and of the D50S native structure (41) allowed the unambiguous determination of the location (Fig. 2A) and conformation of LC in the PTC (Fig. 2B).

LC binding pocket is composed of nucleotides A2602, C2452, A2503, U2504, G2505, U2585, G2061, and U2506 (*E. coli* numbering throughout), and the bound LC is involved in an extensive network of hydrophobic interactions with most of these nucleotides. Additionally, LC is positioned within hydrogen bond distance to N1 and N2 of G2061, the 2’ ribose hydroxyls of A2503, and O5’ of G2505 (Fig. 2B–D). It partially occupies the location of the amino acid attached to the 3’ end of A site tRNA (Fig. 3A) and barely reaches the macrolide binding site (Fig. 3C). The repositioning of the tRNA residues that occurs as a consequence of LC binding creates a unique network of interactions between five reoriented nucleotides: U2506, G2505, G2581, C2610, and G2576. Within this network G2576 stacks upon G2505, C2610 stacks upon G2581, and the exocyclic amine of G2505 is at a hydrogen bonding distance from O2 of C2610. Also, the amino group of G2505 is within a hydrogen bond reach of O4 of U2506, which shifts toward the LC’s 1,3 dicarbonyl system. These newly established contacts stabilize the placement of G2505 and U2506 in a conformation that favors binding of LC. Additionally, similar to pleuromutilins that utilize a network of remote interactions, LC binding is influenced by the second-shell nucleotides, specifically G2576, A2062, C2530, U2531, C2507, U2584, G2581, C2610, and A2059. Thus, LC exploits the PTC’s inherent flexibility for achieving high binding affinity (9, 10).

In its binding site, LC macrolactone ring fits in the shallow depression in the wall of the PTC A site, by forming van der Waals interactions with U2504, G2505 and U2506. The importance of these interactions to the drug binding is manifested by structure–activity studies that showed that hydrogenation of the macrocyclic ring alters its ring conformation and reduces the inhibitory activity of LC (43). The 2-methyl group at the lactone edge of the macrocyclic ring inserts in the opening of the hydrophobic crevice formed by the splayed out bases of A2451 and C2452. This cleft also hosts the aminoacyl moiety of A site-bound aminoacyl-tRNA and is involved in binding of other PTC-targeting antibiotics.

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**Fig. 1.** Chemical structures of antibiotics relevant to this study. Three pairs are shown, in each the compounds that bind to the PTC are in the Upper panel, and their mates that bind to the NPET are in the Lower panel immediately below them. Erythromycin is inserted for size and structure comparisons.

**Fig. 2.** Interaction of lankacidin with D50S PTC. (A) LC (cyan) binding site at the PTC of D50S. (B) Modeling of LC structure in the (Fo-Fc) difference electron density map, contoured at 1.0 σ. (C) Chemical structure diagram of interactions of LC with the PTC nucleotides. (D) A network of induced interactions (in gray) between five PTC nucleotide residues that stabilize LC binding. The wild-type conformations are shown in pink.
including S₄ₕ antibiotic compounds such as dalfopristin and virginiamycin M (3, 13, 44). The overlap of the LC and chloramphenicol binding sites (Fig. 3B) provides the structural basis for their competition for binding to the ribosome (32).

Despite significant size differences (Fig. 1), the position of LC closely resembles those of dalfopristin (6) and virginiamycin M (4). However, substantial differences were observed in the interactions of these compounds with the ribosome. In the D50S-Synercid complex (6) dalfopristin ring extends significantly further toward the P site (Fig. 3A). As LC binds in the PTC center, it causes the flexible base of A2602, which plays a major role in tRNA translocation (42, 45), to undergo a 45° rotation compared to its placement in native D50S or in Synercid-bound complex. U2585, the second flexible nucleotide that also seems to play a role in A-tRNA translocation, undergoes only a minor alteration in D50S-LC complex, while it is rotated by 180° in D50S-Synercid complex. This rotation seems to occur because of steric hindrance of the dalfopristin large macrocyclic ring and to the occupation of the S₉ site by quinupristin, the S₉ component of Synercid. Other important details distinguish binding of LC and dalfopristin. While both drugs are hydrogen-bonded to the G2061 exocyclic amine, an additional H bond links dalfopristin with G2061 2’ hydroxyl (6). Both LC and Synercid induce a conformational change of C2610. However, in the D50S-LC complex C2610 stacks upon G2581 and is H-bonded to G2505, whereas in the D50S-Synercid complex it is flipped away because of steric hindrance caused by quinupristin. Additionally, while both LC and dalfopristin reorient the U2506 base, the shift of this base toward LC is unique.

Consistent with its binding to the functionally critical PTC, LC inhibited bacterial (E. coli) cell-free transcription-translation system, with a respectable IC₅₀ of 1.5 ± 0.1 μM (Fig. 4A).

Furthermore, confirming previous observation (32, 38), we found that the drug readily interfered with the peptide bond formation inhibiting the puromycin reaction catalyzed by either Staphylococcus aureus 70S ribosomes (circle) or D. radiodurans large ribosomal subunits (squares). (C) Competition of LC with 14C-Ery for binding to D. radiodurans 70S subunits. (D) Inhibition of cell-free translation (E. coli) by LM

Lankacidin and Lankamyacin Can Simultaneously Bind to the Ribosome. S. rochei secretes two antibiotics, a 17-member ring macrocyclic LC and a 14-member ring macrolide LM. LM is structurally similar to ERY (Fig. 1) and thus we assumed that it is likely to bind to the ribosome at the site and orientation similar to ERY, namely at the NPET in immediate proximity to the PTC, the LC binding site. However, the comparison of the position of ERY in D50S (3) with the crystal structure of D50S-LC complex (Fig. 3C) revealed that the desosamine sugar of ERY approaches the macrocyclic ring of LC too close for simultaneous binding of both drugs. In agreement with this notion, competition experiments showed that LC displaced 14C-ERY from D50S with IC₅₀ of 355 ± 26 nM (Fig. 4C). These observations raised the question whether similar to ERY, LM will also compete with LC for ribosome binding. If so, why would an antibiotic-producing microorganism synthesize two competing drugs?

Since the lack of radiolabeled LC and LM precluded direct measurement of their binding to the ribosome, we applied an alternative technique to address this puzzle. We first verified that LM binds to the ribosome and inhibits protein synthesis. Indeed, in the E. coli cell-free system, LM inhibited translation (IC₅₀ of 275 ± 36 μM) (Fig. 4D), arguing that the antibiotic does bind to the ribosome, albeit with only moderate affinity. RNA probing was then utilized to follow the binding of LC and LM to the ribosome. For the consistency of structural data, we carried out RNA probing experiments using D. radiodurans large ribosomal subunits. Binding of LC and LM was analyzed using chemical modifying reagents 1-cyclohexyl-3-(2-morpholinoethyl) carbodiimide metho-p-toluene sulfonate (CMCT) and dimethyl sulfate (DMS) (46).

In accord with crystallographic data, association of LC with D50S results in a strong protection of the PTC nucleotide residues U2506 and U2585 from CMCT modification (Fig. 5A). We also noted that upon LC binding, A2059 became partially protected from DMS, whereas modification of A2058 was increased (Fig. 5B). This effect correlated with the crystallographic
binding site of LC and LM partially overlaps with that of SA and SB antibiotics, and because SA and SB act synergistically, we anticipated that despite the difference in their size and chemical properties from streptogramins, LC and LM may also exhibit synergy. To address this issue we performed in vivo and in vitro experiments, using whole cell bacteria as well as a bacterial cell-free system. Synergism was observed by an in vivo assay that utilizes a susceptible strain of Gram-positive S. aureus. We analyzed the minimal inhibitory concentrations (MIC) in a checkerboard fashion and plotted the results as a fraction of MIC (FIC) of individual compounds (Fig. 6). In this assay, antibiotics are considered synergistic if the curve has a concave shape, whereas a linear plot reflects additive action of the drugs, and a convex graph shows antagonistic interaction (47). The experimental MIC plot (solid line in Fig. 6) had a well-pronounced concave character revealing synergy in action of LC and LM. These findings were further verified in an E. coli cell-free transcription-translation system. Thus, similar to streptogramins, the two antibacterial compounds produced by S. rochei bind simultaneously to the neighboring sites in the ribosome and synergistically inhibit sensitive bacteria.

**Discussion**

The crystallographic and biochemical data presented here firmly established the PTC as the site of LC action. The inhibitory effect of the antibiotic upon peptide bond formation is likely achieved by preventing the binding or the proper placement of aminoacyl moiety of aminoacyl-tRNA in the A site (Fig. 3A). As LC trespasses the P site, it may also affect the exact positioning of the peptidyl tRNA C terminus. Although LC is chemically distinct and is less bulky than most S₆-type drugs, its binding site partially overlaps with that of S₆ compounds (4, 6). Furthermore, both, LC and SA drugs and U2506 that is involved in PTC functions (42, 48–50).

As can be concluded from the similarity of chemical structures of LM and ERY and from the overlap of the set of nucleotides protected by these two compounds, LM binds to the NPET macrolide binding site in a fashion similar, albeit not identical, to the other macrolides (Fig. 5). The same NPET region accommodates the B components of streptogramin antibiotics, but owing to differences in chemical nature (Fig. 1), the S₅ compounds and macrolides exploit a different set of interactions (3, 4, 6, 7).

Because the LC and S₅ binding sites in the PTC (Fig. 3C) are adjacent to the macrolides and S₉ binding sites, it is conceivable that compounds acting upon these two sites can either compete or cooperate in binding to the ribosome. Streptogramins are known for their synergistic action (24, 51). Such cooperativity

**Fig. 5.** Chemical probing of interactions of LC (filled arrowheads), LM (open arrowheads) and ERY (open circles) with the ribosome. Protection of 23S rRNA residues from (A) CMCT and (B) DMS modification by LC. (C) Protection of 23S rRNA residues from CMCT modification by LM. (D) Protections from CMCT afforded by LC, LM, and ERY present alone or in combination. Small open circles indicate bands that appeared because of slight nuclease degradation, which were not reproducible between the repeated experiments.

**Fig. 6.** Synergistic inhibitory activity of LC and LM upon S. aureus. The plots represent changes in MIC of individual compounds when both drugs are present in combination. The general shapes of the hypothetical curves corresponding to the additive, synergistic, or antagonistic mode of the drug combinations are shown by broken lines. The experimental curve is shown as a solid line with the MIC values (shown as a fraction of MIC of LM or LC acting alone) indicated by filled circles.
makes evolutionary sense: The same microorganism produces both $S_A$ and $S_B$ components, and their mutually enhanced action should be highly beneficial for the antibiotic producer. This notion initially did not seem to hold true for the macrolide LC and a macrolide LM. Our experiments showed that LC and ERY do not cooperate, but rather compete for binding to the **D. radiodurans** ribosome. Hindrance may result from a direct clash between ERY desosamine sugar and the LC macrolide ring (Fig. 3C) or by allosteric modulation of the binding site of one compound by binding of its counterpart. The altered DMS reactivity of A2058 and A2059, the nucleotides located in the heart of the macrolide binding site (Fig. 5A), upon the LC binding might be a reflection of such allostery. However, the competition between LC and ERY is not so surprising: These drugs are produced by different microorganisms and were not "designed" to work together. In contrast, LM is coproduced with LC. Both drugs appear to be coregulated, and thus similar to the streptogramins case, seem to be intended to work together (39, 40). In agreement with this hypothesis, our results show their simultaneous binding to the ribosome. The small structural differences between ERY and LM (Fig. 1) are likely to cause the variation in their binding properties. One of the important distinctions between LM and ERY is the nature of the C5-linked sugar residue (D-desosamine in ERY vs. D-chalcose in LM). Even small modifications of desosamine in ERY can dramatically alter the antibiotic's activity (52, 53). Replacement of 3'-dimethyl amine of ERY with a methoxy group in LM may facilitate accommodation of the C5-sugar residue in a narrow space left between the A2058/A2059 ridge and the bound LC molecule (see Fig. 3C).

Similarly, minute modifications in the structure of the antibiotics or in their binding sites may have significant effects on their binding and properties (20, 37, 54, 55). LM on its own is a less potent antibiotic than LC, and its activity is compensated by its ability to act synergistically with LC. Both drugs appear to be coregulated, and thus similar to the streptogramins case, seem to be intended to work together (39, 40). In agreement with this hypothesis, our results show their simultaneous binding to the ribosome. The small structural differences between ERY and LM (Fig. 1) are likely to cause the variation in their binding properties. One of the important distinctions between LM and ERY is the nature of the C5-linked sugar residue (D-desosamine in ERY vs. D-chalcose in LM). Even small modifications of desosamine in ERY can dramatically alter the antibiotic's activity (52, 53). Replacement of 3'-dimethyl amine of ERY with a methoxy group in LM may facilitate accommodation of the C5-sugar residue in a narrow space left between the A2058/A2059 ridge and the bound LC molecule (see Fig. 3C).

Thus, it appears that the LM synthetic pathway has been designed to cooperate with other macrolides or in their binding sites may have significant effects on their binding and properties (20, 37, 54, 55). LM on its own is a less potent antibiotic than LC, and its activity is compensated by its ability to act synergistically with LC. Both drugs appear to be coregulated, and thus similar to the streptogramins case, seem to be intended to work together (39, 40). In agreement with this hypothesis, our results show their simultaneous binding to the ribosome. The small structural differences between ERY and LM (Fig. 1) are likely to cause the variation in their binding properties. One of the important distinctions between LM and ERY is the nature of the C5-linked sugar residue (D-desosamine in ERY vs. D-chalcose in LM). Even small modifications of desosamine in ERY can dramatically alter the antibiotic's activity (52, 53). Replacement of 3'-dimethyl amine of ERY with a methoxy group in LM may facilitate accommodation of the C5-sugar residue in a narrow space left between the A2058/A2059 ridge and the bound LC molecule (see Fig. 3C).
Materials and Methods. Crystals of D50S that were grown as in (1) were soaked in solutions containing 0.025mM of lankacidin for 20 h at 20°C, transferred into cryo-buffer and shock-frozen in liquid nitrogen. X-ray data were collected at 85–100 K from shock-frozen crystals at wavelength of 1.0 and 0.837 Å, at crystal to detector distance of 430 mm, using an oscillation range of 0.3° with synchrotron radiation beam at 19ID at the Advanced Photon Source/Argonne National Laboratory and at ID23-2 at the European Synchrotron Radiation Facility (ESRF), respectively. Data were recorded on charge-coupled device and processed with HKL2000 (2). Complete x-ray data sets were obtained from two crystals. The structure of D50S was refined against the structure factor amplitudes of the antibiotic complex D50S-LC using rigid body refinement as implemented in CNS 1.2 (3, 4). For free R-factor calculation, random 5% of the data were omitted during refinement. To obtain an unbiased electron density map a composite omit map of the entire unit cell was calculated. Further refinement was carried out using CNS 1.2 minimization combined with various CCP4 (5) programs, exploiting the available crystal structure of Bundlin-A (lankacidin) (6).

Finally, the complete molecules were subjected to restraint minimization and grouped B factor refinement with CNS. The ribosome-antibiotic interactions were determined with LigPlot (7) and LPC (8). Images were generated using PyMol (9).

Antibiotic binding. D. radiodurans 50S subunits (0.1 μM) were preincubated 15 min at 37°C with 0.1 μM of 14C-erythromycin (48.8 μCi/mmol, Perkin Elmer) in 100 μl binding buffer (20 mM Tris-HCl, pH 7.5, 10 mM MgCl2, 250 mM NaCl, 6 mM β-mercaptoethanol). LC was then added at varying concentrations (0–2 μM) and incubation continued for 30 min. The ribosome-antibiotic complexes were purified by gel filtration in BioGel P30 spin columns as described (10) and the amount of ribosome-bound radioactivity was measured by scintillation counting.

Inhibition of cell-free protein synthesis by LC and LM. The E. coli cell-free transcription-translation system for circular DNA (Promega) was pre-incubated with varying concentrations of antibiotics for 5 min at 20°C. The reactions (10 μl final volume) were initiated by adding 0.64 μg of pBestLuc plasmid DNA (Promega). Reactions were incubated for 40 min at 20°C and stopped by chilling on ice. The activity of firefly luciferase synthesized in the reaction was determined in 96-well plates using Bright-Glo Luciferase Assay System (Promega) as recommended by the manufacturer.

Inhibition of the peptidyl transferase reaction by LC In the reaction catalyzed by large ribosomal subunits, D. radiodurans 50S subunits (200 nM final concentration) were combined in 50 μl of the reaction buffer (20 mM Tris-HCl, pH 8.0, 20 mM MgCl2, 400 mM KCl) with 28 nM [3H]-fMet-tRNA, 1 mM puromycin at varying concentrations of LC. Reactions were initiated by addition of 25 μl methanol and incubated on ice for 30 min. Reactions were stopped and analyzed as described (11). In the ribosome-based puromycin assay, 70S ribosomes of S. aureus (at a final concentration of 200 nM) were preincubated for 15 min at 37°C in 50 μl of polyamine buffer (12) (20 mM HEPES-KOH, pH 7.6, 6 mM Mg-aceatate, 150 mM NH4Cl, 4 mM β-mercaptoethanol, 2 mM spermidine, 0.05 mM spermine) with 600 nM mRNA AAGAGAUAACACAAUGGGG and 28 nM [3H]-fMet-tRNA. After addition of varying concentrations of LC, puromycin was added to the final concentration of 0.5 mM and reactions were incubated for 15 min at 37°C. Reactions were stopped and processed as in (11).

RNA probing. RNA probing was carried out essentially as described (13, 14) using D. radiodurans 50S ribosomal subunits at 200 nM concentration and antibiotics at the following concentrations: LC–50 μM, LM–500 μM, ERY–50 μM. Prior to addition of the modifying reagents, ribosomal subunits were pre-incubated with the drug 10 min at 37°C and then 10 min at 20°C.

Cell-free translation system used for detecting synergism in vitro. Inhibition of cell-free protein synthesis by LC and LM was measured using the E. coli cell-free transcription-translation assay (Fig. S1). Cell free extract was prepared from Escherichia coli (Strain BL21-DE3) in a manner similar to that previously reported (15). An aqueous solution containing amino acid mix (1.5 mM for each amino acid), magnesium acetate (20 mM), NTP mix (0.9 mM), ATP (0.4 mM), potassium glutamate (150 mM), E. coli tRNA mixture (0.17 mg/mL), DTT (1.8 mM), folinic acid (35 μg/mL), cAMP (0.65 mM), NH4OAc (28 mM), creatine phosphate (80 mM), HEPES (pH equals 7.5 at 37°C, 140 mM), 9.5% w/v PEG-8000, tyrosine (0.4 mg/mL), creatine kinase (0.35 mg/mL) and S12 cell free extract (12% v/v) was prepared. PIVEX-6D plasmid encoding wild-type GFP (1 ng/μL) was added to this solution commencing the transcription-translation reaction.

This solution was immediately added to a 96 well plate containing serial dilutions of lankamycin and lankacidin. After incubation for 1 h at 37°C, 50 μL of erythromycin (8 μM) was added to each well to completely stop the translation reaction. The fluorescence data were collected on a TECAN SpectraFluor PlusTM 96-well plate reader (λexcite = 485 nm, λemitt = 535 nm). The data were fitted using the least-squared regression analysis package in Igor ProTM.

A control experiment was performed in the absence of the antibiotics. This experiment was also used for the determination of the time window required for maximum GFP production before the reaction was quenched.


Fig. S1. (A and B): Binding curves for LC and LM respectively. The production of green fluorescent protein in an in vitro transcription-translation reaction was used to determine inhibition. (C): Synergy experiment showing the fractional contributions towards the observed C50 by LC and LM. Points below the dashed blue line indicate synergism.

Table S1. Crystallographic data for the D50S-lankacidin complex

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