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Revisiting the modularity of modular polyketide synthases

Chaitan Khosla[1,2,3], Shiven Kapur[1] and David E Cane[4]

Modularity is a highly sought after feature in engineering design. A modular catalyst is a multi-component system whose parts can be predictably interchanged for functional flexibility and variety. Nearly two decades after the discovery of the first modular polyketide synthase (PKS), we critically assess PKS modularity in the face of a growing body of atomic structural and *in vitro* biochemical investigations. Both the architectural modularity and the functional modularity of this family of enzymatic assembly lines are reviewed, and the fundamental challenges that lie ahead for the rational exploitation of their full biosynthetic potential are discussed.

Addresses
[1] Department of Chemistry, Stanford University, Stanford, CA 94305-5080, United States
[2] Department of Chemical Engineering, Stanford University, Stanford, CA 94305-5080, United States
[3] Department of Biochemistry, Stanford University, Stanford, CA 94305-5080, United States
[4] Department of Chemistry, Box H, Brown University, Providence, RI 02912, United States

Corresponding author: Khosla, Chaitan (khosla@stanford.edu)

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Modularity is a highly sought after feature in engineering design. Large-scale integrated circuits, automobile assembly lines and multipurpose chemical plants are just some examples of the power of modular engineering. (By contrast, notwithstanding its exquisite engineering elegance, a Swiss watch is not known for its modularity.) A modular system may be defined as a multi-component system that can be divided into smaller subsystems, which interact with each other and can be *predictably* interchanged for *functional* flexibility and variety. The two highlighted words merit particular attention as one considers the modularity of catalysts.

Guided by the above definition, the functional flexibility of a catalyst would either refer to its range of chemical transformations or the scope of its substrate tolerance. In the chemical catalysis literature, the term 'modular' is frequently used, although it most often refers to the preparative modularity of a catalyst. (Unlike proteins, the synthesis of man-made catalysts is not necessarily governed by modular principles.) A few man-made catalysts with modular reactive or molecular recognition features are known. In such cases, modular reactivity is achieved by swapping the metal center of an organometallic catalyst [1]. Alternatively, modular substrate range results from systematically altering a particular feature of the ligand structure [2]. In practice, this kind of predictable functional modularity invariably encounters serious limitations due to the intimate interplay between the metal and ligand. The ability to fully decouple catalysis from recognition as is, for example, the case for the ultimate catalytic machine, the ribosome, remains a lofty but elusive goal for man-made catalysts.

Against this general backdrop, it is worth reassessing modular polyketide synthases (PKSs), a family of multifunctional catalysts that has received much attention owing to their ability to synthesize a seemingly endless variety of complex natural products [3,4]. As implied in the above definition, it is the prospect of tapping into the functional modularity of these megasynthases (not merely their architectural modularity) that makes them attractive targets for engineering. Schemes 1 and 2 illustrate two different forms of functional modularity that one desires in a modular PKS. In both schemes E_1, E_2, E_3 and E_4 are sequentially acting catalysts, and B, C and D are intermediates in the polyketide biosynthetic pathway. In Scheme 1, E_2 is replaced with a different catalyst E_2' to alter a targeted functional group or stereocenter without affecting the rest of the natural product or the PKS turnover rate. Examples include replacing a ketoreductase (KR) domain of a PKS module with (i) a KR having different stereospecificity [5], or (ii) a tridomain comprised of a ketoreductase, dehydratase (DH) and enoylreductase [6], or (iii) an aminotransferase (AMT) for reductive amination of the β-ketoacyl intermediate (not yet demonstrated).

Scheme 2 illustrates a distinct modular principle, in which E_1 and E_2 are replaced by E_2' and E_2', respectively, so as to accommodate the processing of an alternative functional group or stereocenter (introduced via the substrate A′) without affecting the PKS turnover rate. Examples include regiospecific introduction of unnatural primer [7–9] or extender units [10,11] into a polyketide backbone.

It should be noted that in both of the above schemes, wild-type E_3 and E_4 have adequately broad substrate tolerance so as to process C′ and D′ with chemical and kinetic fidelity. Hence, modularity is not required of

Scheme 1

E_1 -> E_2 -> E_3 -> E_4 ->
A B C D E

E_1 -> E_2' -> E_3 -> E_4 ->
A B C′ D′ E′

Scheme 2

E_1 -> E_2 -> E_3 -> E_4 ->
A B C D E

E_1'-> E_2'-> E_3 -> E_4 ->
A′ B′ C′ D′ E′

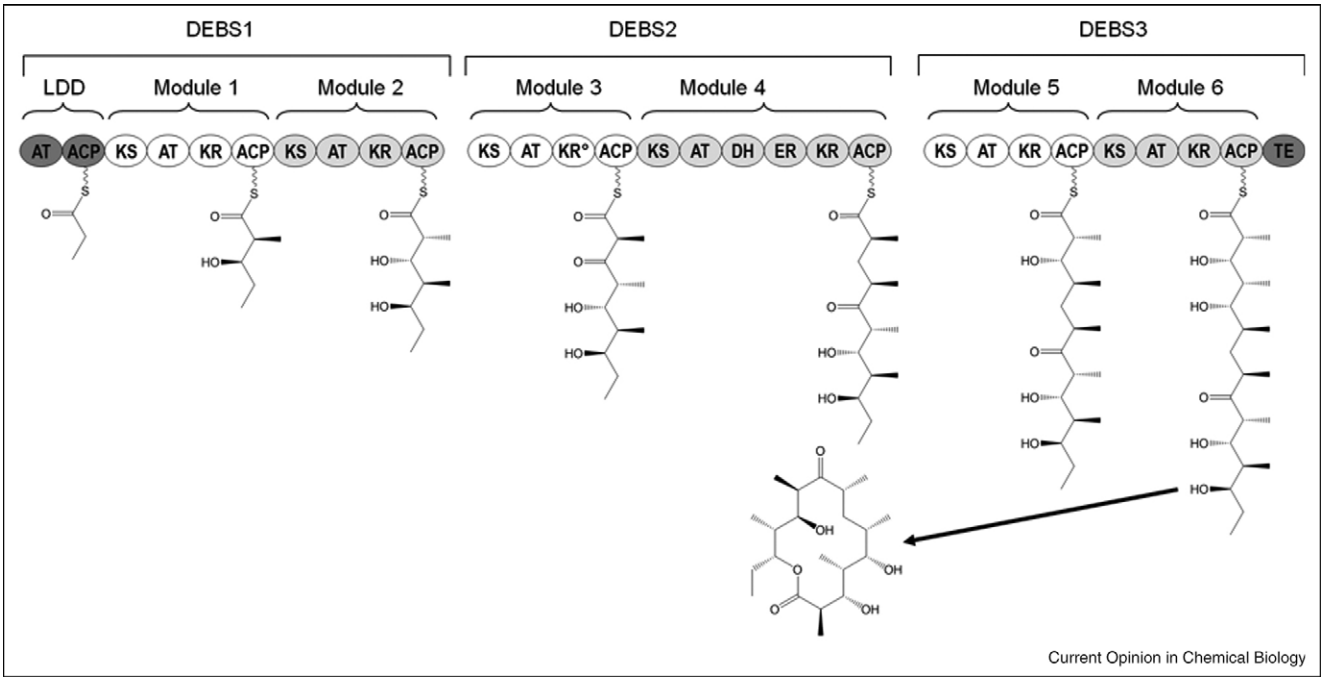
these PKS components. Below we review our current knowledge of the architectural modularity of a modular PKS, followed by a critical assessment of its functional modularity as exemplified in Schemes 1 and 2.

Architectural modularity

The depiction of modular PKS-catalyzed biosynthetic pathways through schemes as in Figure 1 has become common practice. The derivation of such schemes rests upon analysis of the primary sequence of the modular PKS, which reliably identifies individual catalytic centers, generally known as domains, within the megasynthase. The organization of these catalytic domains into modules is governed by the principle that, insofar as possible, active sites clustered along the covalent polypeptide backbone are responsible for catalyzing successive reactions in the polyketide biosynthetic pathway and vice versa. While this approach reliably maps individual enzymes from the 'assembly line' onto corresponding transformations in the biosynthetic pathway, its oversimplification precludes any description of limitations to the architectural (or functional) modularity of the PKS. There are a significant number of examples, for instance, of apparently competent catalytic KR or DH domains that clearly are functionally silent, based on the structure of the resulting polyketide natural product. Unfortunately, there are as yet no clear guidelines for identifying such catalytically silent domains absent knowledge of product structure.

Within the past few years, several high-resolution structures of prototypical components of the 6-deoxyerythronolide B synthase (DEBS) have been solved [12••,13••,14••,15•,16]. Together, these structures have

Figure 1



Modular organization of 6-deoxyerythronolide B synthase (DEBS). Chain elongation occurs minimally through the combined action of the ketosynthase (KS), acyl transferase (AT), and acyl carrier protein (ACP) domains. The final oxidation state of the β-carbon is controlled by the specific combination of ketoreductase (KR), dehydratase (DH) and enoylreductase (ER) domains present in a given module. Once processed, the polyketide chain is either passed to the KS domain of the downstream module or cyclized and released by the thioesterase (TE) domain at the C-terminus of the polyketide synthase. The loading didomain (LDD) is responsible for the selection and subsequent loading of the appropriate priming unit. KR°, inactive ketoreductase domain.