methods. Yeasts such as *Saccharomyces cerevisiae* have been extensively engineered to manufacture high-value products because they are capable of performing many of the complicated transformations that occur late on in biosynthetic pathways. However, difficulty in producing large quantities of dopamine — an important intermediate in the biosynthesis of many metabolites — from simple carbon feedstocks such as glucose, has prevented yeasts from being used to synthesize benzylisoquinoline alkaloids, a group of natural products that include morphine and codeine. The bottleneck in this process is the hydroxylation of L-tyrosine to L-3,4-dihydroxyphenylalanine (L-DOPA), a transformation that is conducted by tyrosine hydroxylases in plants. However, expressing these enzymes in a functional form in bioengineered yeast has proved very challenging.

In an effort to create a tyrosine hydroxylase that is functional in *S. cerevisiae*, a team led by John E. Dueber at the University of Berkeley, California, has now developed an enzyme-coupled biosensor to help them search for such an enzyme that is expressed in an active form. The biosensor uses another plant enzyme, DOPA dioxygenase, which converts L-DOPA to an easily observable yellow fluorescent pigment called betaxanthin. Expressing the biosensor in *S. cerevisiae* along with different tyrosine hydroxylases enabled the team to determine whether any L-DOPA was being produced, and if it was, to quantify how much. Using this approach they were able to find an active tyrosine hydroxylase that was then optimized by PCR mutagenesis.

This tyrosine hydroxylase was then shown to facilitate the biosynthesis of dopamine from glucose — the first demonstration of this in *S. cerevisiae*. The mutations introduced into the tyrosine hydroxylase were also found to limit an unwanted side-reaction. Further bioengineering enabled the biosynthesis of more complicated downstream metabolites with the biosynthetic pathway extended through to (*S*)-reticuline, the final intermediate that is common to the major branches of benzylisoquinoline alkaloids. *RJ*

DNA DUPLEXES

Carefully crafted crosslinks

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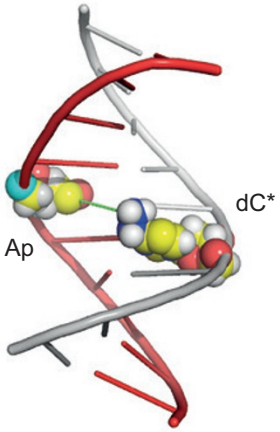
Covalent crosslinking that can occur between the strands of a DNA duplex is of interest both in materials science, where it can impart stability to oligonucleotide-based assemblies, and in biochemistry, as it prevents replication and transcription processes and results in cytoxicity. Studies on crosslinking and the repair processes that naturally occur in cells



have, however, been hindered by the fact that crosslinking agents typically promote the formation of a variety of linkages. Jacqueline Gamboa Varela and Kent Gates, from the University of Missouri, have now devised a simple, high-yield synthesis to crosslink complementary oligonucleotides at a specific location.

The researchers built on previous work that had identified the formation, within a duplex, of a linkage between the abasic site (Ap) of one oligonucleotide — generated by the loss or removal of a coding nucleobase — and a guanine or adenine residue of the opposite strand. Now, Gamboa Varela and Gates have instead crosslinked the Ap residue with a non-natural nucleobase, N^4-amino-2′-deoxycytidine (dC*), installed for this purpose at a defined location. The Ap and dC* residues react through their respective aldehyde and hydrazine moieties to form a covalent hydrazone linkage. The complementary strands containing Ap and dC* were prepared through straightforward treatments of the corresponding deoxyuridine- and cytosine-containing oligonucleotides, using the enzyme uracil DNA glycosylase, and bisulfite and hydrazine in sodium phosphate, respectively. The Ap and dC* moieties are positioned in a manner that facilitates the formation of the hydrazone crosslink: the two residues are offset by one base in the hybridized duplex, and dC* is mispaired.

Characterization by gel electrophoresis analysis showed that the crosslink formation occurred in high yield at the predetermined location. The resulting dC*–Ap hydrazone linkage was stable under physiological conditions, increased the thermal stability of the duplex, and was found to be thermally reversible: repeated heating and cooling resulted in its cleavage and regeneration, respectively. *AP*

Written by Stuart Cantrill, Claire Hansell, Russell Johnson and Anne Pichon.

blogroll

Patently inventive

Toxicology, the odds of discovering a drug and creativity in design.

Spurred on by a tweet from *C&EN*'s Lisa Jarvis (http://go.nature.com/kzo6li), an active discussion has developed on Twitter and in the blogosphere about the odds of a chemist discovering a drug over an entire career. Some number crunching by Derek Lowe, writing at In The Pipeline (http://go.nature.com/7YIjoS), produced a figure around 1%. Ash Jogalekar at The Curious Wavefunction argues (http://go.nature.com/TvLdQR) that the question is more complicated and wonders whether "the patent system has become biased toward chemists". In the protracted, multidisciplinary development of a drug, who is truly responsible for its invention?

Over at The Dose Makes the Poison (http://go.nature.com/sdzWY8), ForensicToxGuy writes of the more illicit innovations in synthetic cannabinoids and what can be considered to be the 'wild west' of drug design. The creativity of these outlaw medicinal chemists in developing chemical diversity never ceases to amaze and is creating a "public health nightmare" of substances with unknown pharmacological and toxicological effects. Meanwhile, writing at amphoteros (http://go.nature.com/1frDgN), Andrei Yudin talks of chemical aesthetics and the merit of elongated molecules in drug design, however ugly they may be.

While the chemical sciences continue to innovate, Vittorio Saggiomo delves into the history of Parafilm — another ugly, yet incredibly handy invention — on his blog Labsolutely (http://go.nature.com/1i8Tjz). Many would shudder to think of a laboratory without that greasy, stretchy film. Finally, the ever-present Kristof Hegedüs at Pictures from an Organic Chemistry Laboratory (http://go.nature.com/W4RmXy) tells a cautionary tale of a nitration gone wrong and the importance of a good lab coat. Even the most commonplace of innovations may well be the most vital.

Written by Luke Gamon, who blogs at https://lukegamon.wordpress.com and tweets as @lgamon