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Engineering modular polyketide synthases for production of biofuels and industrial chemicals

Wenlong Cai[1] and Wenjun Zhang[1,2]

Polyketide synthases (PKSs) are one of the most profound biosynthetic factories for producing polyketides with diverse structures and biological activities. These enzymes have been historically studied and engineered to make un-natural polyketides for drug discovery, and have also recently been explored for synthesizing biofuels and industrial chemicals due to their versatility and customizability. Here, we review recent advances in the mechanistic understanding and engineering of modular PKSs for producing polyketide-derived chemicals, and provide perspectives on this relatively new application of PKSs.

Addresses
[1] Department of Chemical and Biomolecular Engineering, University of California Berkeley, Berkeley, CA 94720, United States
[2] Chan Zuckerberg Biohub, San Francisco, CA 94158, United States

Corresponding author: Zhang, Wenjun (wjzhang@berkeley.edu)

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Introduction

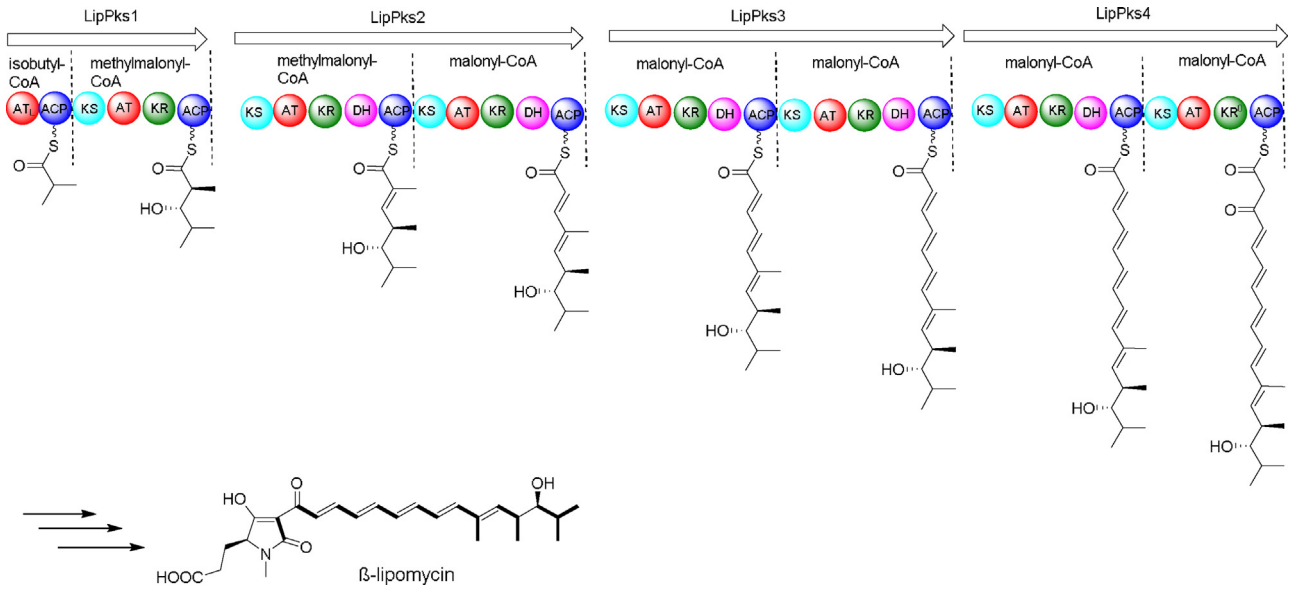
Polyketides are a large family of natural products widely used as drugs, pesticides, herbicides, and biological probes. They are biosynthesized by polyketide synthases (PKSs) that can be catalogued into three groups (type I, type II and type III). Different from type II or III PKSs which are comprised of discrete enzymes used iteratively to generate aromatics [1,2], type I PKSs are mega-synthases with catalytic domains organized into modules that function in an assembly line-like fashion either unidirectionally or iteratively to join together diverse acyl coenzyme A (CoA) building monomers (Figure 1) [3]. A typical type I PKS minimally contains an acyltransferase (AT) domain which is responsible for building monomer selection and loading, an acyl carrier protein (ACP) domain which has a 4′-phosphopantetheine arm to carry nascent polyketide chains, and a ketosynthase (KS) domain which catalyzes decarboxylative Claisen condensations for chain elongation. PKS megasynthases may also contain several optional catalytic domains, such as ketoreductase (KR), dehydratase (DH), enoylreductase (ER), and methyltransferase (cMT). Finally, the full-length polyketide chain is released from the assembly line, typically by a thioesterase (TE) domain, followed by post assembly-line modifications by downstream tailoring enzymes to yield diverse products with complex structures.

The inherent modularity of type I PKSs has led to numerous attempts in re-designing these modular assembly lines, known as combinatorial biosynthesis, to produce polyketides in a predictable manner for drug discovery and development [4,5,6•,7]. Although combinatorial biosynthesis has made encouraging advances over the past two decades, the field remains in its infancy with significant enzymological and technical challenges that must be addressed to efficiently produce un-natural polyketides. While the construction of *de novo* pathways, the ultimate goal of combinatorial biosynthesis, to produce complex polyketides as drug candidates remains a formidable undertaking, compounds with much simpler molecular scaffolds such as biofuels and industrial chemicals could be feasible targets for combinatorial biosynthesis with more achievable designs and implementation strategies [8]. Indeed, *de novo* PKS-based pathways have been designed and successfully implemented to produce a few products in this category (Figure 2). Here we highlight recent modular PKS engineering efforts for the bio-based production of fuels and industrial chemicals, focusing on the new knowledge of modular PKS enzymology to facilitate the efficient combinatorial biosynthesis of polyketide-derived products.

Modular PKS engineering: building monomer selection

Hundreds of distinct monomer blocks have been identified for PKSs, with the greatest diversity found on the loading module. Considering the natural variance in PKS starter units, identification and characterization of enzymes involved in starter unit generation and incorporation may yield a collection of portable biosynthetic machinery that can be utilized to install a functionality of interest through starter unit engineering. One example is a terminal alkyne functionality that has broad applications in chemical synthesis, pharmaceutical science, material science and bio-orthogonal chemistry [9,10]. In a typical type I modular PKS, a loading AT domain is often found in the loading module to select and transfer an acyl group

Figure 1



Activities of modular type I PKSs in the biosynthesis of β-lipomycin. The backbone programmed by PKS is highlighted in the final product, and the substrate specificity of AT domains is labeled. Isobutyl-CoA is used as the starter unit, and methylmalonyl-CoA or malonyl-CoA are used as extender units. The KR domain in the last module is inactive. The KR domain in LipPks1 is an A2-type KR while all other KRs are B-type.

from CoA to the loading ACP (Figure 3). Biochemical analysis of loading ATs has revealed that many of these domains have relaxed substrate specificity toward various acyl groups, and selected loading ATs have been successfully employed in engineered systems to incorporate desired functionalities for fuels and industrial chemicals. For example, as carbon branches are useful to lower the freezing point of biofuels, the loading AT from the lipomycin PKS was scrutinized to reveal a broad substrate specificity toward various short-chain fatty acyl-CoAs, including an unusual pivaloyl-CoA substrate in addition to its native isobutyryl-CoA substrate (Figure 1) [11]. This promiscuous loading AT was subsequently utilized in PKS engineering to introduce terminal carbon branches for β-hydroxyacid and short-chain ketone production, respectively (Figure 2a,b) [12••,13•]. In another example of dicarboxylic acid biosynthesis, a loading AT domain recognizing carboxyacyl-CoAs was needed. Toward this goal, the loading AT from the borrelidin PKS that had been predicted to incorporate a *trans*-1,2-cyclopentanedicarboxylic acid starter unit was biochemically studied to determine its carboxyacyl substrate tolerance [14]. Succinyl-CoA was successfully recognized by this promiscuous loading AT, which led to the production of the commodity chemical adipic acid after one round of chain extension (Figure 2c) [15••].

By contrast to the relaxed substrate specificity of loading ATs, most extending ATs only recognize malonyl-CoA or methylmalonyl-CoA as a substrate. The exact mechanism for substrate selectivity by various ATs is not fully understood, which is also reflected by the current limited success of targeted mutagenesis attempts in engineering AT domain specificity [16]. Alternatively, AT domain swapping has been a classical strategy to change extender unit selectivity, and has been applied to the production of industrially important short-chain ketones to yield either 2-ketones or 3-ketones derived from the terminal malonyl-extender or methylmalonyl-extender unit after decarboxylation (Figure 2b) [12••]. Recently, AT domains that recognize substrates other than malonyl-CoA or methylmalonyl-CoA have been identified, such as a *cis*-acting AT in antimycin biosynthesis [17] and a *trans*-acting AT in kirromycin biosynthesis [18••], providing new opportunities of using un-natural extender units for polyketide chain elongation to introduce a desired functionality for biofuels or industrial chemicals [19]. It is notable that a dedicated enzyme, such as a promiscuous malonyl-CoA synthetase or crotonyl-CoA carboxylase/reductase, is often needed to generate these atypical acylmalonyl-CoA extender units from acylmalonate or α,β-unsaturated acyl-CoA substrates, respectively (Figure 3) [20,21]. In addition to the gate-keeping ATs that select building monomers for PKSs, KS (and possible other domains) on the assembly line may also have substrate preference resulting in the abolished or reduced production of polyketides when an un-natural building monomer is used. While limited success has been achieved to tune the specificity of a KS domain [22], a KS domain with similar substrate specificity is often selected in the current pathway design to minimize the possible issue of KS gatekeeping effect that remains