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Supplementary Material

Elucidation of post-PKS tailoring steps involved in landomycin biosynthesis

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Table S1. List of primers used in this study.

Name of genes	Forward and reverse primers used for PCR amplification (For *in vitro* expression constructs)
lanM2	Forward: 5'-ATCAT ATGGCCGCGAAGCACACCGACACCCAG-3'
	Reverse: 5'-ATGAATTCTCAGCCGGCCGGCCGTCCGTGCA-3'
lanZ4	Forward:5'-ATCATATGTCAGCAAGGGCCGTCGTGC-3'
	Reverse: 5'-ATGAATTCTCATGCTGTGTAGGGCCGTTTCGC-3'
lanZ5	Forward: 5'-ATCATATGAGAACGAACAACGACGTCGGGACAGT-3'
	Reverse: 5'-ATGAATTCCTACTTCGTGTCGGGCAGGTGTGC-3'
lanV	Forward: 5'- TTA CTC ATATGG GCA ATC TCA CCG GAA AAA CGG -3'
	Reverse: 5'- ACT AAG AAT TCG GCT GCATCC GGC GC-3'
lanE	Forward: 5'- GACGACGACAAGATGGACGCTGCGGTGATCATCGCGGGTG-3'
	Reverse: 5'-GAGGAGAAGCCCGGTCAGAAGGCGCGCCCGACCCGCGGTGTA-3'
Name of gene	**Forward and reverse primers used for PCR amplification (for *in vivo* expression constructs)**
lanM2	Forward: 5'- ATA CTA GTG AAG GAG CAG CGCTGA CCATGG CCG CGA AGC ACA C -3'
	Reverse: 5'- ATG AAT TCA TAG CTA GCT CAG CCG GCC GGC CGT CCGTGC AC -3'
lanZ4-lanZ5	Forward: 5'- ATT ACT AGT CTC TCC GCC CGT CAG GAA AGG AAG GAC CGC C-3'
	Reverse: 5'- ATG AAT TCA TAG CTA GCCTACTTC GTGTCG GGC AGGTGT GC -3'
lanE	Forward: 5'- ATA CTA GTA GCCTAT TCA AGG AGC GGA AAATGG ACG CTG CGG -3'
	Reverse: 5'- ATG AAT TCA TAG CTA GCT CAG AAG GCG CGC CCG ACC CGC -3'
lanV	Forward: 5'- TAT GAATTC CAC CGA CAG GAC AGA CAA GCATGG GC -3'
	Reverse: 5'- TATTGG ATC CGG CGCTCA GCC GAG CAGTGT -3 '

Table S2. Summary of the constructs created in this study and the metabolites produced through their expression in *S. lividans* TK64.

Substrates	Order of the genes in the cassettes	Product observed	References
pRab4	*gilA-gilB-gilC-gilF-gilP-JadD-ravG=PKS*	**16***, **17**	Kharel et al.[1]
pLaox1	*PKS-lanZ4-lanZ5*	**16***, **17**	This work
pLaox2	*PKS-lanM2*	**10***, **17**	This work
pLaox3	*PKS-lanE*	**16**	This work
pLaox4	*PKS-lanZ4-lanZ5-lanM2*	**10**	This work
pLaox5	*PKS-lanZ4-lanZ5-lanE*	**17**	This work
pLaox6	*PKS-lanZ4-lanZ5-lanM2-lanE*	**13**	This work
pLaox7	*PKS-lanV*	**16***, **17**	This work
pLaox8	*PKS-lanZ4-lanZ5-lanV*	**16***, **17**	This work
pLaox9	*PKS-lanM2-lanV*	**10**	This work
pLaox10	*PKS-Z4-lanZ5-lanM2-lanV*	**10**	This work
pLaox11	*PKS-lanZ4-lanZ5-lanE-lanV*	**13**	This work
pLaox12	*PKS-lanZ4-lanZ5-lanM2-lanE-lanV*	**8, 13, 10**	This work

* represents the major metabolite in the mixture.

Table S3. Summary of the *in vitro* enzyme assays.

Substrates	Lan post-PKS enzymes	Co-factors used alone or in combination	Products formation
16	LanM2	FAD and NADPH or NADH	N.R.
16	LanZ5	NADPH or NADH and FMN or FAD	N.R.
16	LanZ4, LanZ5	FMN and NADPH	N.R.
16	LanE	NADPH or NADH with or without FMN or FAD	**17**
16	LanM2, LanE	FAD and NADPH or NADH	**17**
16	LanV	NADPH or NADH	N.R.
16	LanM2, LanE, LanV	FAD, NADPH or NADH	**17**
16	LanM2, LanE, LanV, LanZ4, LanZ5	FAD or FMN or FAD or FMN or both (1:1), NADPH or NADH or both in 1:1 ratio	**17**
10	LanM2	FAD, NADPH or NADH	N.R.
10	LanZ5	FAD or FMN, NADPH or NADH	N.R.
10	LanZ4, LanZ5	NADPH and FMN	N.R.
10	LanE	NADPH or NADH with FMN or FAD	**13**
10	LanM2, LanE	FAD or FMN and NADPH, and NADH	**13**
10	LanV	NADPH or NADH	N.R.
10	LanM2, LanE, LanV	NADH with or without FAD and FMN	**4**
10	LanE, LanV	NADH	**4**
10	LanE, LanV	NADPH	**13** (major), **4**[#]
10	LanM2, LanE, LanV, LanZ4, LanZ5	FAD and FMN (1:1), NADPH or NADH or both in 1:1 ratio	**13** (major), **4**[#]
17	LanM2	FAD and NADPH or NADH	N.R.
17	LanZ5	NADPH or NADH and FMN or FAD	N.R.
17	LanZ4, LanZ5	FMN and NADPH	N.R.*
17	LanE	NADPH or NADH with or without FMN or FAD	N.R.
17	LanM2, LanE	FAD and NADPH or NADH	N.R.
17	LanV	NADPH or NADH	N.R.
17	LanM2, LanE, LanV	FAD, NADPH or NADH	N.R.
17	LanM2, LanE, LanV, LanZ4, LanZ5	FAD or FMN or FAD or FMN or both (1:1), NADPH or NADH or both in 1:1 ratio	N.R.*
9	LanM2	FAD and NADPH or NADH	N.R.
9	LanZ5	NADPH or NADH and FMN or FAD	N.R.
9	LanZ4, LanZ5	FMN and NADPH	N.R.
9	LanE	NADPH or NADH with or without FMN or FAD	N.R.
9	LanM2, LanE	FAD and NADPH or NADH	N.R.
9	LanV	NADPH or NADH	N.R.
9	LanM2, LanE, LanV	FAD, NADPH or NADH	N.R.
9	LanM2, LanE, LanV, LanZ4, LanZ5	FAD or FMN or FAD or FMN or both (1:1), NADPH or NADH or both in 1:1 ratio	N.R.
14	LanM2	FAD and NADPH or NADH	N.R.
14	LanZ5	NADPH or NADH and FMN or FAD	**15**[#]
14	LanZ4, LanZ5	FMN and NADPH	**15**

14	LanE	NADPH or NADH with or without FMN or FAD	N.R.
14	LanM2, LanE	FAD and NADPH or NADH	N.R.
14	LanV	NADPH or NADH	N.R.
14	LanM2, LanE, LanV	FAD, NADPH or NADH	N.R.
14	LanM2, LanE, LanV, LanZ4, LanZ5	FAD or FMN or FAD or FMN or both (1:1), NADPH or NADH or both in 1:1 ratio	**15**
5	LanM2	FAD, NADPH or NADH	N.R.
5	LanZ5	FAD or FMN, NADPH	**9**
5	LanZ4, LanZ5	NADPH and FMN	**9**, **8**[#]
5	LanE	NADPH or NADH with or without FMN or FAD	N.R.
5	LanM2, LanE	FAD or FMN and NADPH or NADH	N.R.
5	LanV	NADPH or NADH	N.R.
5	LanM2, LanE, LanV	FAD and NADPH or NADH	N.R.
5	LanM2, LanE, LanV, LanZ4, LanZ5	FAD and FMN (1:1), NADPH or NADH or both in 1:1 ratio	**9**, **8**[#]

N.R.: no reaction; *: color turned into purple from yellow temporarily. Such color change was observed only in the presence of LanZ4, catalytic amount of FAD and NADPH; [#]: produced in very small quantities.

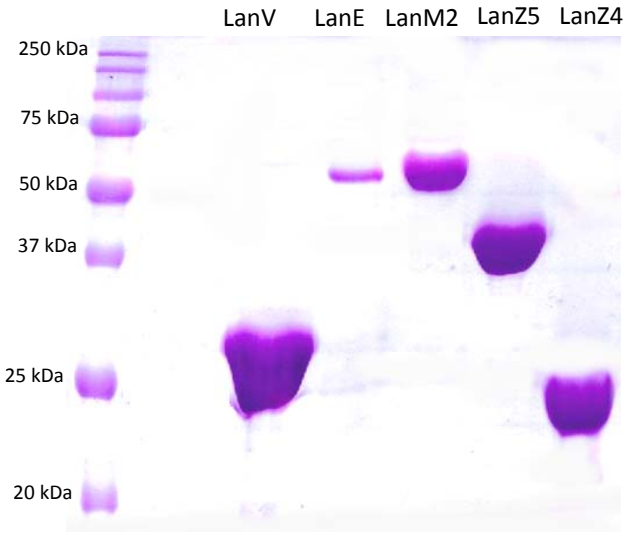

Figure S1. SDS-PAGE analysis of post-PKS landomycin oxygenases. The sizes of most of the proteins observed on the gel were in good agreement with the calculated molecular weights (LanZ4: 23.81 kDa; LanZ5: 43.45 kDa; LanE: 56.96 kDa; LanV: 28.35 kDa) except LanM2 which appeared slightly less (~10 kDa) as compared to the calculated molecular weight (77.47).

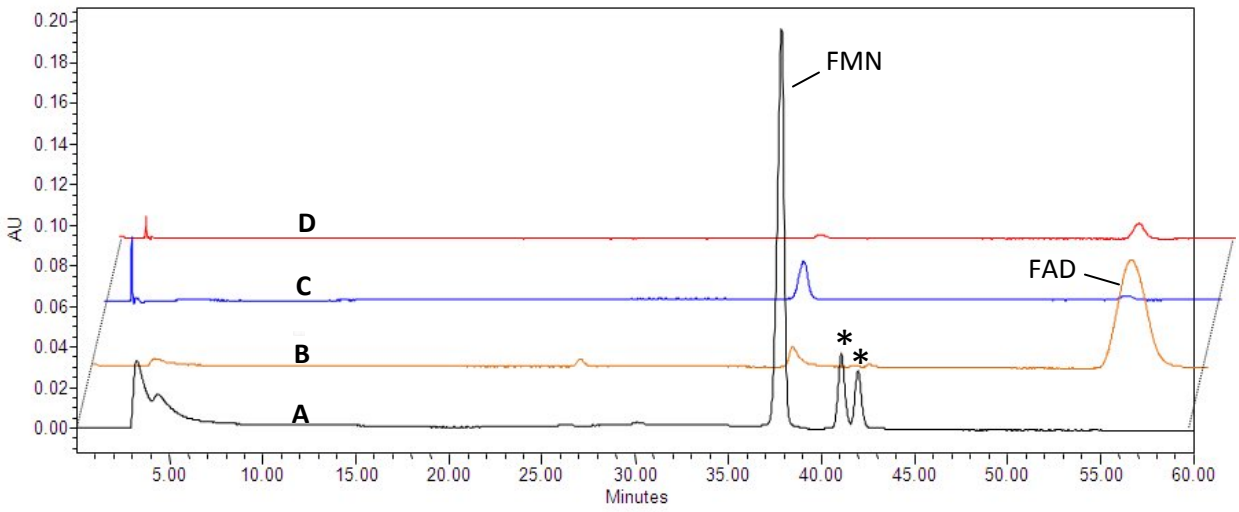

Fig. S2 HPLC analyses of the co-factors released from enzymes. Trace A: standard FMN; trace B: standard FAD; trace C: co-factors released from LanZ4; trace D: co-factors released from LanM2. The peaks pertaining to asterisks represent the impurities present in the commercially available sample.

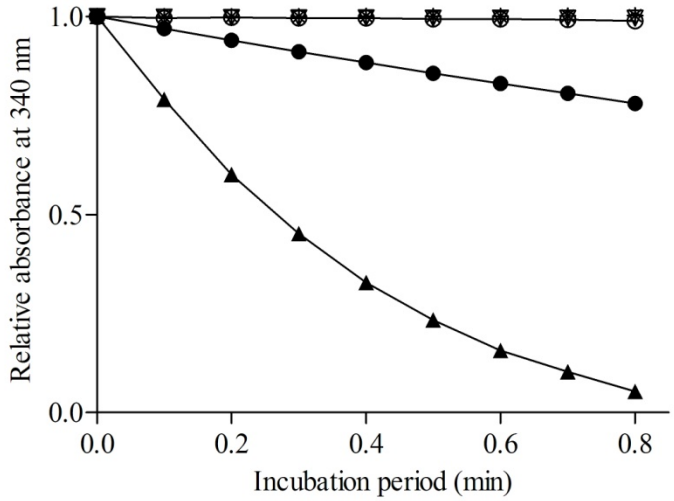

Fig S3. Substrate preference of LanZ4. Line with triangle, solid circle, inverted triangle and asterisk represent the use of FMN and NADPH, FAD and NADPH, FAD and NADH and FMN and NADH, respectively.

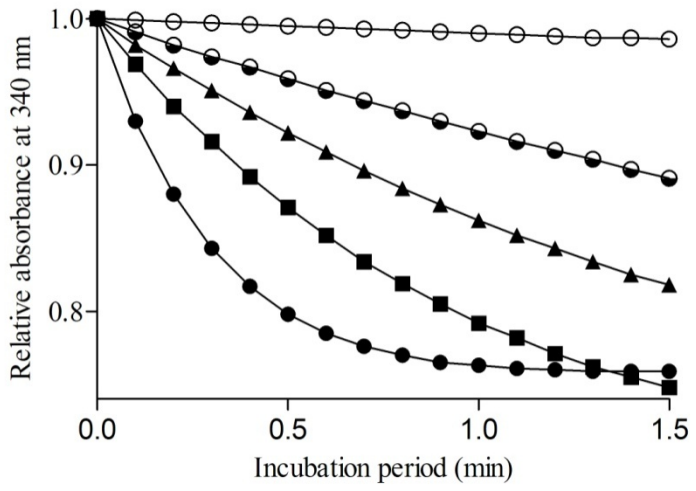

Fig. S4 The rate of NADPH oxidation with various concentrations of LanZ4. Line with solid filled circles, rectangles, triangles, half-filled circles and unfilled circles were observed with the use of 106.48, 35.49, 21.29, 10.64 and 0.71 µg of LanZ4 in the mixture. Each assay mixture was supplied with 100 µM of FMN and 160 µM of NADPH.

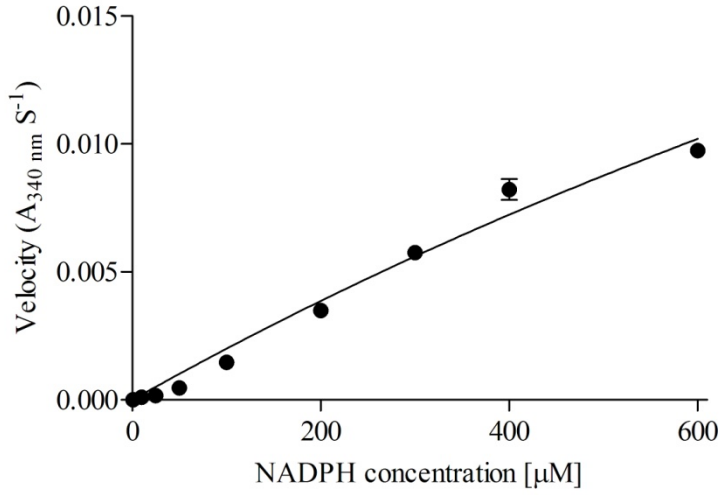

Fig. S5 Kinetics analysis of LanZ4. The curve was fitted to Michaelis-Menten kinetics. Substrate saturation of the enzyme was not obtained which prevented us from determining kinetics parameters.

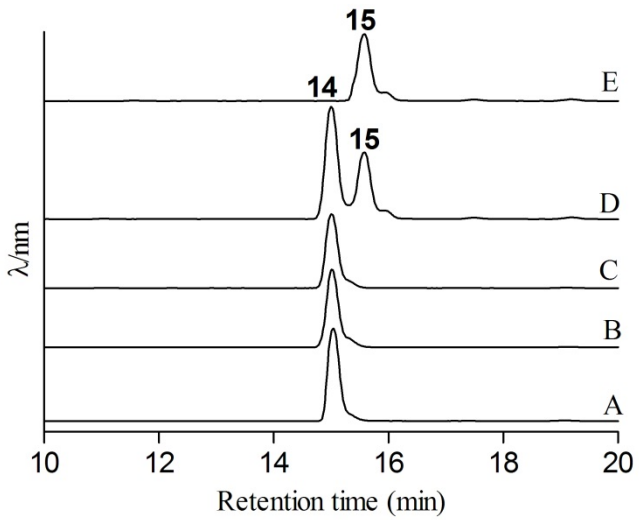

Fig. S6 HPLC analyses of the assay products demonstrating LanZ4 and LanZ5-mediated conversion of tetrangomycin (**14**) to 11-hydroxytetrangomycin (**15**). Traces A: standard tetrangomycin (**14**); trace B: **14**, LanZ5 and NADPH; trace C: **14**, LanZ5, NADPH and FMN; trace D: **14**, NADPH, FMN, LanZ4 and LanZ5; trace E: standard 11-hydroxytetrangomycin (**15**).

References

1. M. K. Kharel, P. Pahari, H. Lian and J. Rohr, *Org. Lett.*, 2010, **12**, 2814-2817.