Hacking nature: genetic tools for reprograming enzymes



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Enzymes have many modern industrial applications, from biomass decomposition in the production of biofuels to highly stereospecific biotransformations in pharmaceutical manufacture. The capacity to find or engineer enzymes with activities pertinent to specific applications has been essential for the growth of a multibillion dollar enzyme industry. Over the course of the past 50–60 years our capacity to address this issue has become increasingly sophisticated, supported by innumerable advances, from early discoveries such as the co-linearity of DNA and protein sequence[1] to modern computational technologies for enzyme design. The design of enzyme function is an exciting nexus of fundamental biochemical understanding and applied engineering. Herein, we will cover some of the methods used in discovery and design, including some 'next generation' tools.

Traditionally, enzymes with useful biochemical properties have been sourced from nature, tapping into the natural diversity generated by evolution. Where known physiological functions are useful in an industrial setting, it is relatively simple to match an enzyme to an application (e.g. amylase-mediated glucose production from starch). Where novel functions are required, enrichment culturing of microbes can be used: for example, the recent isolation of bacteria capable of using nylon intermediates as a nitrogen source with potential utility in nylon manufacture (Figure 1)[3]. Non-culture based methods can also be applied to enzyme discovery, for example by exploiting the explosion of genetic information that followed the 'omics' revolution. Driven by technological advances in DNA sequencing, and computational power, this has provided an enormous resource, accessible by bioinformatic analysis, and leading to the discovery of enzymes with industrial applications, such as novel imine reductases for asymmetric organic synthesis[4].

Methods have also been developed to move beyond the repertoire of enzymes currently known in nature. One of the most prevalent approaches has been to use atomic information about structure and function to rationally redesign enzymes, often to expand or alter substrate range, change stereospecificity or alter physical

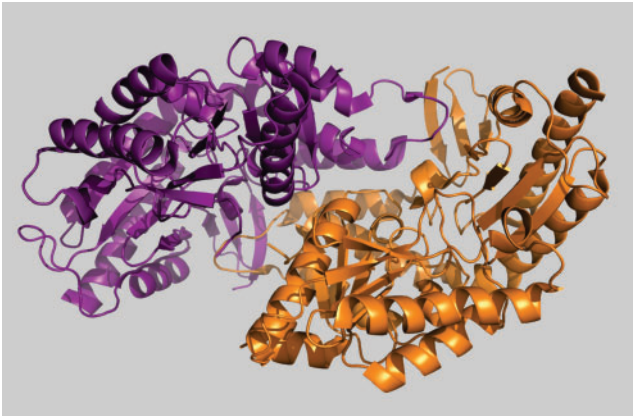

Figure 1. Structure of the *Pseudomonas* sp. strain AAC putrescine transaminase (PDB: 5ti8), which has potential application in nylon manufacture[2].

properties, such as thermostability[5]. Enzyme engineers have also exploited the power of evolution, by applying artificial selection pressures or selective screens to probe libraries of enzyme variants, often generated by low frequency amino acid randomisation[6,7]. This method has been especially effective in accessing the cryptic biochemical diversity available through promiscuous enzyme activities, i.e. physiologically irrelevant 'side-reactions' that do not confer a fitness cost or benefit[8,9]. Combining rational engineering principles with an evolutionary approach has proven particularly powerful: small focused libraries are synthesised, guided by structure-function information, and then screened/selected for properties of interest[10]. Such strategies have been used to alter properties such as stereospecificity, expression level[11] and Michaelis constants (K_M)[12] and to overcome functional constraints, such as inhibition by substrates and/or products leading to improved reaction yields[13].

Impressively, it has also been possible to engineer wholly new catalytic functions for enzymes, such as direct amination of unactivated carbon atoms[14], cyclopropanation[15] and Diels-Alder cycloaddition[16] (albeit, naturally-occurring enzymes for the latter reaction have since been discovered[17]). Several stratagems have been used to introduce 'unnatural' functionality into enzymes, with examples of mechanism-based re-engineering of extant natural enzymes[6,14,15] and computer-aided *de novo* design of new active sites[18–20]. Often, enzymes constructed using the current iteration of such techniques have limited catalytic functionality; however, the catalytic properties of such synthetic and semisynthetic enzymes can be improved by direction evolution and related methodologies[21,22].

In the approaches considered above, enzyme engineers have been content to explore the chemical space provided by the 20 canonical proteinogenic amino acids. However, there have been considerable efforts to expand the chemical repertoire of amino acids and add functionality to enzymes by introducing non-natural amino acids. The synthesis of hundreds of non-natural amino acids have already been reported, with functionalities including halides, cyanides, azides and alkenyl/alkynyl groups for 'click' chemistry, as well as fluorophores and a range of others[23].

Incorporating these amino acids into proteins and living organisms has proven challenging; however, an approach in which components of the protein synthesis apparatus have been re-engineered is proving successful. By reprograming aminoacyl tRNA synthetases to accept non-natural amino acids, rather than their native ones, it is now possible to repurpose codons for the incorporation of non-natural amino acids[23,24]. In some recently reported methods, this approach has been used to incorporate dehydroalanine *via* dephosphorylation of *O*-phosphoserine that had been incorporated using a repurposed tRNA synthase[25–27]. The highly reactive dehydroalanine allows post-translational installation of non-natural side-chains. The potential of such methods is enormous, nonetheless, there is another problem that needs to be addressed. The genetic code is already fully utilised encoding the naturally occurring proteinogenic amino acids. Repurposing a codon for a non-natural amino acid will affect every gene that uses that codon, and modify every protein transcribed by those genes. How, then do we make space for an expanded repertoire of amino acids?

Recent advances in synthetic biology may hold the key. One option, which has recently been reported to be successful, is to recode entire genomes, removing codons from use[28]. In principle, the redundancy of the genetic code can be eliminated so that each amino acid is encoded by a single codon, making available coding space for novel amino acids. More ambitiously, it is possible to expand the genetic code by introducing non-natural nucleotides[29], and in doing so introduce an array of new codons with no natural function. Albeit this additional information is currently 'inaccessible' to the cell, pending reengineering of the cellular machinery to recognise and translate this new coding space.

The rate of advancement in our ability to design and repurpose enzymes has been enormous, with much of our fundamental understanding of protein biochemistry and laboratory and computational tools developed over the course of the past 50 years. In that time, we have progressed from randomly surveying the natural diversity of enzymes in easily cultured organisms, to *de novo* enzyme design and overcoming the limitations of nature's chemical toolbox. As new tools from disciplines such as synthetic biology will support the expanding utility of enzymes, we can expect this field to continue to evolve and play a role in the next industrial revolution.

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Biographies

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Dr Colin Scott obtained his PhD in molecular microbiology from the University of Sheffield in the UK in 2000 before taking up a post-doctoral fellowship with the CSIRO. He currently leads the Biotechnology and Synthetic Biology Group at the CSIRO. He has strong interests in enzyme evolution, biocatalysis, microbial physiology and synthetic biology.