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**In vivo engineering of polyketide synthases**

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**Introduction**

Polyketides form the basic building blocks of numerous natural products, which are in use in pharmaceuticals, food additives and other fine chemicals. Polyketides derived from fungi are formed by type I iterative PKSes (iPKSes). The common domain structure of a non-reducing PKS (NR-PKS) is SAT-AT-PT-ACP-TE, which enables the NR-PKS to produce very complex polyaromatic compounds. Studies have revealed the general catalytic properties of these domains, and for some even the specificity can be predicted based solely by bioinformatics.\(^1\)\(^2\)

Some attempts have been made to investigate and engineer NR-iPKSes, but these have focused on in vitro assays.\(^1\)\(^2\) To speed up construction and screening the present study focuses on in vivo analysis in *S. cerevisiae* of native and engineered iPKSes. To engineer the NR-iPKSes the combination of SAT-AT and PT-ACP-TE tridomain units of different origin should create new compounds. The used linker between the tridomain units has been designed by multiple alignment of all the studied NR-iPKSes and by HMM investigation of the region between the AT and PT domains. This revealed a 12 amino acid long conserved region. This region is used as a uniform linker in the synthetic chimeric iPKSes as it will not extend the overall amino acid chain, thus native protein structure should be conserved.

**Overview of investigated iPKSes**

The domain structure, product length and first ring formation are shown. Right column requires an unloading enzyme as the iPKSes are lacking a TE/RED domain.

**Chemical logic of chimeric iPKSes**

The SAT-AT-TE tridomain is proposed to control chain length while PT-ACP-TE tridomain control the folding pattern. By combining non-native tridomains it may be possible to engineer novel polyketide scaffolds.

**Outlook**

The combination of tridomain modules form vastly different NR-iPKSes will generate insight into the biochemical logic of the NR-iPKSes. As an example, the combination of tridomains from long chain and short chain iPKSes will show whether short chain ACPs can carry long chain products or if the PT-ACP-TE domain dictates premature release. If the designed linker is found to render the enzymes inactive, another design could focus on investigating a longer more flexible linker. This has been previously shown to enable different enzymes to function better in an assembly line fashion.\(^3\) Another option would be to mimic previous in vitro assays and express NR-iPKS domains individually.\(^1\)\(^2\)

**References**


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