Supporting Information for

Protein–Protein Interactions, not Substrate Recognition, Dominates the Turnover of Chimeric Assembly Line Polyketide Synthases

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Running title: Role of ACP-KS interactions in chimeric PKSs

Table S1. Plasmids used for module library

PKS product	Cosmid	Module number	Primer Name	Primer Sequence	Resulting plasmid
Rifamycin	cos2	1	RifM1_N-Term	CCGGGCTGAGCTCCTCGGGATC	pAJ5
			RifM1_C-Term	GTCGGCGGGGAACTCGGTGAC	
	cos2	2	RifM2_N-Term	CCTGTCCCCGGCGATGATCTTCG	pAJ6
			RifM2_C-Term	GGTCCGGGTGGTAGAGGCCGTC	
	cos2	3	RifM3_N-Term	CGTGGAGCTGCCCAGCACCC	pAJ7
			RifM3_C-Term	GCGCGCTGTTCTCCTGCTGAAGC	
	cos2	5	RifM5_N-Term	GGCGAGACGGTGGCGGGTG	pAJ9
			RifM5_C-Term	GTCGGGAAGCTCGACACCGCG	
	cos42	6	RifM6_N-Term	CCGCCACCGGCCTGAAGCTG	pAJ10
			RifM6_C-Term	GCCACCAGCCGCCAGAGGTCAC	
	cos42	7	RifM7_N-Term	GACGCGGGCGAGGAGCGGTC	pAJ11
			RifM7_C-Term	GCGATGGCCCGCTTGAGGTAGTCG	
Rapamycin	KOS002-13 “B1”	4	RapM4_N-Term	GAGTTGTTCACCGGCGAGAACCCTGC	pAJ16
			RapM4_C-Term	CCCGGAACTTGTCAATCGAGATCCCCG	
	KOS002-13 “B1”	6	RapM6_N-Term	CGACGGCTTTGGCTGTTCGGCTG	pAJ18
			RapM6_C-Term	GCATCCGTACCCGACTCGACCAAACG	

Table S2. Plasmid design and primers for bimodular chimeric PKSs

Plasmid Encoded Protein	PCR Fragment	Primer Name	Primer Sequence	PCR Template
pAJ20 (3)RifM1-TE	RifM1	bi_RifM1_Insert_N-Term	GCATCCGCGAGCTGGAATCCGAGCCGATCGCGATCGTGG	pAJ5
		bi_RifM1_Insert_C-Term	TCCCGGGCGGGAGTCCCGCTGAAGAGCTTGGCGCGCAGGT	
	(3)pET28-TE	bi_RifM1_Vector_N-Term	ACCTGCGCGCCAAGCTCTTCAGCGGGACTCCCGCCCG	pBL16
		bi_RifM1_Vector_C-Term	CCACGATCGCGATCGGCTCGGATTCCAGCTCGCGGATGC	
pAJ21 (3)RifM2-TE	RifM2	bi_RifM2_Insert_N-Term	GCATCCGCGAGCTGGAATCCGAGCCGATCGCGATCGTCG	pAJ6
		bi_RifM2_Insert_C-Term	TCCCGGGCGGGAGTCCCGCTGAGCAGTTCGGCCCGCAGG	
	(3)pET28-TE	bi_RifM2_Vector_N-Term	CCTGCGGGCCGAACTGCTCAGCGGGACTCCCGCCCG	pBL16
		bi_RifM2_Vector_C-Term	CGACGATCGCGATCGGCTCGGATTCCAGCTCGCGGATGC	
pAJ22 (3)RifM3+TE	RifM3	bi_RifM3_Insert_N-Term	GCATCCGCGAGCTGGAATCCGAGCCGATCGCGATCGTCG	pAJ7
		bi_RifM3_Insert_C-Term	TCCCGGGCGGGAGTCCCGCTCAGGAGTTCGACGCGCAGGTAGC	
	(3)pET28-TE	bi_RifM3_Vector_N-Term	GCTACCTGCGCGTCGAACTCCTGAGCGGGACTCCCGCCCG	pBL16
		bi_RifM3_Vector_C-Term	CGACGATCGCGATCGGCTCGGATTCCAGCTCGCGGATGC	
pAJ24 (3)RifM5+TE	RifM5	bi_RifM5_Insert_N-Term	GCATCCGCGAGCTGGAATCCGAGCCGATCGCCATCGTCG	pAJ9
		bi_RifM5_Insert_C-Term	TCCCGGGCGGGAGTCCCGCTACCGAGTTCGTCGCGCAGGT	
	(3)pET28-TE	bi_RifM5_Vector_N-Term	ACCTGCGCGACGAACTCGGTAGCGGGACTCCCGCCCG	pBL16
		bi_RifM5_Vector_C-Term	CGACGATGGCGATCGGCTCGGATTCCAGCTCGCGGATGC	
pAJ25 (3)RifM6+TE	RifM6	bi_RifM6_Insert_N-Term	GCATCCGCGAGCTGGAATCCGAGCCGATCGCCATCGTCG	pAJ10

		bi_RifM6_Insert_C-Term	TCCCGGGCGGGAGTCCCGCTGACCAGCCGCGCGCC	
	(3)pET28-TE	bi_RifM6_Vector_N-Term	GGCGCGCGGCTGGTCAGCGGGACTCCCGCCCGGGA	pBL16
		bi_RifM6_Vector_C-Term	CGACGATGGCGATCGGCTCGGATTCCAGCTCGCGGATGC	
pAJ26 (3)RifM7+TE	RifM7-	bi_RifM7_Insert_N-Term	GCATCCGCGAGCTGGAATCCGCGGCCGGCGAGCC	pAJ11
		bi_RifM7_Insert_C-Term	TCCCGGGCGGGAGTCCCGCTGGCGAGTTCCGCCCGC	
	(3)pET28-TE	bi_RifM7_Vector_N-Term	GCGGGCGGAACTCGCCAGCGGGACTCCCGCCCG	pBL16
		bi_RifM7_Vector_C-Term	GGCTCGCCGGCCGCGGATTCCAGCTCGCGGATGC	
pAJ32 (3)RapM4+TE	RapM4	bi_RapM4_Insert_N-Term	GCATCCGCGAGCTGGAATCCGAGCCGTTGGCGATTGTGGG	pAJ16
		bi_RapM4_Insert_C-Term	TCCCGGGCGGGAGTCCCGCTCAACTCGTCCAGCCGGGCG	
	(3)pET28-TE	bi_RapM4_Vector_N-Term	CGCCCGGCTGGACGAGTTGAGCGGGACTCCCGCCCG	pBL16
		bi_RapM4_Vector_C-Term	CCCACAATCGCCAACGGCTCGGATTCCAGCTCGCGGATGC	
pAJ34 (3)RapM6+TE	RapM6	bi_RapM6_Insert_N-Term	GCATCCGCGAGCTGGAATCCGAGCCGCTGGCGATCGTG	pAJ18
		bi_RapM6_Insert_C-Term	TCCCGGGCGGGAGTCCCGCTCAACTCGTCCAACCGAGCAGCC	
	(3)pET28-TE	bi_RapM6_Vector_N-Term	GGCTGCTCGGTTGGACGAGTTGAGCGGGACTCCCGCCCG	pBL16
		bi_RapM6_Vector_C-Term	CACGATCGCCAGCGGCTCGGATTCCAGCTCGCGGATGC	

Table S3. Plasmid design and primers for trimodular chimeric PKSs

Plasmid Encoded Protein	PCR Fragment	Primer Name	Primer Sequence	PCR Template
pAJ40 (3)RifM1(2)	RifM1	tri_RifM1_Insert_N-Term	GCATCCGCGAGCTGGAATCCGAGCCGATCGCGATCGTGG	pAJ5
		tri_RifM1_Insert_C-Term	GCCTCCCCCCGGACCTCGGTGAAGAGCTTGGCGCGCAGGT	
	(3)pET28(2)	tri_RifM1_Vector_N-Term	ACCTGCGCGCCAAGCTCTTCACCGAGGTCCGGGGGGAG	pBL36
		tri_RifM1_Vector_C-Term	CCACGATCGCGATCGGCTCGGATTCCAGCTCGCGGATGC	
pAJ41 (3)RifM2(2)	RifM2	tri_RifM2_Insert_N-Term	GCATCCGCGAGCTGGAATCCGAGCCGATCGCGATCGTCG	pAJ6
		tri_RifM2_Insert_C-Term	GCCTCCCCCCGGACCTCGGTGAGCAGTTCGGCCCGCAGG	
	(3)pET28(2)	tri_RifM2_Vector_N-Term	CCTGCGGGCCGAACTGCTCACCGAGGTCCGGGGGGAG	pBL36
		tri_RifM2_Vector_C-Term	CGACGATCGCGATCGGCTCGGATTCCAGCTCGCGGATGC	
pAJ42 (3)RifM3(2)	RifM3	tri_RifM3_Insert_N-Term	GCATCCGCGAGCTGGAATCCGAGCCGATCGCGATCGTCG	pAJ7
		tri_RifM3_Insert_C-Term	GCCTCCCCCCGGACCTCGGTCAGGAGTTCGACGCGCAGGTAGC	
	(3)pET28(2)	tri_RifM3_Vector_N-Term	GCTACCTGCGCGTCGAACTCCTGACCGAGGTCCGGGGGGAG	pBL36
		tri_RifM3_Vector_C-Term	CGACGATCGCGATCGGCTCGGATTCCAGCTCGCGGATGC	
pAJ44 (3)RifM5(2)	RifM5	tri_RifM5_Insert_N-Term	GCATCCGCGAGCTGGAATCCGAGCCGATCGCCATCGTCG	pAJ9
		tri_RifM5_Insert_C-Term	GCCTCCCCCCGGACCTCGGTACCGAGTTCGTCGCGCAGGT	
	(3)pET28(2)	tri_RifM5_Vector_N-Term	ACCTGCGCGACGAACTCGGTACCGAGGTCCGGGGGGAG	pBL36
		tri_RifM5_Vector_C-Term	CGACGATGGCGATCGGCTCGGATTCCAGCTCGCGGATGC	
pAJ45 (2)RifM6(1)	RifM6	tri_RifM6_Insert_N-Term	GCATCCGCGAGCTGGAATCCGAGCCGATCGCCATCGTCG	pAJ10

		tri_RifM6_Insert_C-Term	GCCTCCCCCCGGACCTCGGTGACCAGCCGCGCGCC	
	(3)pET28(2)	tri_RifM6_Vector_N-Term	ACCTGGGCGCGCGGCTGGTCACCGAGGTCCGGGGGGAG	pBL36
		tri_RifM6_Vector_C-Term	CGACGATGGCGATCGGCTCGGATTCCAGCTCGCGGATGC	
pAJ46 (2)RifM7(1)	RifM7	tri_RifM7_Insert_N-Term	GCATCCGCGAGCTGGAATCCGCGGCCGGCGAGCC	pAJ11
		tri_RifM7_Insert_C-Term	GCCTCCCCCCGGACCTCGGTGGCGAGTTCCGCCCGC	
	(3)pET28(2)	tri_RifM7_Vector_N-Term	CCTGCGGGCGGAACTCGCCACCGAGGTCCGGGGGGAG	pBL36
		tri_RifM7_Vector_C-Term	GATCGGCTCGCCGGCCGCGGATTCCAGCTCGCGGATGC	
pAJ52 (2)RapM4(1)	RapM4	tri_RapM4_Insert_N-Term	GCATCCGCGAGCTGGAATCCGAGCCGTTGGCGATTGTGGG	pAJ16
		tri_RapM4_Insert_C-Term	GCCTCCCCCCGGACCTCGGTCAACTCGTCCAGCCGGGCG	
	(3)pET28(2)	tri_RapM4_Vector_N-Term	CGCCCGGCTGGACGAGTTGACCGAGGTCCGGGGGGAG	pBL36
		tri_RapM4_Vector_C-Term	CCCACAATCGCCAACGGCTCGGATTCCAGCTCGCGGATGC	
pAJ54 (2)RapM6(1)	RapM6	tri_RapM6_Insert_N-Term	GCATCCGCGAGCTGGAATCCGAGCCGCTGGCGATCGTG	pAJ18
		tri_RapM6_Insert_C-Term	GGCTGCTCGGTTGGACGAGTTGACCGAGGTCCGGGGGGAG	
	(3)pET28(2)	tri_RapM6_Vector_N-Term	GCCTCCCCCCGGACCTCGGTCAACTCGTCCAACCGAGCAGCC	pBL36
		tri_RapM6_Vector_C-Term	CACGATCGCCAGCGGCTCGGATTCCAGCTCGCGGATGC	

Table S4. Plasmid design and primers for single amino acid exchanges in PKS modules

Plasmid Encoded Protein	Cloning Method/ Source	Primer Name	Primer Sequence	Primer Purpose	PCR Template
pMK2 (3)DEBSM6+TE-S1577A	Site-directed mutagenesis	TE-knockout.FOR	GTGGCCGGTCACGC GGCGGGGGCACTG	TE active site knockout (FOR)	pBL18
		TE-knockout.REV	CAGTGCCCCCGCCGC GTGACCGGCCAC	TE active site knockout (REV)	
pMK5 (3)RifM2+TE-S1129A	Site-directed mutagenesis	TE-knockout.FOR	GTGGCCGGTCACGC GGCGGGGGCACTG	TE active site knockout (FOR)	pAJ21
		TE-knockout.REV	CAGTGCCCCCGCCGC GTGACCGGCCAC	TE active site knockout (REV)	
pMK9 (3)DEBSM3+TE-S1608A	Restriction site based mutagenesis	pRSG34_EcoRI.REV	GTCGACGGAGCTCG AATTCCCTCCGCCCA GCCAGGC	amplification of restriction sites in TE-mutants (REV)	pRSG34
		pRSG34_MauBI.FOR	CTGGCCGAGCACCA CCGCGCGCGGGGCC GCTCG	amplification of restriction sites in pRSG34 (FOR)	
		TE-knockout.FOR	GTGGCCGGTCACGC GGCGGGGGCACTG	TE active site knockout (FOR)	
		TE-knockout.REV	CAGTGCCCCCGCCGC GTGACCGGCCAC	TE active site knockout (REV)	
pMK10 (3)RAPM4+TE-S1801A	Restriction site based mutagenesis	pAJ32_BstBI.FOR	AGCGACGGCCGCTT CGAAGCGCCACGAC TGACC	amplification of restriction sites in pAJ32 (FOR)	pAJ32
		pRSG34_EcoRI.REV	GTCGACGGAGCTCG AATTCCCTCCGCCCA GCCAGGC	amplification of restriction sites in TE-mutants (REV)	
		TE-knockout.FOR	GTGGCCGGTCACGC GGCGGGGGCACTG	TE active site knockout (FOR)	
		TE-knockout.REV	CAGTGCCCCCGCCGC GTGACCGGCCAC	TE active site knockout (REV)	
pMK11 (3)RAPM6+TE-S1789A	Restriction site based mutagenesis	pAJ34_BamHI.FOR	ATGGCCGCGTCGCTG GATCCGGCACGGGC CGCGGAG	amplification of restriction sites in pAJ34 (FOR)	pAJ34
		pRSG34_EcoRI.REV	GTCGACGGAGCTCG AATTCCCTCCGCCCA GCCAGGC	amplification of restriction sites in TE-mutants (REV)	
		TE-knockout.FOR	GTGGCCGGTCACGC GGCGGGGGCACTG	TE active site knockout (FOR)	

		TE-knockout.REV	CAGTGCCCCCGCCGCGTGACCGGCCAC	TE active site knockout (REV)	
pMK8(5)DEBSM1(2)-E1424K	Restriction site based mutagenesis	pBL13_BstBI.FOR	GTCGCGACGGGGCCGTTCGAACGCGTCCGCAACGC	amplification of restriction sites in pBL13 (FOR)	pBL13
		pBL13_EcoRI.REV	GTCGACGGAGCTCGAATTCGGATCGCCGTCGAGCT	amplification of restriction sites in pBL13 (REV)	
		pBL13_E1424K_DEBSM1ACP.FOR	AAAGCGCTGTTCAAACTCGTGCGCTCG	DEBSACP1 possible chain translocation variant E1424K (FOR)	
		pBL13_E1424K_DEBSM1ACP.REV	CGAGCGCACGAGTTTGAACAGCGCTTT	DEBSACP1 possible chain translocation variant E1424K (REV)	
pBL60(3)DEBSM2+TE-S1598A	Site-Directed Mutagenesis	pBL60_QC_fwd	GTGGCCGGTCACGCGGCGGGGGCACTG	TE active site knockout (FOR)	pBL16
		pBL60_QC_rev	CAGTGCCCCCGCCGCGTGACCGGCCAC	TE active site knockout (REV)	

Table S5. Plasmid design and primers for KR substitution in DEBS M1

Plasmid Encoded Protein	**Cloning Method/ Source**	**PCR Fragment**	**Primer Name**	**Primer Sequence**	**PCR Template**
pMPO25 DEBS (5)M1-KR2(2)	Infusion	KR 2 insert	KR_2 fw	GTTTCCGCGCTGCGCTGGTTCTACCGGGTCGACTGG	pBL16
			KR_2 rv	TTCGGCCGCCGCCTGCGGCGCGGTCGTGACGAT	
		(5)pET 21(2)	BL13_backbone fw	CAGGCGGCGGCCGAACCG	pBL13
			BL13_backbone rv	GCGCAGCGCGGAAACCTCGT	

Table S6. Specific activities of bimodular chimeric PKSs. Real values for bars presented in Figure 6A.[b]

Acceptor module	specific activity [nmol/min/mg total protein]
no Module+TE	0.21 ± 0.09
DEBS M2+TE	11.61 ± 0.77
DEBS M3+TE	0.26 ± 0.05
DEBS M5+TE	0.26 ± 0.05
DEBS M6+TE	1.36 ± 0.12
RIFS M1+TE	0.09 ± 0.02
RIFS M2+TE	0.25 ± 0.05
RIFS M3+TE	0.09 ± 0.04
RIFS M5+TE	0.12 ± 0.06
RIFS M6+TE	0.06 ± 0.03
RIFS M7+TE	0.07 ± 0.02
RAPS M4+TE	0.07 ± 0.02
RAPS M6+TE	0.12 ± 0.04

[b] All initial rate data was obtained at individual PKS protein concentrations of 4 μM and non-limiting concentrations of propionyl-CoA, methylmalonyl-CoA, and NADPH. In assays containing RIFS M2+TE, malonyl-CoA was also included because this module prefers malonyl extender units, although exclusion of malonyl-CoA did not affect the turnover rate of this system. Error bars indicate averages of two measurements (each performed in triplicate).

Table S7. Specific activities of trimodular chimeric PKSs. Real values for bars presented in Figure 6B.[c]

Acceptor module	specific activity [nmol/min/mg total protein]
no Module	0.17 ± 0.03
DEBS M2	1.49 ± 0.02
RIFS M1	0.10 ± 0.00
RIFS M2	0.23 ± 0.01
RIFS M3	0.16 ± 0.01
RIFS M5	0.12 ± 0.02
RIFS M6	0.10 ± 0.01
RIFS M7	0.09 ± 0.00
RAPS M4	0.07 ± 0.01
RAPS M6	0.14 ± 0.02

[c]All initial rate data was obtained at individual PKS protein concentrations of 4 μM and non-limiting concentrations of propionyl-CoA, methylmalonyl-CoA, and NADPH. In assays containing RIFS M2+TE, malonyl-CoA was also included because this module prefers malonyl extender units, although exclusion of malonyl-CoA did not affect the turnover rate of this system. Error bars indicate averages of two measurements (each performed in triplicate).

Table S8. Specific activities of bimodular chimeric PKSs with either DEBS M1 or DEBS M1-KR2 as the upstream module. Real values for bars presented in Figure 9B.[d]

Acceptor module	specific activity with DEBS M1 [nmol/min/mg total protein]	specific activity with DEBS M1-KR2 [nmol/min/mg total protein]
no Module+TE	0.21 ± 0.09	0.21 ± 0.09
DEBS M2+TE	12.15 ± 0.29	1.47 ± 0.02
DEBS M3+TE	0.30 ± 0.03	0.27 ± 0.01
DEBS M5+TE	0.26 ± 0.00	0.20 ± 0.00
DEBS M6+TE	1.23 ± 0.10	1.70 ± 0.04
RIFS M2+TE	0.21 ± 0.10	0.25 ± 0.01
RIFS M5+TE	0.22 ± 0.05	0.12 ± 0.02
RAPS M6+TE	0.19 ± 0.07	0.13 ± 0.02

[d] All initial rate data was obtained at individual PKS protein concentrations of 4 μM and non-limiting concentrations of propionyl-CoA, methylmalonyl-CoA (and malonyl-CoA, in the case of RIFS M2+TE), and NADPH. Errors indicate averages of three measurements.

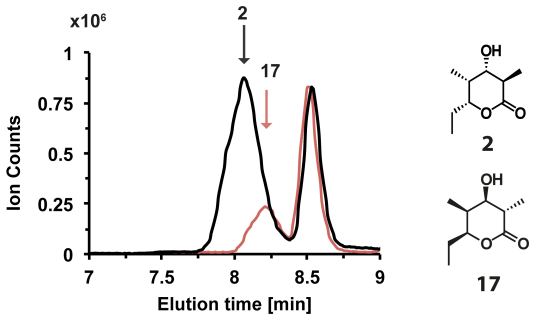

Figure S1. LC-MS analysis of diastereomeric triketide products. Diastereomeric triketide lactones **2** and **17** ($C_9H_{16}O_3$, calculated MW 172.110) are distinguishable by LC-MS analysis. Lactone **2** was detected in reaction mixtures containing the reference module (DEBS (3)M2+TE), while lactone **17** was produced by self-priming of DEBS3 with methylmalonyl-CoA (1). The extracted ion chromatograms, obtained by extraction of the $[M+Na]^+$ species, are shown.

REFERENCES

1. Jacobsen, J. R., Cane, D. E., and Khosla, C. (1998) Spontaneous Priming of a Downstream Module in 6-Deoxyerythronolide B Synthase Leads to Polyketide Biosynthesis. *Biochemistry*. **37**, 4928–4234