The LINKS motif zippers *trans*-acyltransferase polyketide synthase assembly lines into a biosynthetic megacomplex

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ABSTRACT

Polyketides such as the clinically-valuable antibacterial agent mupirocin are constructed by architecturally-sophisticated assembly lines known as *trans*-acyltransferase polyketide synthases. Organelle-sized megacomplexes composed of several copies of *trans*-acyltransferase polyketide synthase assembly lines have been observed by others through transmission electron microscopy to be located at the *Bacillus subtilis* plasma membrane, where the synthesis and export of the antibacterial polyketide bacillaene takes place. In this work we analyze ten crystal structures of *trans*-acyltransferase polyketide synthases ketosynthase domains, seven of which are reported here for the first time, to characterize a motif capable of zippering assembly lines into a megacomplex. While each of the three-helix LINKS (Laterally-INTERacting Ketosynthase Sequence) motifs is observed to similarly dock with a spatially-reversed copy of itself through hydrophobic and ionic interactions, the amino acid sequences of this motif are not conserved. Such a code is appropriate for mediating homotypic contacts between assembly lines to ensure the ordered self-assembly of a noncovalent, yet tightly-knit, enzymatic network. LINKS-mediated lateral interactions would also have the effect of bolstering the vertical association of the polypeptides that comprise a polyketide synthase assembly line.

INTRODUCTION

Complex polyketides are a class of secondary metabolites that provide a competitive advantage for a wide spectrum of bacteria and fungi, often by targeting vital processes within competing organisms^{1,2}. The polyketide synthases (PKSs) responsible for their production are inherently modular, enabling PKS-harboring organisms to explore vast regions of chemical space through the reorganization of enzymatic domains. While the clinical value of the antibiotics erythromycin and mupirocin allude to the potential of polyketides in drug discovery, harnessing the biosynthetic capabilities of PKSs has been hindered by the complexity of their higher-order architecture³⁻⁵.

An enormous molecular assembly-line of the subcellular milieu, a PKS typically contains upwards of 50 independently-folded domains, each responsible for one step in the biosynthesis of a polyketide. The enzymatic modules of PKSs work together in assembly-line fashion to extend and process a growing polyketide chain⁶, each module containing a set of domains responsible for the incorporation and tailoring of a selected α -carboxylated extender unit. The domains employed by Type I PKSs are homologous to those responsible for fatty acid biosynthesis⁷, including acyltransferases (ATs) that select extender units, ketosynthases (KSs) that condense extender units with growing polyketides in a decarboxylative thio-Claisen condensation, ketoreductases (KRs) that stereospecifically reduce newly incorporated β -keto groups, dehydratases (DHs) that eliminate water to form α,β -olefins, enoylreductases (ERs) that stereospecifically reduce those olefins, and acyl carrier proteins (ACPs) that shuttle polyketide intermediates between the domains within a module as well as the following module. Thus, modules containing different sets of KR, DH, or ER domains yield different functional groups

and stereochemistries. A thioesterase (TE) downstream of the final module commonly off-loads the polyketide product via hydrolysis or cyclization.

Modular PKSs are divided into two architecturally-distinct classes: *cis*-AT and *trans*-AT². While *cis*-AT PKSs harbor ATs that are integrated into the multi-domain polypeptide, *trans*-AT systems rely on discretely-encoded AT domains that noncovalently interact with the megasynthase (Figure 1). Each of the independently-folded domains from *cis*-AT PKSs has been structurally characterized⁴, and although structural information has recently become available for domains from *trans*-AT PKSs, if and how the eponymous *trans*-AT domains dock to the megasynthase to charge ACPs with extender units remains to be determined⁸⁻¹⁰. A conserved ~100-residue region C-terminal to KS was hypothesized to facilitate the docking of *trans*-ATs to megasynthases and was named the AT-docking domain, or ATd¹¹. The KS/AT adapters, observed in the crystal structures of KS+AT didomains from the erythromycin PKS and the mammalian fatty acid synthase (FAS), are homologous to ATd on a sequence level and seem to validate this functional assignment^{12,13}. However, *in vitro* experiments examining extender unit transfer from *trans*-ATs to ACPs showed that the inclusion of ATd in constructs decreased the catalytic efficiency of acyl transfer compared to constructs that did not include ATd¹⁴.

The first-discovered, and prototypical, *trans*-AT PKS is encoded within the *Bacillus subtilis* and *Bacillus amyloliquefaciens* genomes (*pksX* and *bae*, respectively)². This hybrid PKS/non-ribosomal peptide synthetase (NRPS) produces bacillaene, a linear polyene diamide that interferes with prokaryotic protein synthesis (Figure 1)¹⁵. Transmission electron micrographs of *B. subtilis* cells have revealed dense masses associated with the plasma membrane, each comprised of several copies of the PksX/Bae megasynthase^{16,17}. This organelle-sized structure, with an approximate mass of 10-100 MDa, includes the five multidomain polypeptides PksJ,

PksL, PksM, PksN, and PksR. At the time of this discovery megacomplex formation was a complete mystery since *trans*-AT PKS polypeptides lack the N- and C-terminal docking domains that linearly organize *cis*-AT PKSs and no PKS architectural scaffolding proteins were known; however, ATd was hypothesized to help stabilize the observed structure (Figure 1)¹⁸. Membrane localization of PKS enzymes has been observed for the mycolactone and actinorhodin pathways, and the biosynthetic machinery for the siderophore pyoverdine is known to accrete into a dense, membrane-bound mass termed the “siderosome”; the co-localization of large enzymatic complexes at the bacterial plasma membrane is emerging as a theme in the biosynthesis of natural products¹⁹⁻²³.

The first ATd to be structurally observed, from a didomain composed of a KS and a β -branching enzyme (KS+B) from the rhizoxin PKS, revealed that ATd is structurally related to the KS/AT adapter of *cis*-AT PKSs⁸. Where the AT domain branches from the KS/AT adapter in *cis*-AT PKSs, only a short loop is present. The second structure of an ATd, from the second KS domain of the *B. subtilis* PksX synthase (PksKS2), showed two additional α -helices where the AT domain branches from the KS/AT adapter in *cis*-AT PKSs⁹. Modeling a *trans*-AT docked to the ATd based on the location of AT within *cis*-AT PKSs indicated that significant restructuring of these helices would be required for docking. Since neither functional nor structural studies supported the role of ATd as a *trans*-AT docking site, it was renamed the “flanking subdomain.”⁹ Comparisons of solved *trans*-AT KS domains have not yet revealed its role.²⁴

Here, we present seven additional crystal structures of *trans*-AT KS domains that provide evidence that the flanking subdomain plays a role in the assembly of PKS megacomplexes. At first considered to be a serendipitous crystal contact, the clearly defined self-association of flanking subdomains published here and elsewhere appears in ten crystal structures (five

different KS domains from three different microbial species harboring *trans*-AT PKSs; seven structures reported here, two structures reported in reference 9, and one reported in reference 24). We introduce the term **Laterally-Interacting Ketosynthase Sequences (LINKS)** to distinguish the three helices that form the observed interaction from the remainder of the flanking subdomain. Bioinformatic analysis of LINKS regions reveals that each is unique to the KS domain possessing them and indicates that PKS assembly lines that form megacomplexes are zippered together by LINKS-containing KSs that possess higher affinity for copies of themselves than other LINKS-containing KSs within the assembly line. These findings suggest an unprecedented mechanism for the assembly of several copies of a PKS megasynthase into a megacomplex.

RESULTS

Structure of the LINKS interaction

Three of the *trans*-AT KS domains deposited into the Protein Data Bank (PDB) prior to this work contain the LINKS region: one from the bacillaene PKS (PksKS2, PDBs 4NA1 and 4NA2) and two from the migrastatin PKS (MgsKS5 and MgsKS7, PDBs 4TL2 and 4TKT, respectively). The LINKS interaction between homodimeric KSs, not apparent from the asymmetric units of wild-type PksKS2 (PDB 4NA1) and MgsKS5 (PDB 4TL2), can be observed by generating the crystallographic symmetry mates. This is opposite in crystals of PksKS2(Cys176Ser) (PDB: 4NA2), in which the asymmetric unit shows two KS monomers associated via the LINKS interaction and the KS dimer is visualized by generating crystallographic symmetry mates. Here we report seven additional crystal structures in which LINKS interactions are observed, including one of PksKS2 in its monomeric form, one of PksKS6, two of PksKS6(Cys169Ser), one of Pks(ACP5+KS6), one of BaeKS1, and one of

BaeKS5 (Table 1). To date, only one crystal structure of a LINKS-containing *trans*-AT KS has been reported that does not show this interaction (MgsKS7, PDB: 4TKT).

The LINKS motif is comprised of ~40 residues (α 17- α 19) that project from the flanking subdomain as a triangular flap (see reference 9 for secondary structure assignments) (Figure 2). While α 19 is structurally equivalent to a helix in the KS/AT adapter of *cis*-AT PKSs and the mammalian FAS, α 17 and α 18 take the place of the AT domain in these synthases. Helices α 17 and α 18 do not make interactions with neighboring secondary structural elements and thus are not rigidly oriented with respect to the flanking subdomain. In the LINKS interaction, α 18 of one flanking subdomain makes contact with a spatially-reversed α 18 of the neighboring flanking subdomain, and α 17 of one flanking subdomain makes contact with α 19 of the neighboring flanking subdomain (a dynamic exploration of the interaction is provided in Supplementary Movie 1).

The observed LINKS interactions form through the burial of hydrophobic residues and exhibit a high degree of shape complementarity ($\sim 700 \text{ \AA}^2$). In the LINKS motif of PksKS2 (the structure possessing the highest-resolution and lowest B-factors for the LINKS region) these hydrophobic residues include I552, F553, M559, L563, W566, and L578 (Figures 2b-d, and Table S1). The LINKS interactions observed from bacillaene synthases (PksKS2, PksKS6, BaeKS1, and BaeKS5) show contact between clusters of charged residues that cap the poles of α 18. The N-terminal end of this helix contains ~3 negatively-charged residues, and the C-terminal end contains ~3 positively-charged residues. Two copies of α 18 are oriented in an antiparallel fashion, and the negatively-charged N-terminal end of α 18 from one flanking subdomain forms salt bridges with the positively-charged C-terminal end of α 18 from the neighboring flanking subdomain (Figure S1). Collectively, the burial of exposed hydrophobic

residues in the center of each LINKS triangular flap and the clustering of complementary ionic interactions at both termini of $\alpha 18$ form a lock-and-key fit uniquely adapted to each LINKS interaction (Figure S3).

The LINKS motifs connect neighboring KS dimers in crystals of MgsKS5 (PDB: 4TL2); however, instead of the twofold axes of these KS dimers being parallel as in each of the other observed LINKS-mediated associations of KS dimers, they are nearly orthogonal (Figure 2a). The MgsKS5 LINKS interaction is distorted relative to all the other crystallographically-observed LINKS interactions - $\alpha 17$ and $\alpha 18$ are skewed, and several ionic interactions potentially made *in vivo* are not formed (e.g., Asp1121/Arg1133 and Glu1122/Arg1129) (Figure S2). The only intact salt bridge, formed by Lys1120 and Asp1138, may provide an example of covariation within the LINKS motif, as both of these residues generally carry the opposite charge in sequence alignments (Figure 3 and Table S2).

The seven crystal structures reported here show the LINKS interaction in seven different contexts. In one crystal form, PksKS2 monomers that do not make the traditional KS homodimer interaction were observed. Remarkably, even though $\sim 5300 \text{ \AA}^2$ is not buried through the formation of the traditional KS homodimer, the LINKS interaction is maintained (Figure 2a). The construct Pks(ACP5+KS6), generated to investigate the interaction between KS and ACP, also yielded crystals in which the LINKS interaction was observed. Although the crystals could not be optimized to diffract beyond 4.0- $\text{\AA}$ resolution and the ACP domain could not be located in the electron density map, the LINKS interaction was observed to be formed by each of the eight KS monomers in the asymmetric unit (Figure S4). In addition to the 2.0 $\text{\AA}$ -resolution structure of PksKS6(Cys169Ser), a construct generated to visualize bound intermediates⁹, two additional crystal forms of PksKS6 were observed that contain the LINKS interaction (wild-type PksKS6,

3.10-Å resolution; PksKS6(Cys169Ser), 2.16-Å resolution) (Table S1). Two crystal structures of KSs from the *B. amyloliquefaciens* bacillaene PKS, BaeKS1 (2.93 Å) and BaeKS5 (4.20 Å), were also determined. While locally-elevated temperature factors prevented the complete modeling of α 17- α 19 in BaeKS1 (Figure S5), the relative positioning of KSs indicates that each of the flanking subdomains form LINKS interactions.

Bioinformatic analysis of LINKS

The lack of sequence conservation of α 17- α 19 is a hallmark of the LINKS motif. While the LINKS motifs shown in Figure 3 are each quite hydrophobic and contain charged residues at the poles of α 18, individual LINKS residues are highly variable (Figures 3 and S6). To further investigate this variability, the sequence conservation of LINKS-containing KS domains was mapped onto a model of a *trans*-AT KS structure (Figure 4). An alignment of 152 *trans*-AT KS sequences with clear LINKS regions was generated, and the degree of conservation of each residue was assessed. A moving average calculation was used to determine the mean conservation for short stretches of sequence, such that the value for the conservation of residue n is equal to the average value of conservation for the 11 residues ranging from $n-5$ to $n+5$. A template KS structure was then colored based on this value: red for 0-25%, yellow for 25-50%, green for 50-75%, and blue for 75-100%. While the active site region of KS appears blue and the remainder of the KS body varies between green and yellow, the longest stretch of red (46 residues) is the LINKS region. The next longest stretch of red sequence is only 17 residues and is located on the face of KS that would be adjacent to processing domains such as DH, KR, and MT. Classes of LINKS motifs other than the one shown in the sequence alignment of Figure 3 likely exist; several LINKS motifs that did not align well with those in Figure 3 are displayed in

Figure S6. Due to the highly variable nature of the LINKS region, it is currently unclear whether these sequences self-associate in the same manner as the more canonical LINKS sequences seen in Figure 3.

LINKS interactions are homotypic

With the exception of MgsKS5, the relative orientation of *trans*-AT KS dimers connected through a LINKS interaction is essentially identical for all of the crystal structures available to date (Figure 2a). We investigated whether heterotypic LINKS interactions were complementary between KSs from different modules. The six LINKS helices (three helices from each KS monomer) were isolated from the observed LINKS interactions (using the structure with the best-resolved LINKS motif for each KS), each of these were superposed, and new coordinate files were created for the heterodimeric LINKS interactions (i.e., PksKS2 with BaeKS1, PksKS6, or MgsKS5). The simulated interactions were investigated to assess the interfacial contacts. In general, heterotypic LINKS interactions were significantly less favorable than homotypic LINKS interactions, displaying much worse shape complementarity with fewer ionic interactions (Figure S7).

DISCUSSION

The docking site of *trans*-ATs had been hypothesized to be generated by an ~100-residue subdomain, formerly termed ATd and now referred to as the “flanking subdomain”, that is associated with the majority of KSs within *trans*-AT PKSs. That an AT domain is integrated into the equivalent subdomain of the mammalian FAS and *cis*-AT PKSs appears to support this role;

however, this subdomain is common to each of these synthases and was most likely present within an ancestral PKS before *cis*- and *trans*-AT PKSs diverged²⁵. Thus, the docking site to which *trans*-ATs dock in *trans*-AT PKSs should not be expected to be equivalent to where ATs are integrated in *cis*-AT PKSs. A ~40-residue LINKS motif is usually found in *trans*-AT PKSs where the AT domain is integrated in *cis*-AT PKSs. In 10 out of 11 crystal structures of constructs containing the LINKS motif it is found mediating a LINKS- interaction, and in 8 of the 10 structures it associates neighboring KS dimers in a parallel orientation (the exceptions being MgsKS5 and the monomeric form of PksKS2). We propose that the LINKS motif makes lateral interactions that help construct megacomplexes like those observed in *B. subtilis* cells (Figure 5; based on the measured dimensions, one bacillaene megacomplex is comprised of 10-20 homodimeric bacillaene PKS assembly lines).

The LINKS hypothesis excludes the site that AT is observed in *cis*-AT PKSs as the docking site for a *trans*-AT. While *trans*-ATs could dock with another region of the flanking subdomain or the KS body, they may only interact with the ACP domains to which they transfer extender units²⁷. Studies have shown that PksE, a *trans*-AT in the PksX bacillaene pathway, is present in concentrations 10–100 fold greater than the assembly line polypeptides¹⁷. Additionally, superstoichiometric *trans*-AT concentrations have a large effect on the rate of polyketide production by the virginiamycin *trans*-AT PKS²⁷. If *trans*-ATs were stably associated with each *trans*-AT PKS module, increased *trans*-AT expression would have little effect. Several condensation-incompetent *trans*-AT KSs harbor flanking subdomains yet have no need to interact with a *trans*-AT (e.g., BTKS10, ChiKS18, DszKS10, and OnnKS4). Similarly, several *trans*-AT KSs that catalyze polyketide chain elongation do not contain a flanking subdomain, yet the corresponding ACPs from these modules must interact with a *trans*-AT (e.g., ChiKS16,

LnKS3, MlnKS8, and DifKS10). Studies have shown that *trans*-ATs are capable of charging ACP domains in the absence of a KS or flanking subdomain and that a *trans*-AT from the kirromycin PKS (KirCII) is quite specific for its cognate ACP^{14,26,28-30}. This degree of *trans*-AT specificity for an ACP is surprising if the flanking subdomain or KS body is selective for docking with a particular *trans*-AT.

The structure of the homodimeric KS+B didomain from the rhizoxin *trans*-AT PKS shows that the longest dimension of the B dimer is rotated 90° relative to the longest dimension of the KS dimer⁸. This orientation, also anticipated for the *trans*-AT KS+DH didomain based on the structural homology of the B and DH domains, would be compatible with the higher-order architecture of a PKS megacomplex formed through LINKS interactions (Figure 5). While several contacts between the KS and B dimers from a *trans*-AT PKS fix their relative orientation, the KS and DH dimers of the mammalian FAS rotate freely relative to one another^{13,32}. The crystal structure of the mammalian FAS shows no contact between the two dimers (Figure S8), and cryo-electron microscopy studies also report flexibility between the KS and DH dimers.

Even if LINKS motifs do not make high affinity interactions, significant avidity could be generated through the several LINKS motifs interacting along the length of the synthase (Figure S9). Within the megacomplex, the lateral LINKS interactions would have the effect of bolstering vertical interactions, thought to be mediated by the N- and C-terminal regions of *trans*-AT PKS polypeptides. The flexibility of the LINKS interaction, illustrated by the MgsKS5 crystal structure, could aid megacomplex formation and maintenance. Many *trans*-AT PKS assembly lines harbor several LINKS motifs (e.g., the bacillaene PKS contains 12, the chivosazol PKS contains 9, the mupirocin PKS contains 7), such that the dissociation of a single LINKS interaction would have little effect on the stability of the entire megacomplex. LINKS

276 interactions may be difficult to biophysically characterize using methods other than x-ray
277 crystallography. If the dissociation constant for an individual LINKS interaction is in the
278 millimolar range, it may not be observable through techniques such as small-angle x-ray
279 scattering (SAXS) or analytical ultracentrifugation³². To accurately determine the dissociation
280 constant of the LINKS interaction, the natural KS dimerization interface may need to be
281 disrupted by site-directed mutagenesis such that only the LINKS-mediated association of KS
282 monomers is measured.

283 Several structures of *trans*-AT PKS KS domains have been deposited in the PDB that do
284 not contain a LINKS motif, including the KS+B didomain from the rhizoxin PKS⁸. However,
285 only one reported crystal structure of a LINKS-containing KS does not display the LINKS
286 interaction (MgsKS7). Favorable crystal contacts made elsewhere may preclude this interaction
287 in the same manner that LINKS-mediated crystal contacts within the monomeric crystal structure
288 of PksKS2 are favored over natural homodimer interactions.

289 The prevalence of the LINKS motif within many *trans*-AT systems suggests that
290 megacomplex formation may be a more common occurrence than is currently recognized. The
291 mode through which LINKS interactions could stabilize *trans*-AT PKS megacomplexes has not
292 been observed in the architectures of other biosynthetic assemblies. The crystal structures
293 presented here reveal a defined binding interface formed by hydrophobic and ionic interactions
294 mediated by three helices on the surface of the flanking subdomain. Bioinformatic analysis
295 suggests that the LINKS interaction is unique for each KS, such that the in-register, homotypic,
296 lateral interactions made by them also confer stability to the vertical interactions between the
297 assembly lines of the megacomplex through avidity. While *trans*-AT systems represent an
298 excellent template for the rational engineering of synthetic PKSs for the exploration of new

medicines, it may be necessary to consider the implications of modifying the LINKS network when genetically relocating modules or domains within a *trans*-AT PKS megasynthase.

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AUTHOR CONTRIBUTIONS

D.G. solved and refined or assisted in the solution and refinement of each crystal structure, realized that LINKS mediate megacomplex assembly, wrote the manuscript, and generated the figures. D.W. solved and refined or assisted in the solution and refinement of the crystal structures for PksKS6 and its mutant, BaeKS1, and BaeKS5. J.M., C.Z., and G.G. assisted in the solution and refinement of the BaeKS5, BaeKS1, and PksKS2 crystal structures, respectively. A.K.C. provided experimental and crystallographic insight, and assisted in composing the

manuscript and figures. All authors contributed to editing the manuscript and figures, and declare no competing financial interests.

FIGURE LEGENDS

Figure 1. *Trans*-AT vs. *cis*-AT PKS architecture.

The modular assembly for the archetypal *trans*-AT (PksX) and *cis*-AT (6-dEBS) PKSs are shown, responsible for the biosynthesis of bacillaene and erythromycin, respectively. For each system, the fourth PKS module has been magnified to reveal the organization of domains. Although much of the higher-order architecture remains unknown, the schematics represent hypothetical models based on available crystal structures (Ery(KS+AT)₃ (PDB: 2QO3), EryDH4 (PDB: 3EL6), Spn(KR+ER)₂ (PDB: 3SLK), PksKS2 (PDB: 4NA1), and Rhi(KS+B)₁₁ (PDB: 4KC5). The presence of a LINKS contact is shown as lateral red extensions for each of the modules in PksX that harbor this region. N- and C-terminal docking domains for 6-dEBS are shown as vertical blue extensions. ACP, acyl carrier protein; AL, acyl-CoA ligase; AT, acyltransferase; DH, dehydratase; ECH, enoyl-CoA hydratase; ER, enoylreductase; FSD, flanking subdomain; HCS, HMG-CoA synthase; HYD, hydrolase; KS, ketosynthase; KR, ketoreductase; LD, loading didomain; M, module; NRPS, non-ribosomal peptide synthetase; P450, cytochrome P450; TE, thioesterase.

Figure 2. The LINKS interaction.

The LINKS interaction that forms between *trans*-AT KS domains is mediated by three α -helices (α 17- α 19) that bind to a spatially-reversed copy of the same structure. (a) A model for the LINKS interaction between two KS homodimers is magnified, revealing the orientation of the

LINKS helices. The angle above the structures indicates the relative angle between KS monomers forming the interaction (Figure S2). Each individual LINKS sequence is colored blue at the N-terminal end of $\alpha 17$ and red at the C-terminal end of $\alpha 19$, and the N-terminal end of $\alpha 17$ always forms favorable interactions with the C-terminal end of $\alpha 19$ from the neighboring KS ($\alpha 19'$). The coloring scheme used for the cartoon model has been repeated in each of the displayed structures to highlight the conservation of the LINKS interface. The structure shown for PksKS2 (monomeric form) reveals two KS monomers that do not form the traditional homodimer interaction; however, the LINKS interaction is crystallographically maintained. The relatively high thermal factors for the LINKS helices in BaeKS1 did not permit the complete construction of $\alpha 17$ - $\alpha 19$ for each of the KS monomers, but the relative orientation of the KS bodies indicates that the LINKS interaction is conserved. (b) The three helices forming the LINKS interaction for PksKS2 are shown in cartoon format (green), and the remainder of this PksKS2 monomer has been hidden for clarity. The neighboring KS forming the complementary LINKS interaction is represented with a transparent surface (cyan). The angle of the image is set from the interior of the flanking subdomain, to reveal the collection of centrally-located hydrophobic residues that would be surface-exposed in the absence of the LINKS interaction. (c) A 90° rotation of the viewing angle shown in panel B reveals the series of ionic interactions formed at the poles of $\alpha 18$. The aspartate and glutamate residues at the N-terminal end of $\alpha 18$ form well-defined ionic interactions with the lysine residues at the C-terminal end of $\alpha 18$ from the neighboring structure. Met559 of the blue monomer can be observed in the center of the image, extending into the hydrophobic cavity of the green monomer (Figure S1a). (d) The three LINKS helices for PksKS2 have been modeled linearly to reduce the complexity of the image. LINKS contacts between residues ranging from 2.0 - 3.0 Å are shown as black dots, and those

ranging from 3.1 – 4.0 Å are shown as grey dots. The corresponding sequence for the region is also shown, and residues represented by grey letters have been hidden from the cartoon and do not directly contribute to the LINKS interaction.

Figure 3. Sequence alignment of the LINKS region.

Over 100 *trans*-AT KS sequences were aligned, and a subset of the alignment is shown. Residues in the alignment have been colored according to the following scheme: red for acidic residues, blue for basic residues, and yellow for hydrophobic residues. Helices α 17- α 19 (grey) delineate the boundaries of the LINKS region. If a KS in the alignment has been structurally characterized, the associated PDB identifier is shown in parentheses. Although strict conservation of the LINKS region is poor, the conservation of LINKS attributes are generally maintained (i.e., charged poles of α 18 flanked by hydrophobic residues). For those KSs that do not contain LINKS (e.g., RhiKS11 and OzmKS9, bottom of alignment), the available structures do not reveal any crystallographic homotypic interactions mediated by this region. The only structure available that contains LINKS but does not exhibit the crystallographic interaction observed in all other *trans*-AT KS structures is of MgsKS7. The structure of MgsKS5 reveals the LINKS interaction, yet the LINKS attributes differ from what has been observed in other structures. A seemingly anomalous basic residue at the N-terminal end of α 18 (Lys1120) forms an ionic interaction with an acidic residue at the N-terminal end of α 19 (Asp1138) that is almost invariably basic in the other sequences (both residue positions are marked with asterisks). Accession numbers for the sequences are available in the supporting information (Table S4).

Figure 4. Simple moving average calculation of sequence conservation.

To determine which regions of *trans*-AT KSs are the most susceptible to sequence variation, a simple moving average (SMA) calculation was performed based on sequence conservation and residues were colored according to the corresponding quartiles. The five regions that received the highest SMA conservation scores all map to the active site of the KS body (blue), while the lowest scoring region corresponds to the LINKS helices (red). A representative KS structure is shown colored accordingly. N- and C-termini are depicted by cyan and red spheres, respectively, and residue numbering is based on the structure of PksKS2.

Figure 5. Vertical and lateral interactions stabilize the PKS megacomplex.

The electron micrographs observed by Straight et al. revealed a dense mass associated with the membrane of *B. subtilis* cells, identified to consist of numerous copies of the PksX megasynthase¹⁷. An image from that work is reprinted here, revealing that the observed superstructure measures approximately 150 × 150 nm. The arrow points to the cell membrane, and the arrowhead points to gold nanoparticles engineered to target PksX proteins. For visualizing how this mass of proteins may self-associate, a diagram shows the assembly line subunits (PksJ, PksL, PksM, PksN, and PksR) interacting vertically through domain-domain interactions, and laterally through the LINKS network. The estimations for megacomplex dimensions that flank the cartoon are calculated based on available crystal structures of individual PKS domains. In the vertical dimension, 175 nm is represented by 15 PKS modules and 2.5 NRPS modules (10 nm each). In the lateral dimension, 160 nm is estimated by the total length of 15 KS homodimers that polymerize through LINKS interactions.

TABLES

414 **Table 1. Crystallographic Data and Refinement Statistics**

	PksKS2 (4NA1) (monomeric)	PksKS6 (5E5N) (Cys169Ser)	PksKS6 (5E6K)(Cys169Ser)	PksKS6 (5ERF)	Pks(ACP5+KS6) (SENY)	BaeKS1 (5ELP)	BaeKS5 (5ERB)
Data Collection							
Wavelength (Å)	0.9999	1.0332	0.9765	0.9765	1.0332	1.0332	1.0332
Space group	C2	P 2 ₁	P 2 ₁	P 1	P 1	P 1	P 2 ₁
Cell dimensions, <i>a</i> , <i>b</i> , <i>c</i> (Å); α , β , γ (°)	197.1, 75.1, 134.1; 90.0, 132.9, 90.0	89.4, 108.2, 151.2; 90.0, 96.4, 90.0	76.7, 105.5, 89.3; 90.0, 96.8, 90.0	69.0, 77.3, 85.3; 77.7, 70.3, 64.2	63.1, 112.7, 211.4; 105.0, 90.1, 106.3	61.2, 100.0, 100.8; 91.9, 88.2, 96.0	70.8, 319.1, 103.5; 90.0, 110.3, 90.0
Resolution (Å)	47.25-3.02	150.89 -2.00	36.55 - 2.16	39.73 - 3.10	39.77 - 4.00	99.35 - 2.93	97.07 – 4.20
<i>R</i> _{merge}	0.048 (0.663)	0.120 (0.570)	0.092 (0.481)	0.058 (0.359)	0.102 (0.373)	0.148 (0.641)	0.114 (0.483)
<i>I</i> / σ (<i>I</i>)	32.2 (1.8)	28.1 (2.5)	12.3 (2.0)	13.3 (1.7)	6.1 (2.7)	15.7 (1.5)	14.2 (2.8)
Completeness (%)	99.7 (98.4)	99.2 (96.0)	99.2 (93.5)	94.1 (75.6)	98.7 (99.0)	94.9 (89.75)	79.9 (59.3)
Redundancy	3.7 (3.8)	2.1 (2.1)	1.9 (1.8)	1.9 (1.9)	3.3 (3.5)	1.1 (1.1)	3.2 (2.8)
Wilson B value (Å ²)	119.7	35.3	42.5	77.3	157.2	62.4	196.4
Refinement							
Resolution (Å)	47.25-3.02	150.89 -2.00	36.55 - 2.16	39.73 - 3.10	39.77 - 4.00	99.35 - 2.93	97.07 – 4.20
No. of reflections	26825 (1879)	183336 (13049)	71285 (4947)	24128 (1403)	39874 (2246)	46279 (3209)	23712 (1314)
<i>R</i> _{work} / <i>R</i> _{free}	0.223/0.268	0.201/0.231	0.224/0.258	0.218/0.262	0.322/0.344	0.262/0.288	0.288/0.341
No. of atoms							
Protein	8938	17314	8463	8944	34416	16904	18315
Water	-	1358	88	-	-	-	-
Average <i>B</i> factors (Å ²)							
Protein	119.7	44.3	42.7	77.8	157.3	63.1	196.4
Water	-	27	42.4	-	-	-	-
RMS deviations							
Bond lengths (Å)	0.0018	0.0186	0.0083	0.0180	0.0013	0.0127	0.0130
Bond angles (°)	1.084	1.352	1.342	1.390	0.716	1.937	2.651
Ramachandran Statistics (%)							
Preferred Regions	91.8	97.3	96.5	94.0	95.8	92.6	93.3

Allowed Regions	8.2	2.7	3.5	5.9	4.2	6.7	6.6
Outliers	0.0	0.0	0.0	0.1	0.0	0.7	0.1

METHODS

All protein structures described in this work were cloned, purified, and the corresponding structures refined using identical methods to those previously described for PksKS2⁹. Therefore, only the primers used for gene amplification of new constructs, crystallization conditions, phasing methods, and any other modifications will be reported here.

Crystals of PksKS2 in which the KS domains did not crystallize in the native dimeric form grew over a period of 5-7 days by sitting drop vapor diffusion at 4 °C. Drops were formed by mixing 2 μ L protein solution (4.5 mg/ml PksKS2, 150 mM NaCl, 10% glycerol, 10 mM HEPES, pH 7.5) with 1 μ L crystallization buffer (30% PEG 400 (v/v), 0.2 M $MgCl_2$, 0.1 M HEPES, pH 7.5). Crystals were frozen in liquid nitrogen after a 20 min soak in crystallization buffer modified with 20% glycerol, and the diffraction data collected at ALS Beamline 5.0.3. The structure was solved to 3.0 Å resolution by molecular replacement with PhaserMR in the CCP4 suite, using a single chain of the dimeric structure of PksKS2 (PDB: 4NA1) as the search model.

[illegible]

437 2 μ L protein solution (15 mg/mL PksKS6, 150 mM NaCl, 10 mM HEPES, pH 7.5) with 1 μ L
438 crystallization buffer (150 mM $(\text{NH}_4)_2\text{SO}_4^{-2}$, 15% PEG 4000 (v/v), 100 mM MES, pH 6.0).
439 Crystals were frozen in liquid nitrogen after a 20 min soak in the crystallization buffer modified
440 with 10% (v/v) ethylene glycol. Diffraction data, collected at ALS Beamline 5.0.3, were
441 processed by HKL2000. The structure was solved to 3.00 Å resolution by molecular replacement
442 using the KS2 monomer from the PksX synthase of *B. subtilis* as the search model (PDB:
443 4NA1).

444 The PksKS6(Cys169Ser) expression plasmid was generated by PCR amplification from
445 the PksKS6 construct described above using primers 5'-
446 AGACGGCCTCGAGCTCTTCATTGGTCGGCACTCATTTAGCGCGCCAGGCACTTATAA
447 ACAAAG-3' and 5'-
448 AGAACTGCTCGAGGCGGTATCAATAGGAATCGCTGGCCCTTTTAAATTTAGAAAATA
449 CGG-3' (XhoI site underlined). The PCR product was digested with XhoI and ligated. Crystals
450 of PksKS6(Cys169Ser) grew over 2-10 days by sitting drop vapor diffusion at 22 °C. Drops were
451 formed by mixing 2 μ L protein solution (15 mg/mL PksKS6(Cys169Ser), 150 mM NaCl, 10 mM
452 HEPES, pH 7.5) with 1 μ L crystallization buffer (1.7 M LiSO_4 , 100 mM Tris, pH 8.0) (both
453 crystal forms grew in the same crystallization condition). Crystals were frozen in liquid nitrogen
454 after a 20 min soak in crystallization buffer modified with 10% (v/v) ethylene glycol and the
455 diffraction data were collected at APS Beamline 23-ID-D. The structure was solved by molecular
456 replacement using a single chain of the dimeric structure of PksKS2 (PDB: 4NA1) as the search
457 model.

458 *Bacillus amyloliquefaciens* FZB42 genomic DNA was used as template DNA to PCR
459 amplify BaeKS1 with KAPA High-Fidelity DNA polymerase (KAPA) using the forward primer

460 5'-GCGGCCTGGTGCCGCGCGGCTCTAGCTTTCCCGATTATTACGAGGACAG-3' and the
461 reverse primer 5'- GTGGTGGTGGTGGTGGTGATGTTAGTTCTTTTCTGCTTTTTTCGGCG-
462 3' (pGAY28 LIC cloning regions underlined). The eluted protein was concentrated to 14 mg/mL
463 in the equilibration buffer and stored at -80 °C until needed. Crystals of BaeKS1 grew over 4-15
464 days by sitting drop vapor diffusion at 22 °C. Drops were formed by mixing 2 µL protein
465 solution (14 mg/mL BaeKS1, 150 mM NaCl, 10 mM HEPES, pH 7.5) with 1 µL crystallization
466 buffer (32% PEG 400 (v/v), 0.2 M MgCl₂, 100 mM HEPES, pH 7.5). Crystals were frozen in
467 liquid nitrogen after a 20 min soak in crystallization buffer modified with 40% PEG 400 (v/v)
468 and the diffraction data were collected at APS Beamline 23-ID-D. The structure was solved by
469 molecular replacement using a single chain of the dimeric structure of PksKS2 (PDB: 4NA1) as
470 the search model.

471 The gene corresponding to Pks(ACP5+KS6) was PCR amplified using primers 5'-
472 GCGGCCTGGTGCCGCGCGGCTCTAGCGCTGAAGAAACGATTCAATATGC-3' and 5'-
473 GTGGTGGTGGTGGTGGTGATGTTAGCCTTCTTGAGTTGGCAGC-3' (pGAY28 LIC
474 cloning regions underlined). Crystals grew over a period of 2-4 days by sitting drop vapor
475 diffusion at 22 °C. Drops were formed by mixing 2 µL protein solution (25 mg/ml
476 Pks(ACP5+KS6), 150 mM NaCl, 10% glycerol, 10 mM HEPES, pH 7.5) with 1 µL
477 crystallization buffer (30% PEG 4000 (v/v), 0.2 M sodium acetate, 0.1 M Tris, pH 8.5). Crystals
478 were frozen in liquid nitrogen after a 20 min soak in crystallization buffer modified with 20%
479 glycerol, and the diffraction data collected at ALS Beamline 5.0.2. Phases were obtained by
480 molecular replacement using PksKS6 (wild-type) as a search model. The structure was solved to
481 4.0 Å resolution by molecular replacement using a single chain from the dimeric structure of
482 PksKS6 as the search model. Electron density for the eight ACPs in the asymmetric unit could

not be located despite exhaustive inspection of the map.

The DNA encoding BaeKS5 was amplified using primers: 5'-
GCGGCCTGGTGCCGCGCGGCTCTAGCCCAGCAGCTGACAGAGCGTG-3' and 5'-
GTGGTGGTGGTGGTGGTGGTGATGTTAATCAGCTGATGTGTCGATCCAATAAC-3'
(pGAY28 LIC cloning regions underlined). The eluted protein was concentrated to 9.9 mg/mL in
the equilibration buffer. Crystals of BaeKS5 grew in 2 days by sitting drop vapor diffusion at 22
°C. Drops were formed by mixing 1.75 µL protein solution (9.9 mg/mL BaeKS5, 150 mM NaCl,
15 mM HEPES, pH 7.5) with 0.5 µL crystallization buffer (1.1 M LiSO₄, 0.1 M Tris, pH 8.0).
Crystals were frozen in liquid nitrogen after a 20 min soak in the crystallization buffer modified
with 10% (v/v) glycerol. Diffraction data, collected at APS Beamline 23-ID-B, were processed
by HKL2000. The structure was solved to 4.20 Å resolution by molecular replacement using the
KS2 monomer from the PksX synthase of *B. subtilis* as the search model (PDB: 4NA1).

REFERENCES

1. Hertweck, C. The biosynthetic logic of polyketide diversity. *Angew. Chem. Int. Ed. Engl.* **48**, 4688-716 (2009).
2. Piel, J. Biosynthesis of polyketides by trans-AT polyketide synthases. *Nat. Prod. Rep.* **27**, 996-1047 (2010).
3. Walsh, C.T. The chemical diversity of natural-product assembly lines. *Acc. Chem. Res.* **41**, 4-10 (2008).

506

507 4. Keatinge-Clay, A.T. The structures of type I polyketide synthases. *Nat. Prod. Rep.* **29**, 1050-

508 1073 (2012).

509

510 5. Dutta, S. *et al.* Structure of a modular polyketide synthase. *Nature* **510**, 512-517 (2014).

511

512 6. Khosla, C., Herschlag, D., Cane, D.E., & Walsh, C.T. Assembly line polyketide synthases:

513 mechanistic insights and unsolved problems. *Biochemistry* **53**, 2875-2883 (2014).

514

515 7. Smith, S. & Tsai, S.C. The type I fatty acid and polyketide synthases: a tale of two

516 megasynthases. *Nat. Prod. Rep.* **24**, 1041-1072 (2007).

517

518 8. Bretschneider, T. *et al.* Vinylogous chain branching catalyzed by a dedicated polyketide

519 synthase module. *Nature* **502**, 124-128 (2013).

520

521 9. Gay, D. *et al.* A close look at a ketosynthase from a *trans*-acyltransferase modular polyketide

522 synthase. *Structure* **22**, 441-451 (2014).

523

524 10. Piasecki, S.K., Zheng, J., Axelrod, A.J., Detelich, M E., & Keatinge-Clay, A.T. Structural

525 and functional studies of a *trans*-acyltransferase polyketide assembly line enzyme that catalyzes

526 stereoselective α - and β -ketoreduction. *Proteins* DOI: 10.1002/prot.24561 (2014).

527

11. Tang, G.-L., Cheng, Y.-Q., & Shen, B. Leinamycin biosynthesis revealing unprecedented architectural complexity for a hybrid polyketide synthase and nonribosomal peptide synthetase. *Chem. Biol.* **11**, 33-45 (2004).
12. Tang, Y., Chen, A.Y., Kim, C.Y., Cane, D.E., & Khosla, C. Structural and mechanistic analysis of protein interactions in module 3 of the 6-deoxyerythronolide B synthase. *Chem. Biol.* **14**, 931-943 (2007).
13. Maier, T., Leibundgut, M., & Ban, N. The crystal structure of a mammalian fatty acid synthase. *Science* **321**, 1315-1322 (2008).
14. Aron, Z.D., Fortin, P.D., Calderone, C.T., & Walsh, C.T. FenF: Servicing the mycosubtilin synthetase assembly line *in trans*. *ChemBioChem* **8**, 613-616 (2007).
15. Patel, P.S. *et al.* Bacillaene, a novel inhibitor of procaryotic protein synthesis produced by *Bacillus subtilis*: Production, taxonomy, isolation, physico-chemical characterization and biological activity. *J. Antibiot.* **48**, 997-1003 (1995).
16. Butcher, R.A. *et al.* The identification of bacillaene, the product of the PksX megacomplex in *Bacillus subtilis*. *Proc. Natl. Acad. Sci. U.S.A.* **104**, 1506-1509 (2007).

17. Straight, P.D., Fischbach, M.A., Walsh, C.T., Rudner, D.Z., & Kolter, R. A singular enzymatic megacomplex from *Bacillus subtilis*. *Proc. Natl. Acad. Sci. U.S.A.* **104**, 305-310 (2007).
18. Broadhurst, R.M., Nietlispach, D., Wheatcroft, M.P., Leadlay, P.F., & Weissman, K.J. The structure of docking domains in modular polyketide synthases. *Chem. Biol.* **10**, 723-731 (2003).
19. Xu, X.-P. *et al.* Localization of the ActIII actinorhodin polyketide ketoreductase to the cell wall. *FEMS Microbiol. Lett.* **287**, 15-21 (2008).
20. Imperi, F. & Visca, P. Subcellular localization of the pyoverdine biogenesis machinery of *Pseudomonas aeruginosa*: A membrane-associated “siderosome”. *FEBS Lett.* **587**, 3387-3391 (2013).
21. Porter, J.L. *et al.* The cell wall-associated mycolactone polyketide synthases are necessary but not sufficient for mycolactone biosynthesis. *PLoS ONE* **8**(7): e70520 (2013).
22. Simunovic, V. *et al.* Myxovirescin A biosynthesis is directed by hybrid polyketide synthases/nonribosomal peptide synthetase, 3-hydroxy-3-methylglutaryl-CoA synthases, and *trans*-acting acyltransferases. *ChemBioChem* **7**(8), 1206-1220 (2006).
23. Hantash, F.M. & Earhart, C.M. Membrane association of the *Escherichia coli* enterobactin synthase proteins EntB/G, EntE, and EntF. *J. Bacteriol.* **182**, 1768-1773 (2000).

572

573 24. Lohman, J.R. *et al.* Structural and evolutionary relationships of “AT-less” type I polyketide
574 synthase ketosynthases. *PNAS* **112**, 12693-12698 (2015).

575

576 25. Nguyen, T. *et al.* Exploiting the mosaic structure of *trans*-acyltransferase polyketide
577 synthases for natural product discovery and pathway dissection. *Nat. Biotechnol.* **26**, 225-233
578 (2008).

579

580 26. Ye, Z., Musiol, E.M., Weber, T., & Williams, G.J. Reprogramming acyl carrier protein
581 interactions of an acyl-CoA promiscuous *trans*-acyltransferase. *Chem. Biol.* **21**, 636-646 (2014).

582

583 27. Pulsawat, N., Kitani, S., & Nihira, T. Characterization of biosynthetic gene cluster for the
584 production of virginiamycin M, a streptogramin type A antibiotic, in *Streptomyces virginiae*.
585 *Gene* **393**, 31-42 (2007).

586

587 28. Wong, F.T., Jin, X., Mathews, I.I., Cane, D.E., & Khosla, C. Structure and mechanism of the
588 *trans*-acting acyltransferase from the disorazole synthase. *Biochemistry* **50**, 6539-6548 (2011).

589

590 29. Lopanik, N.B. *et al.* In vivo and in vitro *trans*-acylation by BryP, the putative bryostatin
591 pathway acyltransferase derived from an uncultured marine symbiont. *Chem. Biol.* **15**, 1175-
592 1186 (2008).

593

- 594 30. Musiol, E.M. *et al.* Supramolecular templating in kirromycin biosynthesis: the
595 acyltransferase KirCII loads ethylmalonyl-CoA extender onto a specific ACP of the *trans*-AT
596 PKS. *Chem. Biol.* **18**, 438-444 (2011).
- 597
- 598 31. Brignole, E.J., Smith, S., Asturias, F.J. Conformational flexibility of metazoan fatty acid
599 synthase enables catalysis. *Nat. Struct. Mol. Biol.* **16**, 190-197 (2009).
- 600
- 601 32. Davison, J. *et al.* Insights into the function of *trans*-acyl transferase polyketide synthases
602 from the SAXS structure of a complete module. *Chem. Sci.* **5**, 3081-3095 (2014)
- 603
- 604 33. Gay, G., Wagner, D., Keatinge-Clay, A.T., & Gay, D.C. Rapid modification of the pET-28
605 expression vector for ligation-independent cloning using homologous recombination in
606 *Saccharomyces cerevisiae*. *Plasmid* **76**, 66-71 (2014).
- 607

(PksX, *Bacillus subtilis*)

(PksX, *Bacillus subtilis*)

***Cis* - AT PKS**

(6-dEBS, *Saccharopolyspora erythraea*)

Bacillaene

610

611

■ Anionic
■ Cationic
■ Hydrophobic

BaeKS1 (5ELP)	---	AVT	-	GCFRGE	KQQA	---	---	I	AWL	S	DDDD	S	AEL	I	KWLA	G	KGP	K	LCE	M	VS	K	GVA	-	I	NW	H	K	L	K	H	---	---	P	K	R	I	S	L	P																																							
PksKS2 (4NA1)	---	TDE	---	FFRGQS	HRN	---	---	K	TQT	I	F	T	A	E	D	M	A	L	A	D	A	W	I	R	K	R	K	Y	A	K	L	A	D	L	W	V	K	G	V	S	-	I	Q	N	T	L	Y	G	---	---	T	K	P	R	L	I	S	L	P																				
BaeKS5 (5ERB)	---	S	I	D	Q	---	---	L	Y	R	G	V	K	Q	N	---	---	K	D	T	M	A	L	F	T	A	E	D	M	E	K	T	I	A	W	L	E	K	G	K	A	A	V	L	E	L	W	V	K	G	L	P	---	---	L	N	W	K	L	Y	Q	M	---	---	G	R	P	O	K	I	S	L	P						
PksKS6 (5ERF)	---	N	A	S	---	I	F	A	A	H	V	K	T	---	---	K	S	E	I	K	L	F	T	D	H	A	K	A	L	L	Q	T	W	I	---	---	K	K	R	L	E	K	V	A	E	L	W	V	K	G	L	Q	---	---	I	D	W	N	K	L	Y	G	---	---	Y	T	P	R	F	I	S	L	P						
MgsKS7 (4TKT)	---	S	G	L	P	A	G	---	---	A	Y	F	N	P	G	G	---	---	G	G	A	V	K	F	L	T	D	E	D	F	Q	T	A	V	R	W	A	R	R	G	K	P	A	P	L	A	E	A	W	T	S	G	L	A	---	---	V	D	W	A	R	L	H	T	E	G	P	K	---	---	P	R	K	V	A	L	P		
MgsKS9 (4TL2)	S	---	---	A	E	A	E	V	P	W	S	R	G	R	V	R	A	T	---	---	R	E	T	L	A	A	L	A	E	K	D	E	L	R	A	L	V	T	R	W	I	N	R	G	W	H	D	L	A	A	F	W	A	K	G	M	P	---	---	L	D	W	T	R	L	H	A	G	A	---	---	T	P	A	R	V	H	L	P
BaeKS9	---	S	A	G	---	L	H	R	G	T	E	E	N	---	---	K	R	M	L	A	V	F	S	A	D	D	L	E	T	I	I	A	W	I	S	K	R	K	Y	S	K	L	L	E	L	W	V	K	G	L	Q	---	---	I	D	W	T	K	L	Y	G	---	---	R	P	P	A	R	I	S	L	P							
BaeKS2	---	N	I	A	G	---	---	L	Y	T	G	Q	S	H	R	N	---	---	K	D	T	F	A	L	F	T	A	E	D	M	I	V	I	A	W	I	K	K	G	F	A	K	L	A	E	V	W	V	K	G	V	---	---	F	N	W	N	R	L	Y	E	K	---	---	G	T	P	R	L	L	S	L	P						
GUKS5	---	S	F	D	---	L	Y	R	G	Q	A	K	R	---	---	K	E	T	F	S	Q	F	A	I	D	E	L	Q	K	A	V	D	N	W	I	S	K	R	K	Y	E	K	L	L	D	F	W	V	R	G	L	S	---	---	I	N	W	N	K	I	Y	D	---	---	R	K	P	V	R	I	S	L	P						
GUKS11	---	D	T	E	D	---	---	L	Y	K	G	N	V	K	R	---	---	N	D	S	A	W	L	Y	A	V	D	E	D	M	A	K	T	I	A	W	V	S	K	G	K	Y	A	R	L	L	E	L	W	V	K	G	M	H	---	---	F	D	W	N	R	L	Y	G	A	---	---	A	K	P	R	R	I	G	L	P			
GUKS9	---	D	L	E	H	---	---	L	H	K	G	H	V	K	R	---	---	N	D	S	A	W	L	Y	A	V	D	E	D	M	A	K	T	I	A	W	V	S	K	G	K	Y	A	R	L	L	D	L	W	V	K	G	L	N	---	---	F	D	W	N	R	L	Y	G	A	---	---	A	K	P	R	H	I	S	L	P			
GUKS10	---	D	L	E	G	---	---	L	S	M	G	Q	V	K	R	---	---	N	D	M	L	A	V	F	T	A	D	D	D	M	Q	E	A	I	G	K	W	I	R	K	R	K	Y	S	K	V	L	D	L	W	V	Q	G	I	P	---	---	V	D	W	N	V	L	Y	G	A	---	---	S	T	P	R	R	I	S	L	P		
BaeKS11	---	H	A	D	---	L	Y	R	G	R	I	D	---	---	---	---	K	G	T	L	Q	M	L	T	E	D	E	E	I	Q	E	A	V	K	M	M	A	R	G	K	L	S	K	V	L	E	V	W	T	R	G	M	P	---	---	V	D	W	A	K	L	H	G	---	---	A	Q	L	R	R	R	S	L	P					
TaKS10	---	L	A	D	S	---	---	V	Y	R	G	H	V	K	R	---	---	K	E	F	L	S	V	F	A	D	A	E	M	Q	E	A	V	A	K	M	M	A	R	G	K	L	S	K	V	L	E	V	W	T	R	G	M	P	---	---	V	D	W	A	K	L	H	G	---	---	A	Q	L	R	R	R	S	L	P				
GUKS8	---	G	I	E	K	---	---	L	Y	R	G	Q	V	K	P	N	---	---	K	E	A	L	S	V	F	S	M	D	E	E	L	Q	E	A	I	E	K	M	M	E	R	G	K	Y	S	R	L	L	G	L	W	R	G	Q	---	---	F	D	W	N	K	L	Y	B	---	---	R	K	P	R	R	V	S	L	P				
TaKS9	---	A	G	G	A	---	---	V	F	R	G	H	V	K	R	N	---	---	K	D	A	L	S	V	F	T	G	D	D	L	Q	E	A	I	A	S	W	L	S	K	G	K	Y	H	K	L	L	D	L	W	T	K	G	L	S	---	---	F	D	W	N	R	L	Y	G	A	---	---	Q	R	P	O	R	L	S	L	P		
DiffKS4	---	T	D	G	---	C	F	R	G	K	S	K	Q	Y	---	---	K	D	I	L	A	V	F	---	---	D	E	D	M	Q	E	A	V	R	K	M	K	R	K	F	A	K	L	L	E	L	W	V	K	G	L	Q	---	---	I	D	W	S	E	L	Y	N	G	---	---	S	L	P	H	R	I	S	L	P					
DiffKS2	---	H	A	H	---	I	W	R	G	R	T	A	K	H	---	---	H	S	L	A	A	S	L	T	S	D	E	D	M	A	L	T	I	N	A	W	L	K	G	K	Y	G	K	L	L	D	L	W	V	K	G	L	Q	---	---	V	D	W	S	A	L	Y	Q	---	---	R	N	P	G	R	I	S	L	P					
DiffKS3	---	T	G	Q	N	---	---	F	F	T	G	R	N	K	A	M	---	---	K	Q	L	F	T	E	M	T	D	N	A	E	M	S	G	T	V	Q	W	I	E	A	G	K	Y	A	M	L	A	K	L	W	V	Q	G	L	H	---	---	V	D	W	N	K	L	Y	B	---	---	R	P	K	K	V	S	L	P				
BaeKS6	---	G	E	G	---	I	Y	E	A	H	I	K	T	A	---	---	K	K	E	I	K	A	F	E	T	D	D	D	L	Q	L	V	R	S	W	F	D	K	K	K	Y	A	K	I	A	E	L	W	A	K	G	L	Q	---	---	M	D	W	O	R	L	Y	Q	G	---	---	R	K	P	R	R	I	S	L	P				
GUKS4	---	S	S	G	---	V	F	S	G	R	V	Q	E	G	---	---	K	N	E	A	G	L	F	S	S	D	E	N	G	K	A	L	L	T	W	I	Q	K	A	R	P	A	K	I	A	E	A	W	A	N	G	L	N	---	---	F	D	W	S	G	V	Y	G	A	---	---	E	T	P	R	T	I	S	L	P				
DiffKS9	---	S	E	D	---	I	I	S	G	R	K	K	R	---	---	---	---	N	N	T	I	S	M	F	S	D	D	E	G	A	A	D	L	V	S	A	W	L	E	K	K	K	Y	R	K	V	L	R	L	W	A	N	G	F	---	---	T	D	W	E	Q	L	Y	T	---	---	S	K	P	R	R	I	S	L	P				
TaKS8	---	S	A	A	---	V	L	S	G	Q	A	K	R	T	---	---	A	A	S	I	S	A	L	A	A	D	E	D	G	R	V	L	F	E	S	W	L	H	K	G	K	L	R	F	V	A	D	L	W	T	Q	G	V	---	---	V	D	W	R	R	L	Y	A	T	---	---	H	T	P	R	R	L	S	L	P				
TaKS7	Q	P	K	S	K	G	K	---	R	V	M	R	G	R	V	E	R	V	---	---	S	N	G	L	T	D	A	L	V	D	E	M	Q	Q	T	V	D	A	W	V	A	K	R	K	F	D	K	L	L	D	L	W	T	A	G	V	S	---	---	I	D	W	S	R	L	H	G	---	---	S	K	P	R	K	V	S	L	P	
MmpKS7	---	A	L	T	S	G	---	V	Q	V	G	S	A	R	Q	D	---	---	E	N	P	L	V	R	L	L	G	E	D	L	S	A	M	V	A	Q	W	A	A	T	G	Q	Q	K	L	L	A	L	W	S	G	G	---	---	I	D	W	Q	A	L	L	P	G	---	---	R	W	G	R	I	I	S	L	P					
PedKS8	---	T	Q	T	E	G	---	V	Q	Q	G	L	V	T	Q	E	A	---	---	E	E	Q	F	D	T	V	L	G	D	E	R	A	A	A	V	E	R	W	V	E	K	Q	Y	E	K	L	L	D	A	W	T	R	G	W	A	---	---	I	D	W	N	V	L	Y	G	T	---	---	R	T	P	R	R	I	G	L	P		
BTKS10	S	G	A	Q	A	S	---	G	V	L	R	G	V	R	A	A	---	---	D	A	Q	H	A	P	A	V	D	E	A	T	---	---	L	A	R	W	P	A	A	A	A	I	A	S	A	W	V	R	G	A	---	---	I	D	W	M	R	L	R	R	A	P	---	---	S	V	R	---	---	R	M	S	L	P					
OnnKS5	---	R	I	S	---	G	C	Y	T	G	S	A	R	H	A	G	---	---	N	V	L	R	I	L	G	D	E	A	H	E	L	I	Q	R	W	T	E	T	G	K	H	A	K	L	A	E	L	W	N	G	I	A	---	---	I	D	W	K	A	L	Y	R	G	H	S	---	---	P	R	R	I	S	A	P					
ChiKS11	A	---	---	---	V	L	R	G	R	A	R	S	D	---	---	---	---	D	R	A	L	L	P	G	D	D	E	G	V	R	S	L	I	A	Q	W	I	G	M	R	L	Q	Q	V	A	R	L	W	S	G	A	---	---	I	D	W	E	A	L	P	R	P	S	---	---	A	C	R	---	---	R	I	S	L	P				
ChiKS18	I	D	---	---	D	L	A	R	G	V	G	T	T	P	---	---	A	A	S	R	L	L	D	G	R	E	G	---	---	A	Y	L	R	I	V	I	E	E	R	K	L	R	K	L	A	Q	L	W	S	G	V	---	---	V	D	W	P	L	L	Y	P	---	---	T	P	P	---	---	L	M	Y	L	P						
DszKS10	V	D	---	---	D	L	H	V	G	S	A	A	A	S	---	---	---	---	L	S	A	L	L	L	D	G	R	E	G	---	---	A	F	L	S	I	L	V	E	D	G	R	H	D	K	L	A	R	L	W	S	G	A	---	---	I	D	W	T	L	Y	G	S	---	---	T	P	R	---	---	P	L	S	L	P				
OnnKS3	---	D	Q	I	A	D	---	C	Y	R	G	T	V	I	K	Q	N	---	---	I	E	T	F	S

614

615

Bacillaene Megacomplex

Bacillus subtilis

Assembly Line Domains

● Condensation (NRPS)	● Ketoreductase
● Adenylation (NRPS)	● Methyltransferase
● Dehydratase	● Thioesterase
● Ketosynthase and flanking subdomain	● Acyl Carrier Protein

100 nm