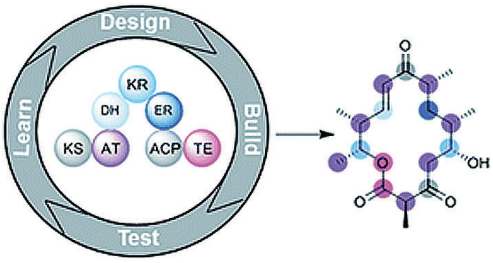
Accelerating the Development of Designer Polyketide Molecules

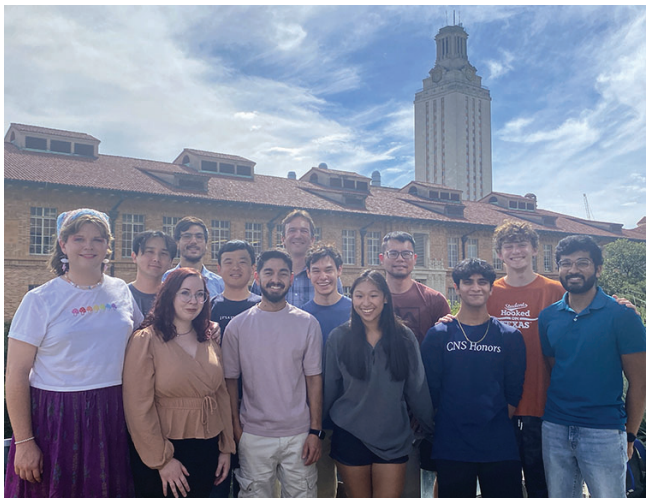
Elegen's cell-free ENFINIA™ DNA accelerates projects with high sequence complexity from the drawing board to the incubator, saving weeks to advance research goals faster.

Polyketides are a source of bio-active molecule representing approximately 20% of top-selling drugs. As valuable as previously isolated polyketides have proven to be, the rise of antibiotic-resistant microorganisms and the demand for novel anti-infective and anticancer treatments underscore the necessity for additional polyketides. Given their diverse and targeted biological effects, exploring new polyketides remains an important and active area of research.

Complex polyketides are a class of natural product synthesized by large, multi-domain enzyme complexes known as modular polyketide synthases (PKSs). The domains within a synthase are organized into functional modules that carry out extension and modification of the growing polyketide product. The polyketide molecule is passed from domain to domain; each domain either extends it by one polyketide unit or performs one chemical modification.



The design-build-test-learn (DBTL) iterative cycle is inherent in the bio-discovery process. It can take weeks or even months to manufacture highly complex DNA sequences, including designs with high or low GC content, long hairpins, and repeat regions.



The Keatinge-Clay Lab at The University of Texas at Austin

Within diverse microbes, the DNA-encoding modules assemble into gene clusters that produce synthases that biosynthesize a diverse range of compounds used for medicinal, agricultural, and industrial purposes (for example, the antibiotic erythromycin). Because polyketides are often used by microbes as chemical weapons against other microbes, they are a good source of bio-active molecules.

TAKING ON THE CHALLENGES OF PKS GENE CLUSTER SYNTHESIS

Until recently, the ability to engineer polyketide biosynthesis for the discovery of new compounds has been limited by a lack of technology and know-how. With the availability of

BY THE NUMBERS

14,713 bp

de novo plasmid synthesis

3

weeks turnaround for delivery

thousands of bacterial genomes and an improved understanding of how PKS modules are organized, researchers like those in the Keatinge-Clay lab at the University of Texas at Austin use synthetic DNA to engineer and heterologously express PKSs for the *in vivo* biosynthesis of designer polyketides. The Keatinge-Clay lab primarily works with PKSs from *Streptomyces* bacteria, which are encoded by long, repetitive, GC-rich sequences difficult to amplify from genomic DNA.

The high GC-content and length of PKS gene clusters have historically proved challenging for conventional gene synthesis suppliers. Even with modern codon-optimization tools, sequences are often very complex and littered with near-repeats that lead to assembly errors. Codon optimization can also have detrimental effects on protein translation and product yield, making native-like sequences paramount for reconstitution.

To mitigate these risks, the Keatinge-Clay lab prefers to isolate the gene clusters directly from genomic DNA. When that is not feasible, the lab asks external gene synthesis providers to produce synthetic gene fragments to their longest length with as little alteration as possible. Even with this approach, the lab typically must order multiple 5 kb gene fragments to build PKS gene clusters for insertion into their custom expression vectors. Due to the length and complexity of each fragment order, it can take three or more weeks from the time of order to receive the set of fragments from external suppliers, and another one to two weeks to build the final expression vector internally.

FROM THE DRAWING BOARD TO THE INCUBATOR IN WEEKS

The Keatinge-Clay lab ordered *de novo* synthesis of a 14,713 bp plasmid containing a PKS gene cluster. After receiving the full-length ENFINIA™ DNA plasmid just three weeks later, the Keatinge-Clay lab transferred the gene cluster into a custom plasmid for biosynthesis. Elegen's rapid, full-length plasmid synthesis saved the lab one to two weeks of assembly time and labor when compared to traditional approaches. Furthermore, Elegen synthesized the entire sequence with minimal changes to avoid the risks of yield loss.

Having observed successful biosynthesis in their heterologous host of choice, the Keatinge-Clay lab plans to expand their pool of synthases for testing. With Elegen's ability to synthesize regions of high complexity, the Keatinge-Clay lab is able to move projects with difficult, complex sequences, including repetitive GC-rich regions, from the drawing board to the incubators in a matter of weeks.

LEARN MORE

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