Electronic Supplementary Material (ESI) for Medicinal Chemistry Communications

Supplementary material

Discrete acyltransferases involved in polyketide biosynthesis

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Contents

I Abbreviations

The following abbreviations are used in the publication and supporting information:

A	adenylation
ACP	acyl carrier protein
ADH	acyl-dehydrogenase
AL	acyl-AMP ligase
AMT	aminotransferase
AT	acyltransferase
B	unknown domain
C	condensation
CoA	coenzyme A
CP	carrier protein
Cy	cyclization
D	docking domain
DH	dehydratase
E	epimerization
ECH	enoyl-CoA dehydratase
ER	enoylreductase
F	formylation
FkbH*	FkbH homolog
GNAT	*N*-acetyl transferase
GT	glycosyltransferase
HADH	3-hydroxyacyl-CoA dehydrogenase
HCS	HMG-CoA synthase homolog
HMGS	3-hydroxy-3-methylglutaryl-CoA synthase
KR	ketoreductase
KR^0	inactive ketoreductase
KS	ketosynthase
MT	methyltransferase
NRPS	nonribosomal peptide synthetase
Ox	oxidation/oxidoreductase
PCP	peptidyl carrier protein
PKS	polyketide synthase
Pr	C39A protease
PS	pyran synthase
TE	thioesterase

*FbkH-like enzymes are involved in the biosynthesis of three-carbon loading units from 1,3-bisphosphoglycerate (for example: methoxy- and hydroxymalonate)

II Genetically characterized discrete ATs

1 Related to chapter 2.1: MmpC involved in mupirocin biosynthesis

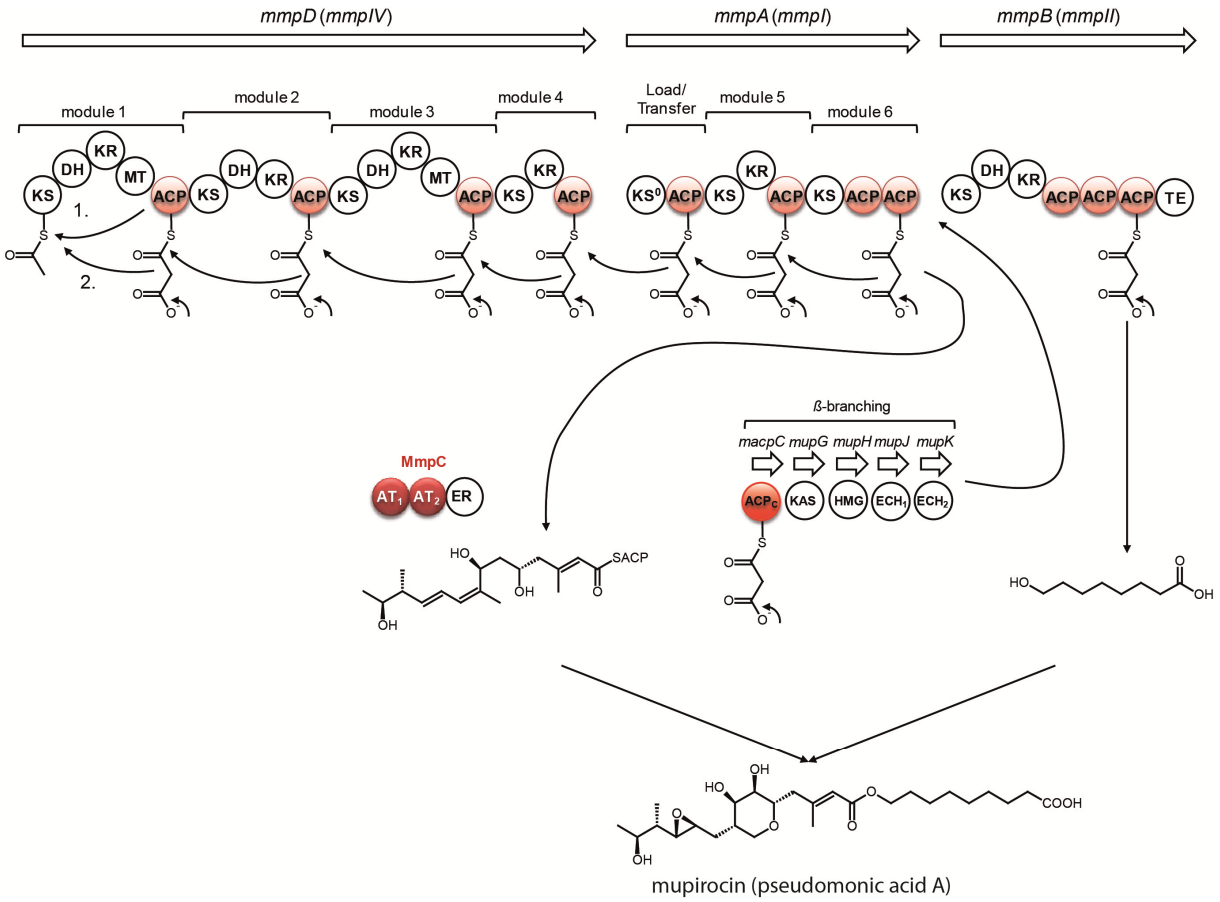

Figure S1: The mupirocin assembly line according to El Sayed et al.[1]

2 Related to chapter 2.2: OzmM and OzmC involved in oxazolomycin biosynthesis

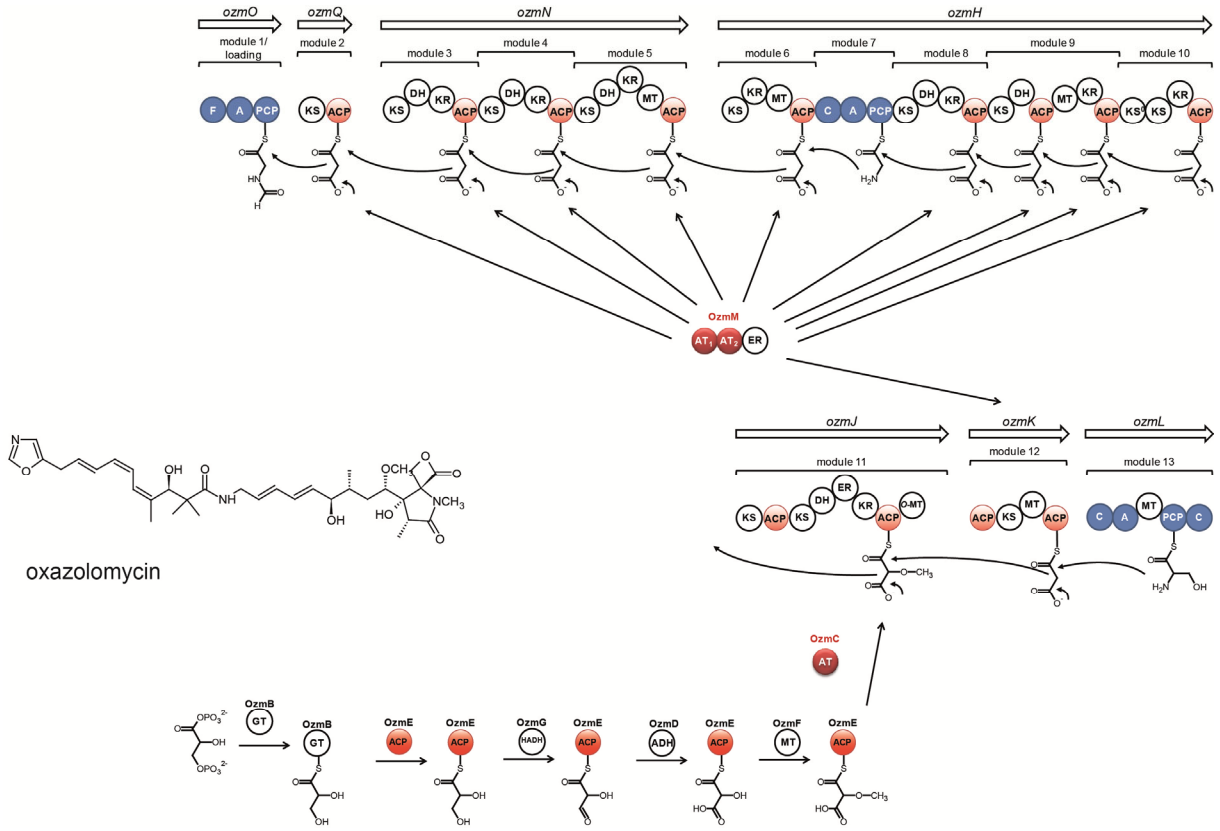

Figure S2: The oxazolomycin assembly line according to Zhao et al.[2] refined with antiSMASH[3] and manual sequence analysis.

3 Related to chapter 2.3: RhiG involved in Rhizoxin biosynthesis

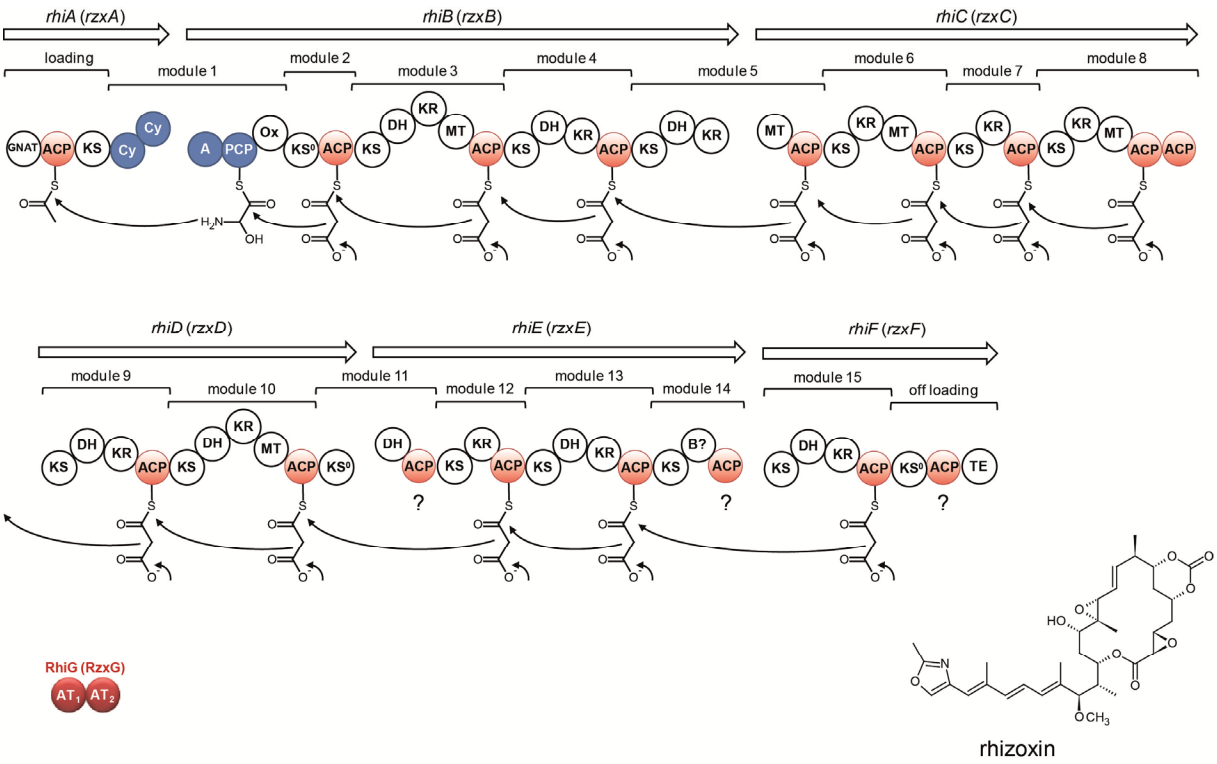

Figure S3: The rhizoxin assembly line according to Partida-Martinez et al.[4] / Piel[5].

4 Related to chapter 2.4: VirI involved in virginiamycin M biosynthesis

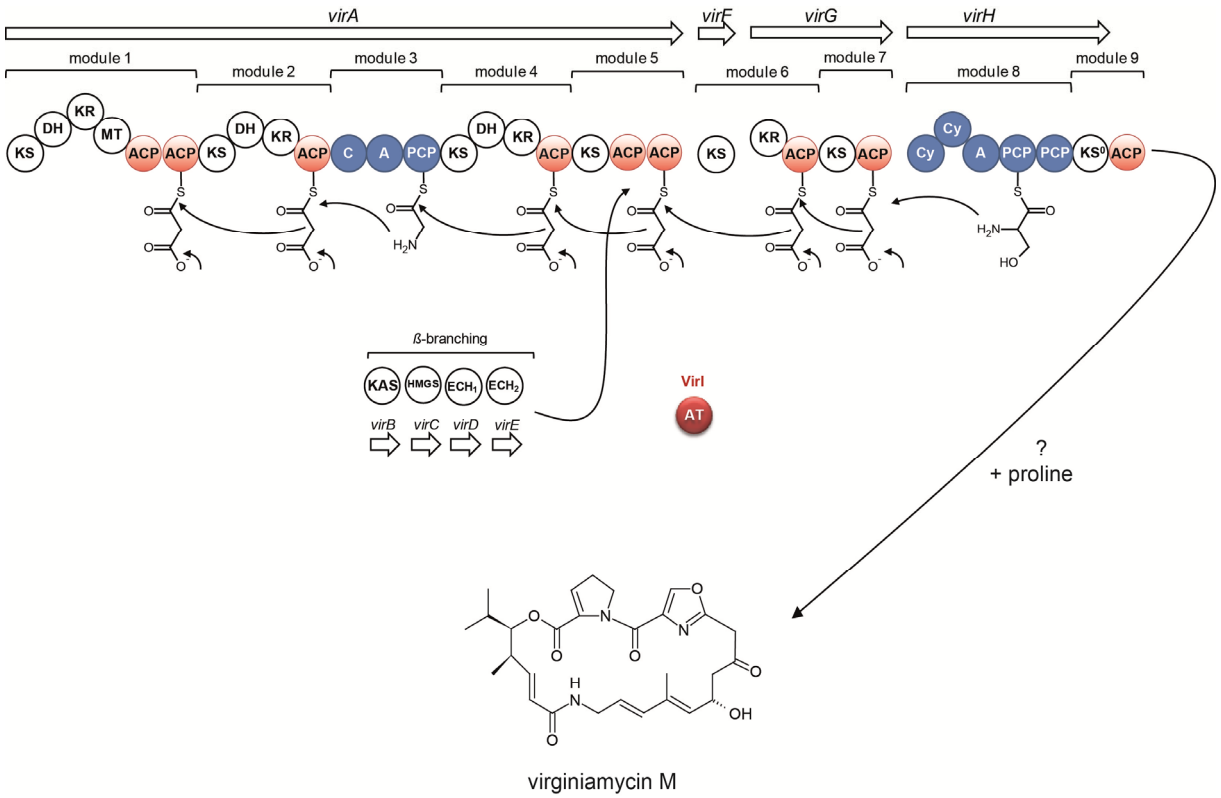

Figure S4: The virginiamycin M assembly line according to Pulsawat et al.[6]

5 Related to chapter 2.5: LkcD involved in lankacidin biosynthesis

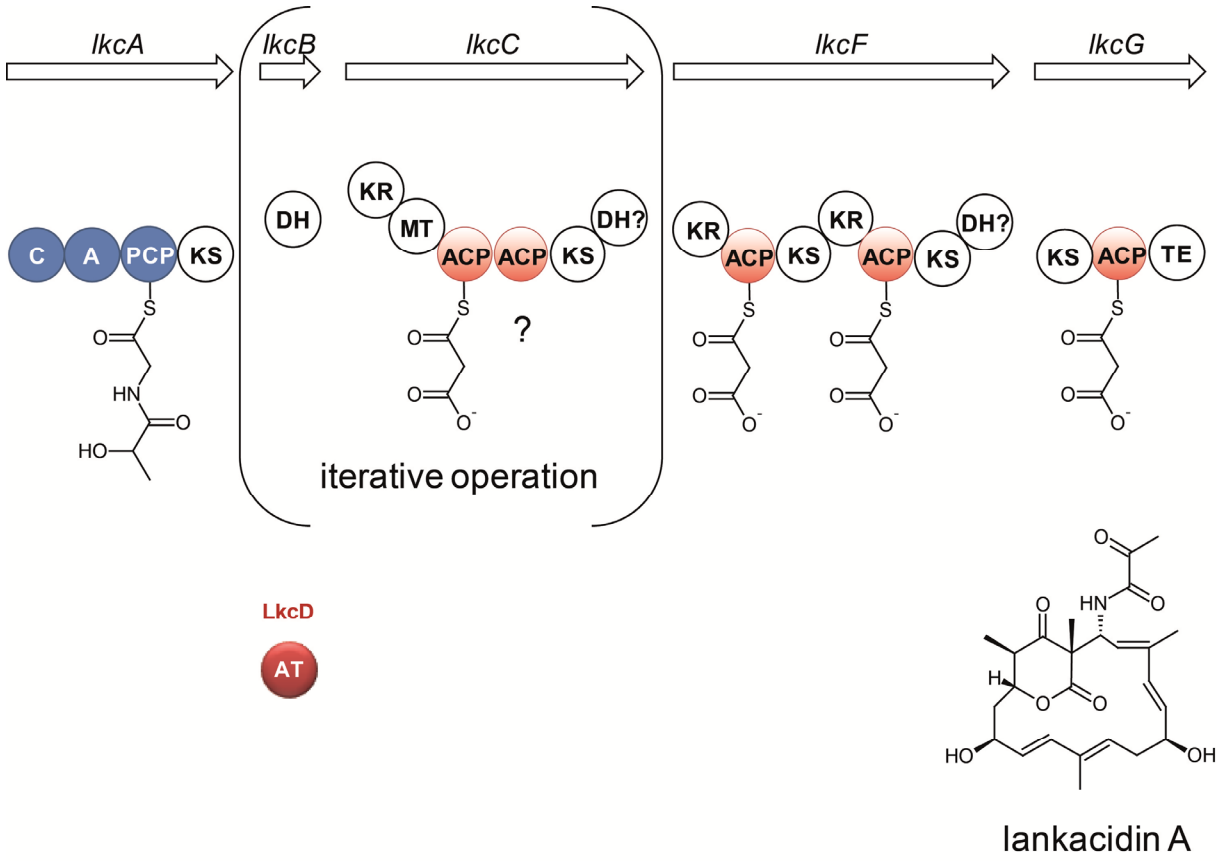

Figure S5: The lankacidin assembly line according to Dickschat al.,[7] revised with antiSMASH.[3]

III Biochemically characterized discrete ATs

1 Related to chapter 3.1: LnmG involved in leinamycin biosynthesis

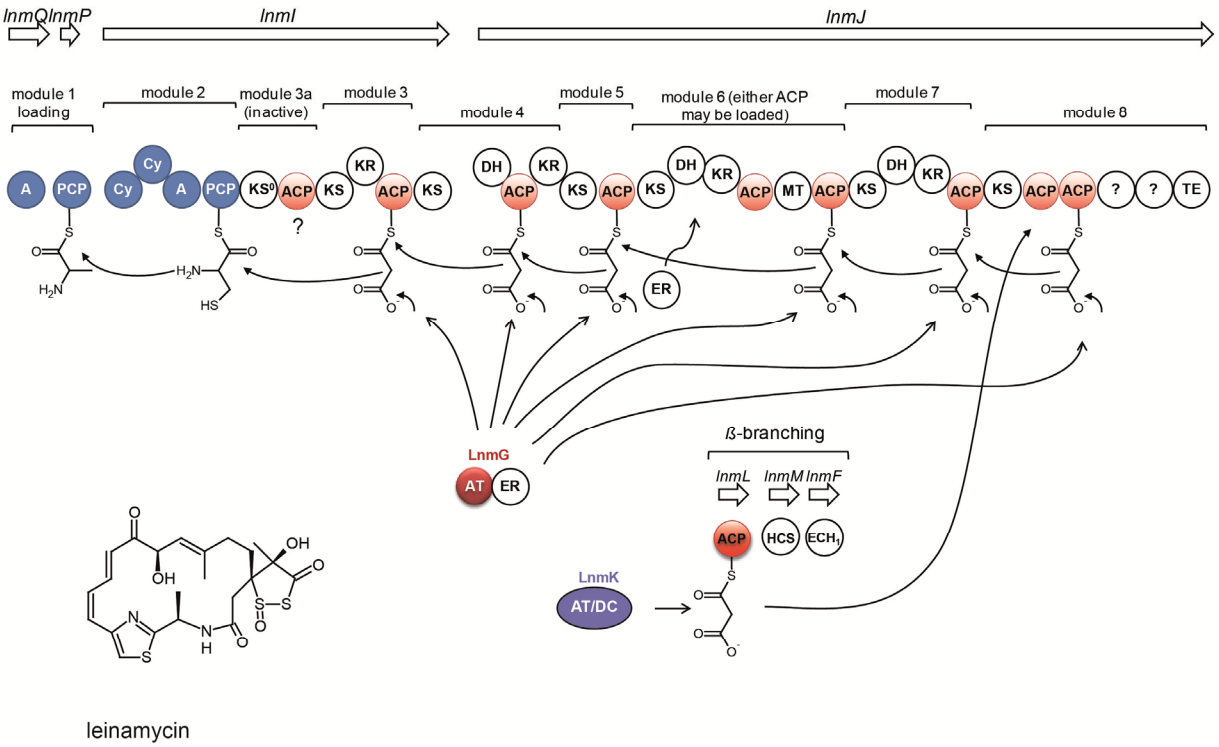

Figure S6: The leinamycin assembly line according to Tang et al.[8]/Piel[5].

Table S1: Substrate specificity of LnmG tested in ACP-loading assays. Based on the study of Shen and coworkers.[9]

Acyltransferase protein	Acyl-CoA substrate	Carrier protein or PKS module	Results
-	[2-^{14}C]malonyl-CoA	holo-LnmP-PCP	no loading
-	[2-^{14}C]malonyl-CoA	holo-LnmI-ACP$_3$	no loading
-	[2-^{14}C]malonyl-CoA	holo-LnmJ-ACP$_4$-holo-LnmJ-ACP$_8$*	no loading
LnmG	[2-^{14}C]malonyl-CoA	-	binding
LnmG	[2-^{14}C]malonyl-CoA	holo-LnmP-PCP	no loading
LnmG	**[2-^{14}C]malonyl-CoA**	**holo-LnmI-ACP$_3$**	**loading (also confirmed by HPLC and ESI-MS)**
LnmG	**[2-^{14}C]malonyl-CoA**	**holo-LnmJ-ACP$_4$-holo-LnmJ-ACP$_8$***	**loading** (also confirmed by HPLC and ESI-MS)**
LnmG	[2-^{14}C]malonyl-CoA	apo-LnmJ-(DH-ACP-KR) (of module 4)	no loading
LnmG	**[2-^{14}C]malonyl-CoA**	**holo-LnmJ-(DH-ACP-KR) (of module 4)**	**loading**

* holo-LnmJ-ACP$_4$-holo-LnmJ-ACP8 comprise: holo-LnmJ-ACP$_4$, holo-LnmJ-ACP$_5$, holo-LnmJ-ACP$_{6\text{-}1}$, holo-LnmJ-ACP$_{6\text{-}2}$, LnmJ-ACP$_7$, LnmJ-ACP$_8$. The carrier proteins (CPs) are numbered consecutively according to the module position in the leinamycin NRPS/PKS hybrid. Module 6 contains two ACPs. To distinguish between this CPs, they were termed LnmJ-ACP$_{6\text{-}1}$ and LnmJ-ACP$_{6\text{-}2}$.
** The loading on LnmJ-ACP$_{6\text{-}1}$ by LnmG with malonate occurred with lower efficiency, compared to the LnmJ-ACP$_{6\text{-}2}$.
Those reactions, which confirm the proposed carrier protein loading by LnmG in the model for leinamycin biosynthesis, are indicated with red color.

2 Related to chapter 3.2: LnmK a bifunctional acyltransferase/ decarboxylase involved in precursor production for polyketide biosynthesis

Table S2: Substrate specificity of LnmG tested in ACP-loading assays. Based on the study of Liu et al.[10]

Acyltransferase protein	Acyl-CoA substrate	Carrier protein or PKS module	Results
LnmK	[1,3-^{14}C$_2$]methylmalonyl-CoA	-	binding
LnmK	[^{14}C]acetyl-CoA	holo-LnmL	no loading
LnmK	[1,3-^{14}C$_2$]malonyl-CoA	holo-LnmL	no loading
LnmK	**[1,3-^{14}C$_2$] methylmalonyl-CoA**	**holo-LnmL**	**loading**
LnmK	**methylmalonyl-CoA (HPLC and ESI-MS analysis)**	**holo-LnmL**	**loading (identified product: propionyl-*S*-LnmL)**
LnmK	[^{14}C]propionyl-CoA	holo-LnmL	no loading

Those reactions, which confirm the predicted steps in leinamycin biosynthesis, are indicated with red color.

3 Related to chapter 3.3: PksC (BaeC) and PksE (BaeE) involved in bacillaene biosynthesis

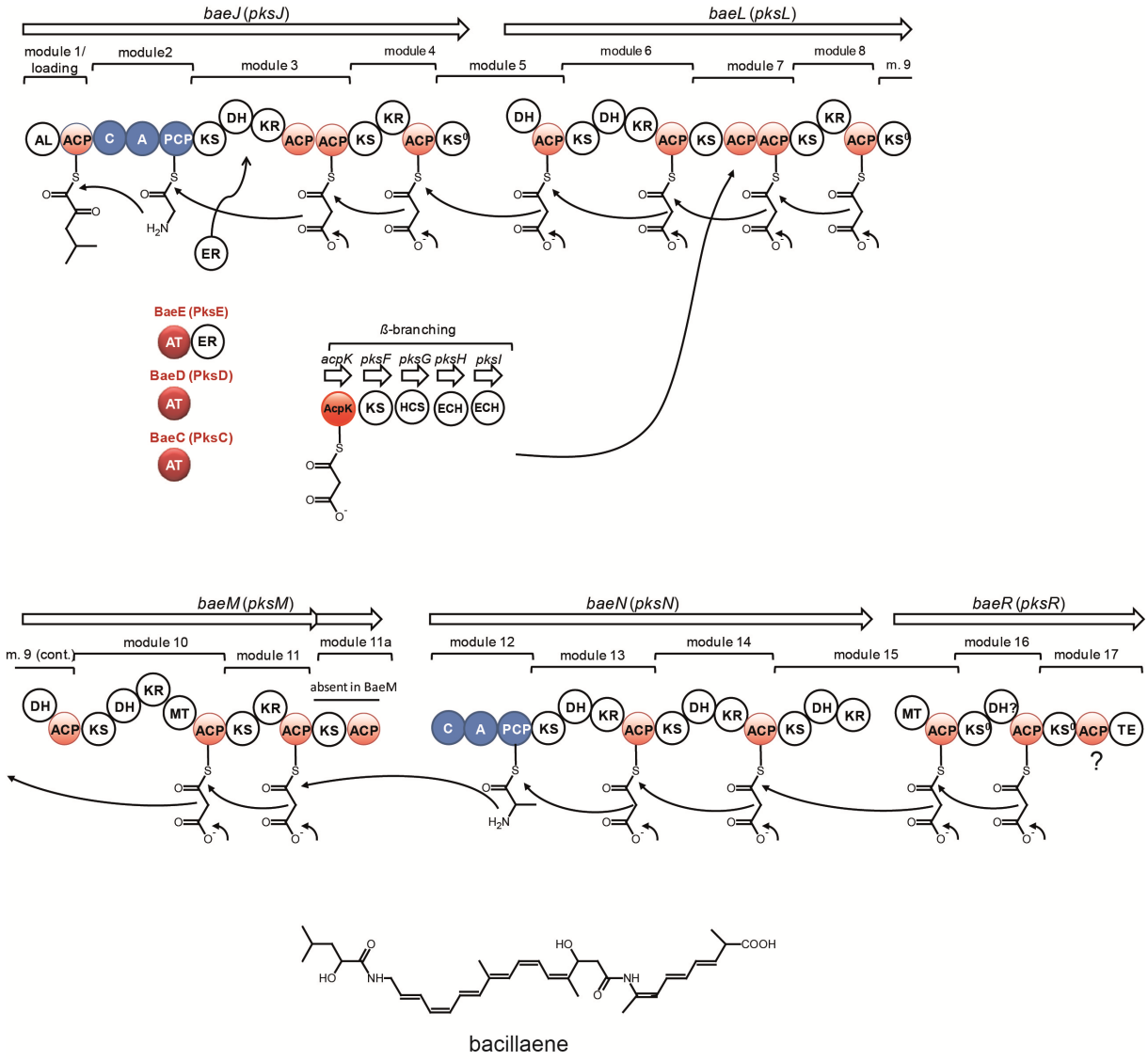

Figure S7: The bacillaene assembly line according to Butcher et al.[11] and Piel[5].

Table S3: Substrate specificity of PksC and PksE tested in ACP-loading assays. Based on the study of Calderone et al. and Bumpus et al.[12, 13]

Acyltransferase protein	Acyl-CoA substrate	Carrier protein or PKS module	Results
PksC	malonyl-CoA	-	binding
PksC	acetyl-CoA	-	no binding
PksC	methylmalonyl-CoA	-	no binding
PksC	**malonyl-CoA**	**holo-AcpK**	**loading** product: Mal-*S*-AcpK (loading rate shown by radioactivity assays: 4.2 µMxmin^{-1})
PksC	**[2-^{14}C]malonyl-CoA**	**holo-PksJ-ACP-ACP**	**loading**
PksC	**[2-^{14}C]malonyl-CoA**	**holo-PksL-ACP-ACP**	**loading**
PksE*	[2-^{14}C]malonyl-CoA	holo-PksJ-ACP-ACP	loading
PksE*	[2-^{14}C]malonyl-CoA	holo-PksL-ACP-ACP	loading
PksE*	[2-^{14}C]malonyl-CoA	holo-AcpK	loading

* PksE consists of an AT and ER domain. The enoylreductase activity in bacillaene biosynthesis was confirmed by Bumpus et al.[12]

Those reactions, which confirm the proposed carrier protein loading by PksC in the model for bacillaene biosynthesis, are indicated with red color.

4 Related to chapter 3.4: TaV involved in myxoviresicin biosynthesis

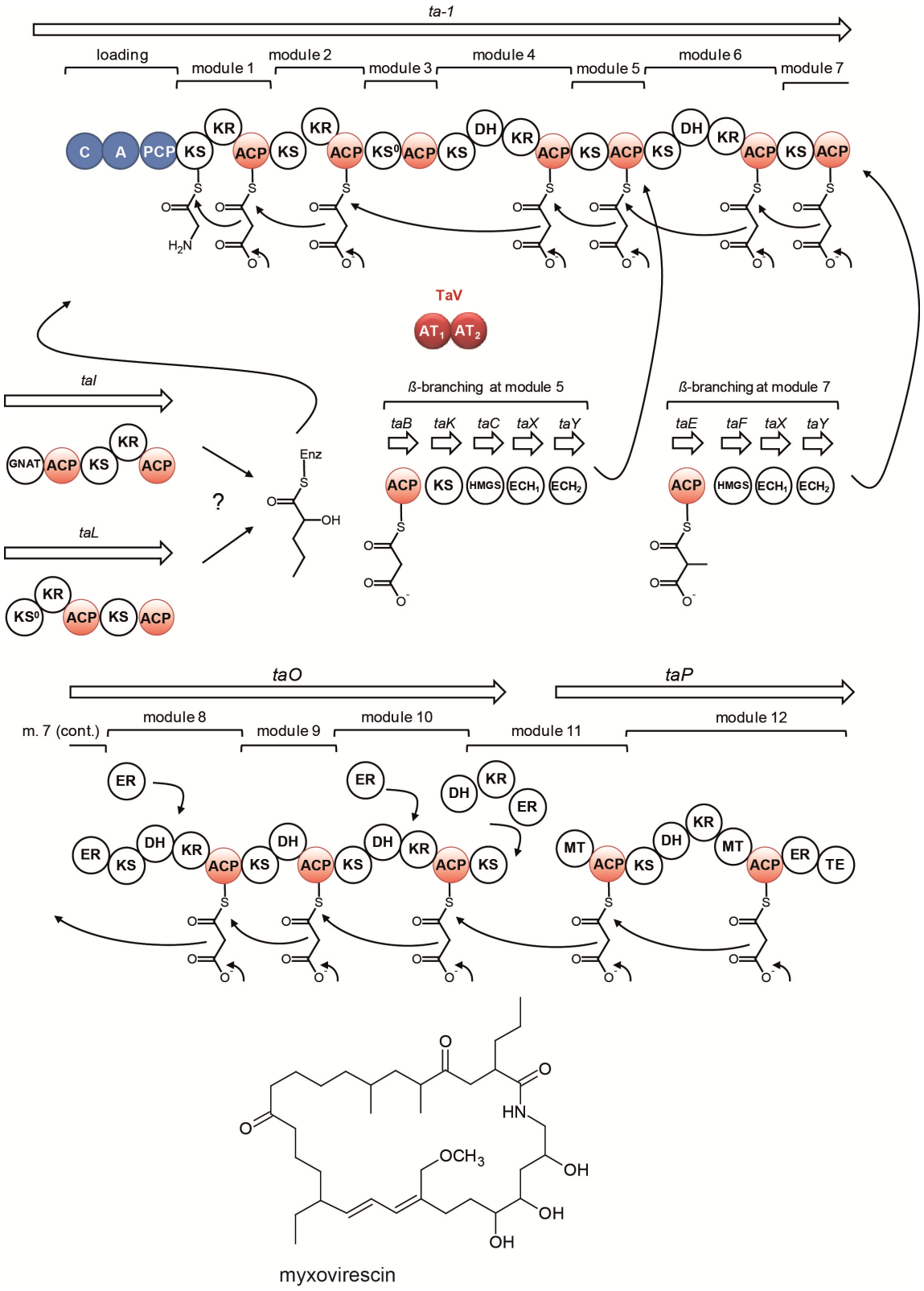

Figure S8: The myxovirescin assembly line according to Simunovic et al.[14]/Piel[5].

Table S4: Substrate specificity of TaV tested in ACP-loading assays. Based on the study of Calderone et al.[15]

Acyltransferase protein	Acyl-CoA substrate	Carrier protein or PKS module	Results
TaV-AT_2*	[^{14}C]acetyl-CoA	holo-TaB	no loading
TaV-AT_2*	**([^{14}C])malonyl-CoA**	**holo-TaB**	**loading** (selective loading of malonate was confirmed by mass spectrometry using a mixture of acyl-CoA substrates)
TaV-AT_2*	[^{14}C]methylmalonyl-CoA	holo-TaB	no loading
TaV-AT_2*	[^{14}C]propionyl-CoA	holo-TaB	no loading
TaV-AT_2*	[^{14}C]acetyl-CoA	holo-TaE	no loading
TaV-AT_2*	**[^{14}C]malonyl-CoA**	**holo-TaE**	**loading** (at higher malonyl-CoA concentrations)
TaV-AT_2*	[^{14}C]methylmalonyl-CoA	holo-TaE	no loading
TaV-AT_2*	[^{14}C]propionyl-CoA	holo-TaE	no loading
TaV-AT_2*	**([^{14}C])malonyl-CoA**	**holo-Ta1-ACP_5** (published as holo-Ta1-T6)	**loading** (selective loading of malonate was confirmed by mass spectrometry using a mixture of acyl-CoA substrates)
TaV-AT_2*	**([^{14}C])malonyl-CoA**	**holo-Ta1-ACP_6** (published as holo-Ta1-T7)	**loading** (selective loading of malonate was confirmed by mass spectrometry using a mixture of acyl-CoA substrates)
TaV-AT_2*	**([^{14}C])malonyl-CoA**	**holo-Ta1-ACP_7** (published as holo-Ta1-T8)	**loading** (selective loading of malonate was confirmed by mass spectrometry using a mixture of acyl-CoA substrates)

*TaV-AT_2 is the C-terminal domain of the protein TaV and was originally termed TaV_C.[15]
Those reactions, which confirm the proposed carrier protein loading by TaV-AT_2 in the model for myxovirescin biosynthesis, are indicated with red color.

5 Related to chapter 3.5: BryP involved in bryostatin biosynthesis

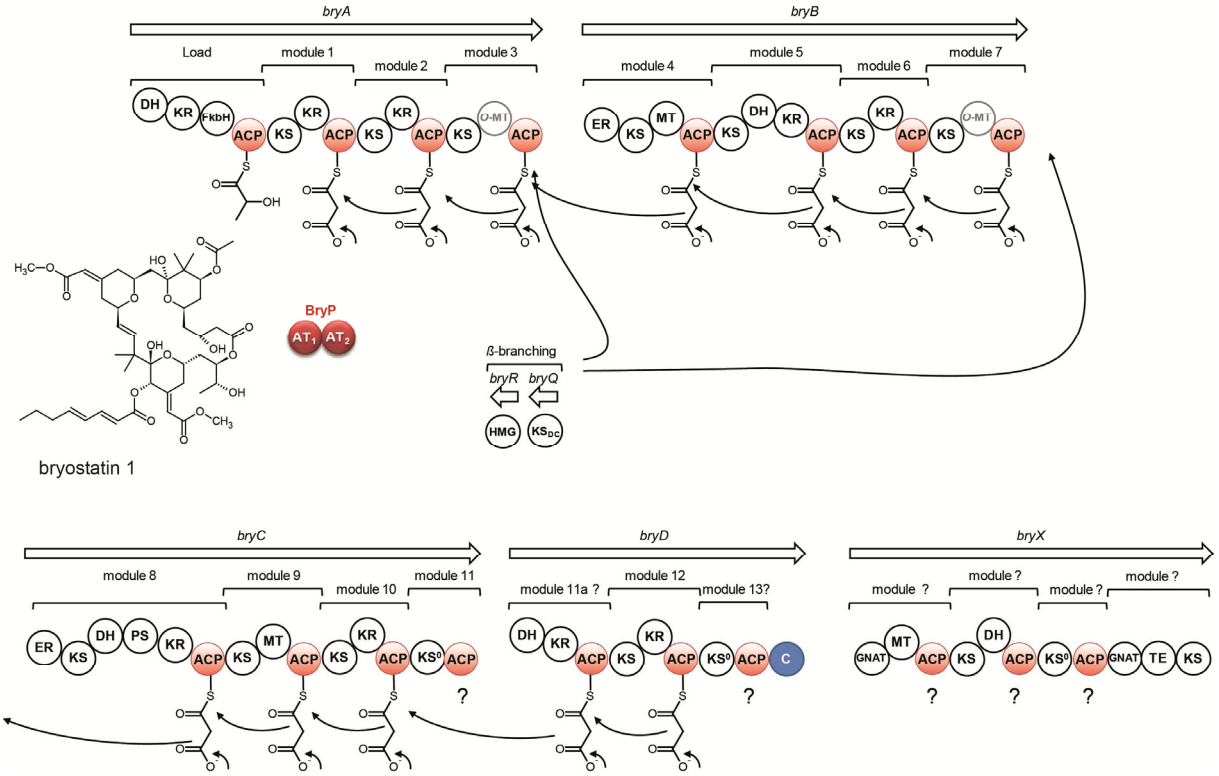

Figure S9: The bryostatin assembly line according to Sudek et al.[16]/Piel.[5]

Table S5: Substrate specificity of BryP tested in ACP-loading assays. Based on the study of Sherman, Thomas and coworkers.[17]

Acyltransferase protein	Acyl-CoA substrate	Carrier protein or PKS module	Results
BryP-AT_1	malonyl-CoA	-	binding
BryP-AT_1	methylmalonyl-CoA	-	binding
-	malonyl-CoA	holo-BryB M3 ACP	no self-loading
-	methylmalonyl-CoA	holo-BryB M3 ACP	no self-loading
BryP-AT_1	**malonyl-CoA**	**holo-BryB M3 ACP**	**loading**
BryP-AT_1	methylmalonyl-CoA	holo-BryB M3 ACP	loading with lower efficiency
BryP-AT_1	**malonyl-CoA**	**holo-BryB M4**	**loading**
BryP-AT_1	methylmalonyl-CoA	holo-BryB M4	no loading
BryP-AT_1AT_2	**malonyl-CoA**	**holo-BryB M4**	**loading**
BryP-AT_1AT_2	methylmalonyl-CoA	holo-BryB M4	no loading
BryP-$AT_1{}^{o}AT_2$	**malonyl-CoA**	**holo-BryB M4**	**loading**
BryP-$AT_1{}^{o}AT_2$	methylmalonyl-CoA	holo-BryB M4	no loading
BryP-$AT_1AT_2{}^{o}$	**malonyl-CoA**	**holo-BryB M4**	**loading**
BryP-$AT_1AT_2{}^{o}$	methylmalonyl-CoA	holo-BryB M4	no loading
BryP-$AT_1{}^{o}AT_2{}^{o}$	malonyl-CoA	holo-BryB M4	no loading
BryP-$AT_1{}^{o}AT_2{}^{o}$	methylmalonyl-CoA	holo-BryB M4	no loading
BryP-AT_2(37)	**malonyl-CoA**	**holo-BryB M4**	**loading**
BryP-AT_2(37)	methylmalonyl-CoA	holo-BryB M4	no loading
-	malonyl-CoA	holo-BryB M7 ACP	no self-loading
-	methylmalonyl-CoA	holo-BryB M7 ACP	no self-loading
BryP-AT_1	**malonyl-CoA**	**holo-BryB M7 ACP**	**loading**
BryP-AT_1	methylmalonyl-CoA	holo-BryB M7 ACP	loading with lower efficiency
BryP-AT_1AT_2	**malonyl-CoA**	**holo-BryB M7 ACP**	**loading**
BryP-AT_1AT_2	methylmalonyl-CoA	holo-BryB M7 ACP	loading with lower efficiency
BryP-$AT_1{}^{o}AT_2$	**malonyl-CoA**	**holo-BryB M7 ACP**	**loading**
BryP-$AT_1AT_2{}^{o}$	**malonyl-CoA**	**holo-BryB M7 ACP**	**loading**
BryP-$AT_1{}^{o}AT_2{}^{o}$	malonyl-CoA	holo-BryB M7 ACP	no loading
BryP-AT_1	malonyl-CoA	holo-CloN5 (PCP)	loading
BryP-AT_1AT_2	malonyl-CoA	holo-CloN5 (PCP)	loading
BryP-$AT_1{}^{o}AT_2$	malonyl-CoA	holo-CloN5 (PCP)	loading
BryP-$AT_1AT_2{}^{o}$	malonyl-CoA	holo-CloN5 (PCP)	loading
BryP-$AT_1{}^{o}AT_2{}^{o}$	malonyl-CoA	holo-CloN5 (PCP)	no loading
BryP-AT_1	malonyl-CoA	holo-PikAIII M5 ACP	loading
BryP-AT_1AT_2	malonyl-CoA	holo-PikAIII M5 ACP	loading
BryP-$AT_1{}^{o}AT_2$	malonyl-CoA	holo-PikAIII M5 ACP	loading
BryP-$AT_1AT_2{}^{o}$	malonyl-CoA	holo-PikAIII M5 ACP	loading
BryP-$AT_1{}^{o}AT_2{}^{o}$	malonyl-CoA	holo-PikAIII M5 ACP	no loading
-	malonyl-CoA	holo-PikAIV M6 WT	no self-loading
-	methylmalonyl-CoA (is the native substrate for PikAIV M6 WT)	holo-PikAIV M6 WT	self-loading
BryP-AT_1	malonyl-CoA	holo-PikAIV M6 AT^{o}	no loading
BryP-AT_1	methylmalonyl-CoA	holo-PikAIV M6 AT^{o}	no loading
-	malonyl-CoA	holo-EryAIII M6 WT	no self-loading
-	methylmalonyl-CoA (is the native substrate for EryAIII M6 WT)	holo-EryAIII M6 WT	self-loading
BryP-AT_1	malonyl-CoA	holo-EryAIII M6 AT^{o}	loading
BryP-AT_1	methylmalonyl-CoA	holo-EryAIII M6 AT^{o}	no loading
BryP-AT_1AT_2	malonyl-CoA	holo-EryAIII M6 AT^{o}	loading

Acyltransferase protein	Acyl-CoA substrate	Carrier protein or PKS module	Results
BryP-AT_1AT_2	methylmalonyl-CoA	holo-EryAIII M6 AT^o	no loading
BryP-$AT_1{}^oAT_2$	malonyl-CoA	holo-EryAIII M6 AT^o	loading
BryP-$AT_1{}^oAT_2$	methylmalonyl-CoA	holo-EryAIII M6 AT^o	no loading
BryP-$AT_1AT_2{}^o$	malonyl-CoA	holo-EryAIII M6 AT^o	loading
BryP-$AT_1AT_2{}^o$	methylmalonyl-CoA	holo-EryAIII M6 AT^o	no loading
BryP-$AT_1{}^oAT_2{}^o$	malonyl-CoA	holo-EryAIII M6 AT^o	no loading
BryP-$AT_1{}^oAT_2{}^o$	methylmalonyl-CoA	holo-EryAIII M6 AT^o	no loading
BryP-AT_2(construct 37)	malonyl-CoA	holo-EryAIII M6 AT^o	loading
BryP-AT_2(construct 37)	methylmalonyl-CoA	holo-EryAIII M6 AT^o	loading with lower efficiency

* In this study different constructs with a variety of start and stop sites into the expression vectors were cloned and tested for the expression of soluble BryP proteins. Some of the selected BryP variants were used in the *in vitro* loading assay. The two enzymes BryP-AT_1AT_2 and BryP-AT_2(construct 37), listed in this table span the residues 1-574 and 294-574, respectively.
BryP-$AT_1{}^oAT_2{}^o$ is the BryP protein where the active site serine residues, S88A in AT_1 and S410A in AT_2, were inactivated by site directed mutagenesis. Accordingly, one of these active site serine residues was inactivated in the mutants BryP-$AT_1{}^oAT_2$ and BryP-$AT_1AT_2{}^o$.
Those reactions, which confirm the proposed carrier protein loading by the AT domains of BryP in the model for bryostatin biosynthesis, are indicated with red color. Lopanik et al. concluded from the results of the *in vitro* assays that the AT_1 of BryP is more active than the AT_2.[17]

6 Related to chapter 3.6: DSZS AT involved in disorazole biosynthesis

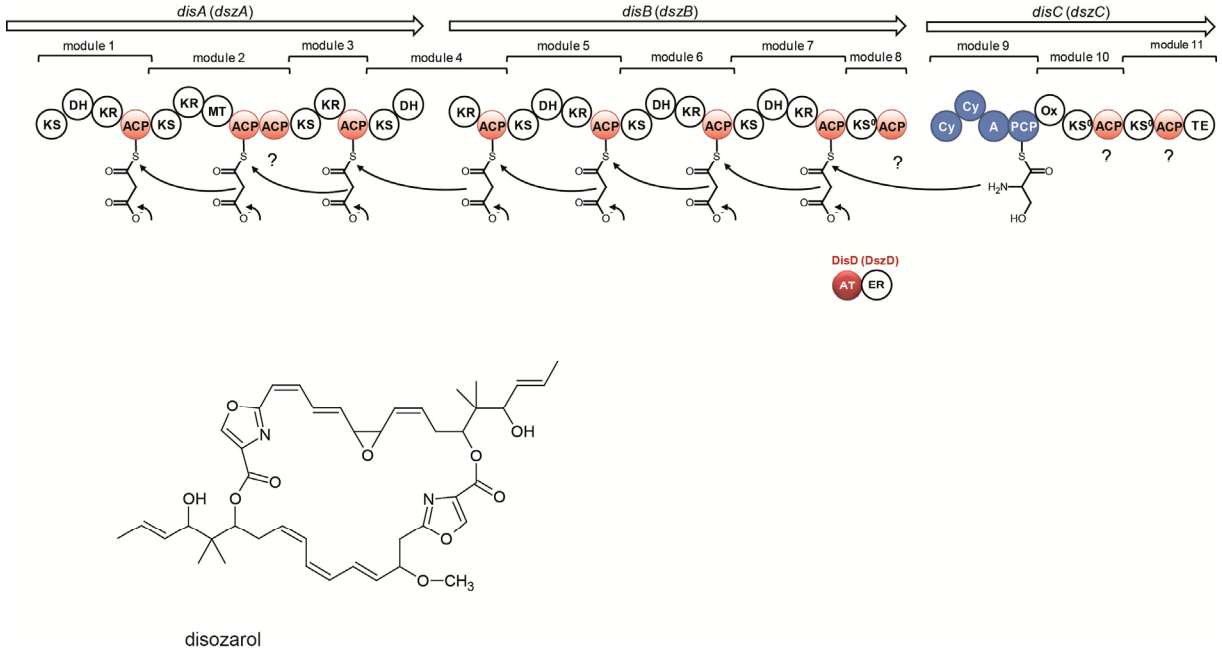

Figure S10: The disorazol assembly line according to Kopp et al.[18]

Table S6: Substrate specificity of the DisD AT domain tested in ACP-loading assays. Based on the study of Khosla, Cane and coworkers.[19, 20]

Acyltransferase protein*	Acyl-CoA substrate	Carrier protein or PKS module	Results
DSZS AT_S	[^{14}C]malonyl-CoA	-	binding
DSZS AT_L	[^{14}C]malonyl-CoA	-	binding
DSZS AT_S*	**[^{14}C]malonyl-CoA**	**holo-DSZS-ACP_1**	**loading** (k_{cat}/K_M= (87±13) x 10^3)
DSZS AT_S	[^{14}C]malonyl-CoA	holo-DEBS-M3+TE**	loading
DSZS AT_S	[^{14}C]malonyl-CoA	holo-DEBS-M6+TE**	loading
DSZS AT_L	[^{14}C]malonyl-CoA	holo-DEBS-M3+TE**	loading
DSZS AT_L	[^{14}C]malonyl-CoA	holo-DEBS-M6+TE**	loading
DSZS AT_S	[^{14}C]methylmalonyl-CoA	holo-DEBS-M3+TE**	no loading
DSZS AT_S	[^{14}C]methylmalonyl-CoA	holo-DEBS-M6+TE**	no loading
DSZS AT_L	[^{14}C]methylmalonyl-CoA	holo-DEBS-M3+TE**	no loading
DSZS AT_L	[^{14}C]methylmalonyl-CoA	holo-DEBS-M6+TE**	no loading

Based on the ACP loading assay, Wong et al. assumed that DSZS AT transacylates extender units onto all ACPs of the disorazole PKSs.[19]

* DisD has a AT-(linker)-ER domain architecture. DSZS AT_L does include the linker regin, whereas DSZS AT_S does not.

**holo-DEBS-M3+TE and holo-DEBS-MS+TE are the activated module 3 and module 6 of DEBS with a thioesterase domain and mutated AT domains. In each module the AT domain was inactivated by replacement of a Ser to Ala residue in the active site.

The reaction, which confirms the ACP loading by the DSZS AT in disorazole biosynthesis, is indicated with red color.

7 Related to chapter 3.7: KirCII involved in kirromycin biosynthesis

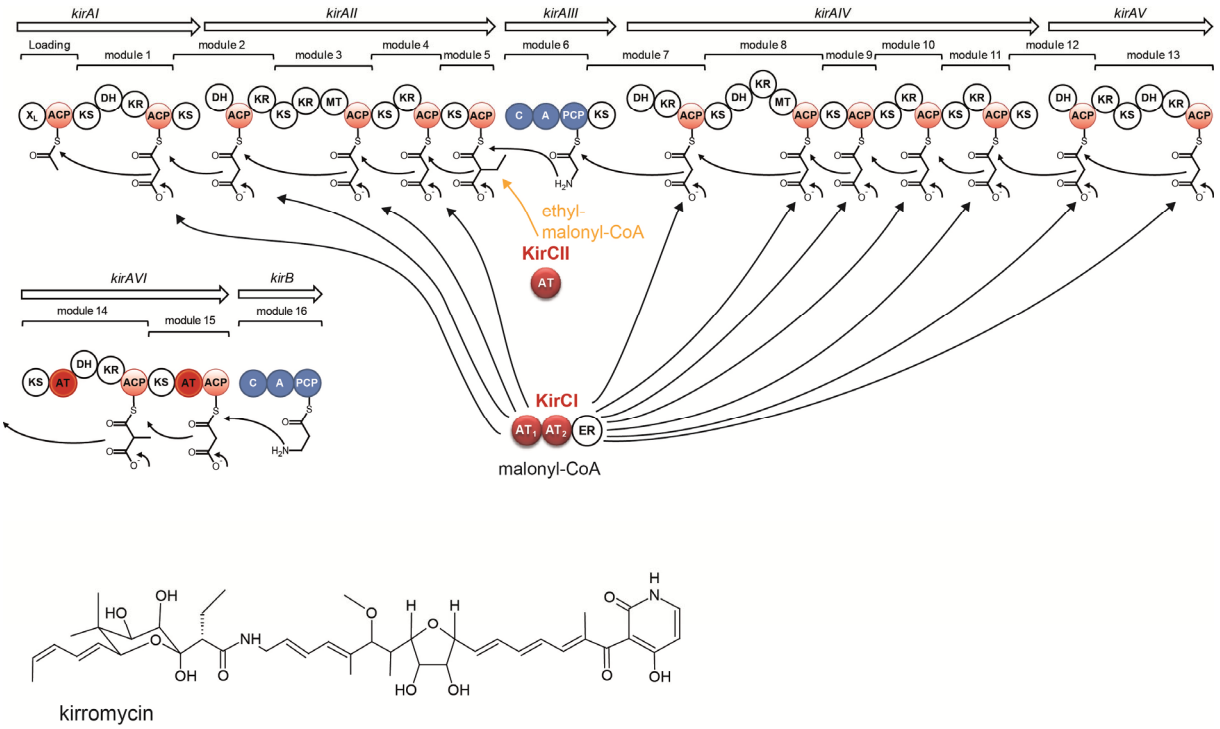

Figure S11: The kirromycin assembly line according to Weber et al.[21]

Table S7: Substrate specificity of KirCII tested in ACP-loading assays. Based on the study of Wohlleben, Weber and coworkers.[22]

Acyltransferase protein	Acyl-CoA substrate	Carrier protein or PKS module	Results
-	malonyl-CoA	holo-ACP_4	no self-loading
-	methylmalonyl-CoA	holo-ACP_4	no self-loading
-	ethymalonyl-CoA	holo-ACP_4	no self-loading
-	malonyl-CoA	holo-ACP_5	no self-loading
-	methymalonyl-CoA	holo-ACP_5	no self-loading
-	ethylmalonyl-CoA	holo-ACP_5	no self-loading
KirCII	malonyl-CoA	holo-ACP_4	no loading
KirCII	methylmalonyl-CoA	holo-ACP_4	no loading
KirCII	ethymalonyl-CoA	holo-ACP_4	no loading*
KirCII	malonyl-CoA	holo-ACP_5	no loading
KirCII	methymalonyl-CoA	holo-ACP_5	no loading
KirCII	**ethylmalonyl-CoA**	**holo-ACP_5**	**loading**

*Small mass peaks of loaded holo-ACP_4 were detected manually. No significant loading was observed.
The ACPs are numbered consecutively according to the module position in the kirromycin PKS/NRPS hybrid.
The reaction, which confirms the selective ACP loading by KirCII in kirromycin biosynthesis, is indicated with red color.

IV Non-canonical PKS/NRPS pathways

1 Related to chapter 4.1: ZmaA involved in zwittermicin biosynthesis

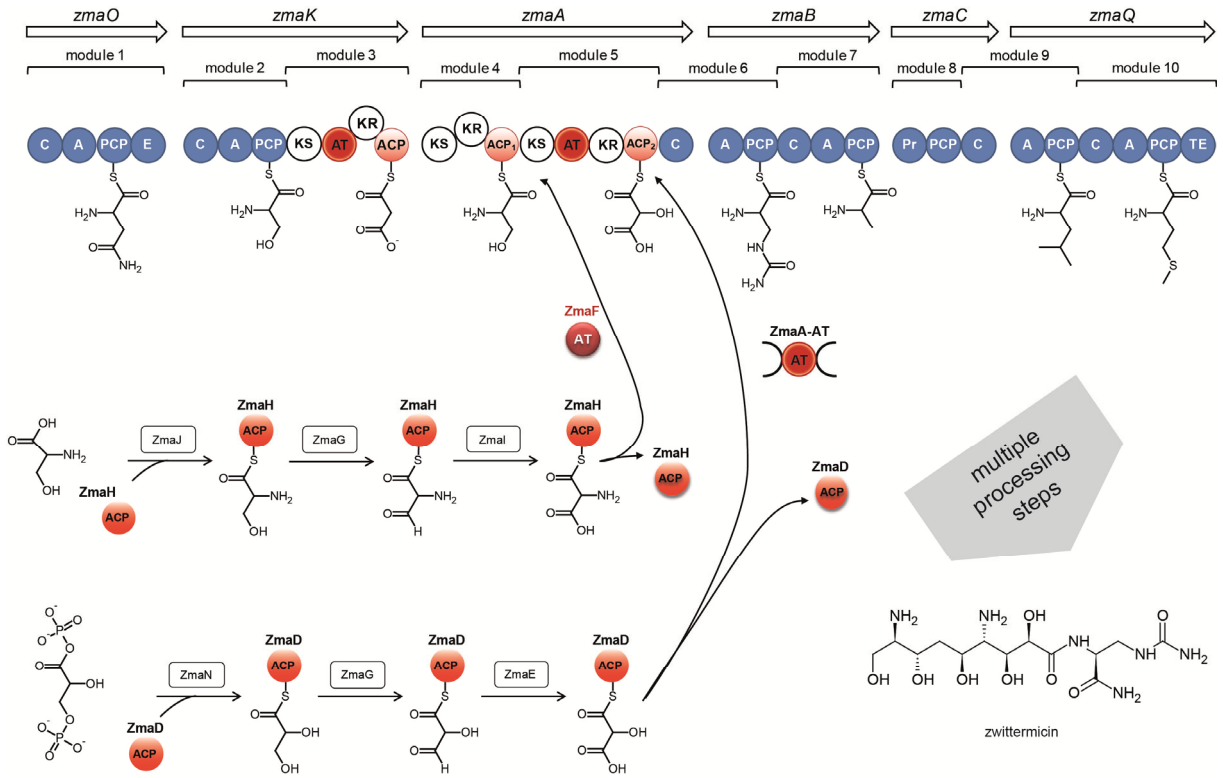

Figure S12: The zwittermicin assembly line according to Kevany et al.,[23] refined with antiSMASH[3] and manual sequence analysis.

Table S8: Substrate specificity of ZmaF and ZmaA tested in ACP-loading assays. Based on the investigations done by Chan and Thomas.[24]

Acyltransferase protein	Acyl-CoA or ACP-linked substrate	Carrier protein or PKS module	Activity	Detected product/ intermediate
ZmaF	[^{14}C]malonyl-CoA	-	no binding	-
ZmaF	[^{14}C]-(2*S*)-methylmalonyl-CoA	-	no binding	-
ZmaF	[^{14}C]-(2*S*)-aminomalonyl-ZmaH (is the preferred substrate)	-	binding	(2*S*)-aminomalonyl-ZmaF
ZmaF	[^{14}C]-(2*S*)-seryl-ZmaH	-	binding (the binding activity of ZmaF depends on ZmaH concentration/no binding at low ZmaH concentrations)	(2*S*)-seryl-ZmaF
ZmaF	(2*S*)-2-amino-3-oxopropionate-ZmaH	-	binding with lower efficiency ("misacylation") (the binding activity of ZmaF depends on ZmaH concentration/no binding at low ZmaH concentrations)	(2*S*)-2-amino-3-oxopropionate-ZmaF
ZmaF	(2*S*)-seryl-ZmaH	holo-ZmaA-ACP$_1$	no loading	-
ZmaF	(2*R*)-hydroxymalonyl-ZmaD	holo-ZmaA-ACP$_1$	no loading	-
ZmaF	(2*R*)-hydroxymalonyl-ZmaD	holo-ZmaA-ACP$_2$	no loading	-
ZmaF	**(2*S*)-aminomalonyl-ZmaH** (is the native substrate for ZmaF)	**holo-ZmaA-ACP$_1$**	**loading**	**(2*R*)-aminomalonyl-ZmaA-ACP$_1$** (also formation of a side-product: glycyl-ZmaA-ACP$_1$, which is the decarboxylated form of (2*S*)-aminomalonyl-ZmaA-ACP$_1$)
ZmaF	(2*S*)-aminomalonyl-ZmaH	holo-ZmaA-ACP$_2$	no loading	-

Acyltransferase protein	**Acyl-CoA or ACP-linked substrate**	**Carrier protein or PKS module**	**Activity**	**Detected product/ intermediate**
ZmaA-AT*	[^{14}C]malonyl-CoA	-	no binding	-
ZmaA-AT*	[^{14}C]-(2*S*)-methylmalonyl-CoA	-	no binding	-
ZmaA-AT*	malonyl-CoA	ZmaA-ACP$_2$	no loading	-
ZmaA-AT*	methylmalonyl-CoA	ZmaA-ACP$_2$	no loading	-
ZmaA-AT*	[^{14}C]-(2*S*)-aminomalonyl-ZmaH	-	binding at 5 μM [^{14}C]-(2*S*)-aminomalonyl-ZmaH (the binding activity of ZmaA-AT depends on ZmaH concentration/ no binding at low ZmaH concentrations (1 μM)	[^{14}C]-(2*S*)-aminomalonyl-ZmaA-AT
ZmaA-AT*	(2*S*)-aminomalonyl-ZmaH	holo-ZmaA-ACP$_1$	no loading	-
ZmaA-AT*	(2*S*)-aminoymalonyl-ZmaH	holo-ZmaA-ACP$_2$	no loading	-
ZmaA-AT*	(2*R*)-hydroxymalonyl-ZmaD	holo-ZmaA-ACP$_1$	no loading	-
ZmaA-AT*	2-hydroxy-3,3-dihydroxypropionyl-ZmaD	-	no binding	-
ZmaA-AT*	**(2*R*)-hydroxymalonyl-ZmaD**	**holo-ZmaA-ACP$_2$**	**loading**	glycyl-ZmaA-ACP$_2$, which is the decarboxylated form of **(2*R*)-hydroxymalonyl-Zma-ACP$_2$**

*The ZmaA-AT domain is not a discrete AT. This domain is a part of a hybrid NRPS/PKS megaenzyme (ZmaA). The sequence encoding ZmaA-AT was excised and cloned onto a vector for expression, purification and analysis of its function.
The reactions, which confirm the proposed selective ACP loading by ZmaF and the cognate ZmaA-AT in the model for zwittermicin biosynthesis, are indicated with red color.

2 Related to chapter 4.2: FenF involved in mycosubtilin biosynthesis

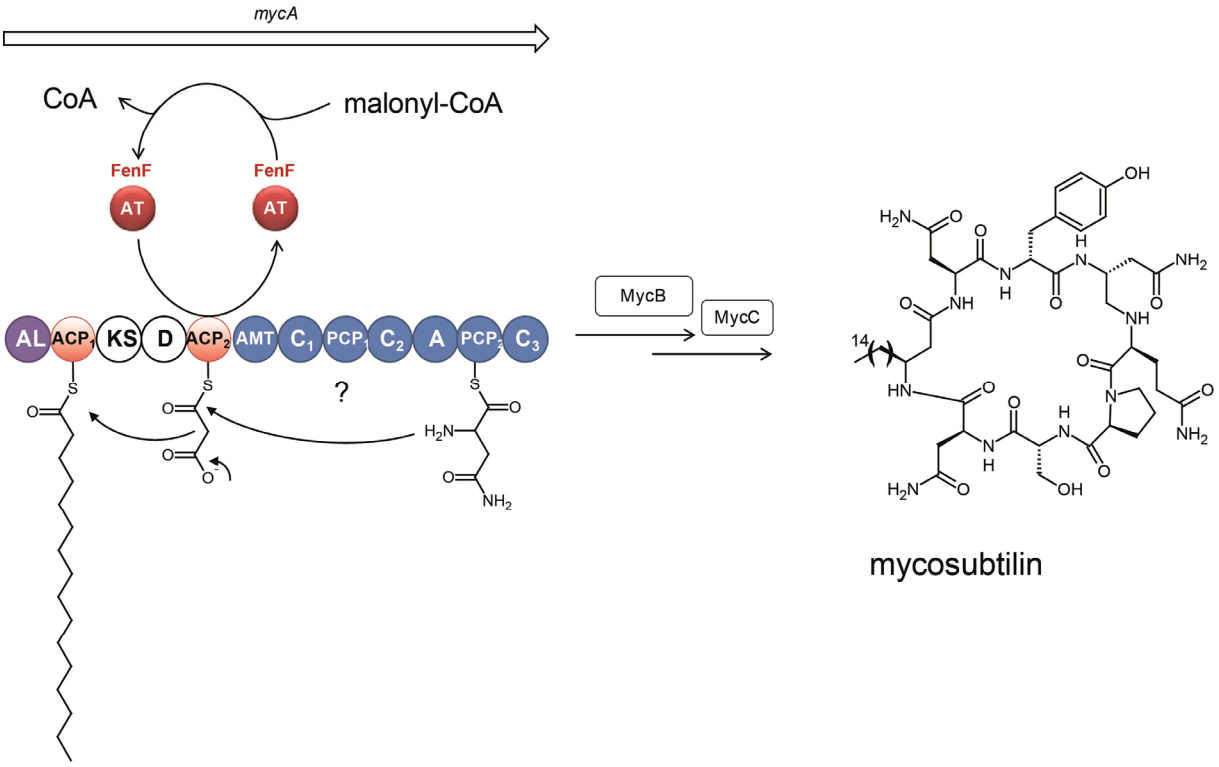

Figure S13: The mycosubtilin assembly line according to Aron et al.[25]

Table S9: Substrate specificity of FenF tested in ACP-loading assays. Based on the results of Walsh and coworkers.[25]

Acyltransferase protein	Acyl-CoA substrate	Carrier protein or PKS module	Activity
FenF	malonyl-CoA	holo-ACP_1	loading (does not comply with the postulated model of mycosubtilin biosynthesis)
FenF	**malonyl-CoA**	**holo-ACP_2**	**loading**
FenF	methylmalonyl-CoA	holo-ACP_2	loading with lower efficiency versus malonyl-CoA
FenF	acetyl-CoA	holo-ACP_2	loading with lower efficiency versus malonyl-CoA
FenF	malonyl-CoA	holo-D-ACP_2	loading
FenF	malonyl-CoA	holo-KS-D-ACP_2	loading
FenF	malonyl-CoA	holo-PCP_1*	loading with low efficiency
FenF	malonyl-CoA	holo-AcpK (from bacillaene biosynthesis)[13, 26]	loading
FenF	malonyl-CoA	holo-PksL-ACP-ACP ($PksL\text{-}T_2$ from bacillaene biosynthesis)**	loading

*The function of PCP_1 is unknown.
The reaction, which confirms the proposed ACP loading by FenF in the model for mycosubtilin biosynthesis, is indicated with red color.

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