Redesigning nature’s catalysts

Harnessing the power of natural enzymes to perform reactions outside their normal abilities is adding powerful tools to the synthetic chemist’s armoury. James Mitchell Crow investigates.
Enzymes are the molecule-making masters. Nature’s elegant answer to catalysis carry out a dazzling array of different transformations, with a selectivity and specificity that we can often only wonder at.

Rather than trying to compete with this kind of performance, synthetic chemists are increasingly co-opting it instead. Using enzymes to catalyse our own reactions, though, is not the simple solution it might first sound.

‘Nature has done a fantastic job at making a very sophisticated arsenal of catalysts, but there are not natural catalysts for every conceivable chemical reaction that chemists or biologists might like to promote,’ says Donald Hilvert, who develops biocatalysts at the Swiss Federal Institute of Technology (ETH) in Zurich. Over the past 10–15 years, however, researchers in the field have developed an increasingly sophisticated array of tools to re-engineer enzymes, to adapt their specificity to the reaction in question.

‘This whole field has moved on quite significantly over the past few years,’ says Nick Turner, director of the Centre of Excellence for Biocatalysis, Biotransformations and Biocatalytic Manufacture in Manchester, UK. ‘There are still tremendous developments being made in the academic community, but we’re really seeing the application of these engineered enzymes in industry now as well.’

As the power of enzyme catalysis becomes clearer, the pace of development has picked up – especially since industry has begun to take a serious interest in the last two to three years, Turner adds. ‘Every biocatalyst that gets developed for a commercial application has probably now been through a protein engineering phase. More and more, enzymes are engineered for practical applications.’

Recent advances in the field were brought into focus in July last year, by a pair of papers that appeared together in the same issue of Science. One paper revealed the remarkable potential of computational chemistry to design enzymes with totally new functionality not seen in nature. But the other paper was arguably the more significant.

Industrial need
Sitagliptin is the active ingredient in the type 2 diabetes drug Januvia, made by US pharmaceutical company Merck & Co. The final step in the large-scale manufacture of sitagliptin involves converting a ketone into a chiral amine, a troublesome step involving the rhodium-catalysed asymmetric hydrogenation of an intermediate enamine. The stereoselectivity of the transformation was inadequate, and additional purification steps were needed to remove the rhodium.

‘As Merck’s Jacob Janey explains, the desire to improve sitagliptin manufacture was not the only reason why the company decided to invest the time to try to develop a biocatalyst-based alternative to the rhodium reaction. ‘Within Merck we had identified the transformation of a ketone to a chiral amine to be a key piece of technology that we thought was worth developing, not only for sitagliptin but as a general methodology,’ says Janey, a senior research chemist based in Rahway, New Jersey, US. ‘Hence we took this initial risk to develop the chemistry.’

Merck researchers worked with enzyme specialists Codexis to search for a biocatalytic answer to the problem. What is remarkable about this research, for which the scientists won one of the US Environmental Protection Agency’s 2010 Presidential Green Chemistry Challenge awards, is the lengths that the team had to go to in order to develop an active transaminase biocatalyst. The enzyme that they started with was a known transaminase, but its activity towards Merck’s starting molecule was essentially zero – the substrate was too large to fit within the enzyme’s active site.

From zero to hero
To re-engineer such an enzyme, where do you start? Enzymes are proteins, long chains of amino acids folded up to form a 3D structure, within which forms the reactive pocket known as the active site. Adapting an enzyme for a new substrate means switching around some of those individual amino acids – changing the shape of the pocket to fit.

But which to switch? A quick look at the numbers shows the extent of the challenge, says Hilvert. ‘A small protein, say 100 amino acids, has 20000 different isomers, and we can’t look at all of those – that’s more atoms than there are in the universe. It’s hard to look at even a tiny fraction of those possibilities.’

Traditionally, two different approaches have been developed to deal with this problem. Rational design deliberately targets particular amino acids in the protein structure. But predicting how changes to the amino acid sequence of a protein will affect its three dimensional structure is notoriously difficult to do. So the other approach is make random mutations, and use high-throughput screening to pick out the best one. This best candidate can then be subjected to further rounds of mutagenesis, picking out the best example from each generation, and so gradually evolving the enzyme’s structure. It’s an approach known as directed evolution.

Increasingly, the most effective way forward has been to use a hybrid of the two approaches – as was the case with sitagliptin. The team used computational modelling to map the enzyme’s binding pocket, identifying two regions that would need to be adjusted for the substrate to fit.

They then used directed evolution targeted at the amino acids lining the binding pocket, working on the two problematic regions one at a time.

‘The tough part in this entire activity was developing that initial activity – going from zero to

Developing a biocatalyst improved efficiency and eliminated metal impurities in the synthesis of Merck’s Januvia

In short

- Two recent papers have shown that we are beginning to be able to design new enzymes from scratch
- Merck & Co’s enzyme amination catalyst is helping make drug synthesis greener and more efficient
- US researchers fitted a new active site onto an existing enzyme to perform unnatural Diels–Alder reactions
- Total design of entirely new enzymes could help us understand nature’s catalysts and unlock powerful new ones
Artificial enzymes

If you’re going to re-engineer an enzyme, why limit yourself to the same narrow range of building blocks that nature uses?

Unnatural amino acids
Nature uses just 20 amino acids to build virtually all of its proteins. However, many of them rely on non-amino acid cofactors for their particular functionality. Researchers looking to re-engineer enzymes are freer in their choices. Why not introduce new properties by expanding the range of amino acids that the protein itself is built from? Scientists such as Peter Schultz at the Scripps Research Institute in La Jolla, US, have shown how enzymes can be tailored and their reactivity changed by introducing unnatural amino acids into the protein sequence.

Artificial enzymes
Chemists have tapped a rich vein of catalytic activity in the transition metal region of the periodic table, from the palladium catalysts of Heck, Suzuki and Sonogashira fame to Robert Grubbs’ eponymous ruthenium compounds. Nature, on the other hand, is limited to the handful of metals that are readily bioavailable. One band of biocatalyst pioneers is developing hybrid enzymes that harness the best of both worlds, combining the reactivity of palladium and the like with the selectivity and specificity of enzymes.

Organocatalyst-inspired
Another new branch of catalysis that has progressed dramatically over the past decade is organocatalysis – the use of small organic molecules to catalyse reactions. Certain amino acids, including proline and phenylalanine, have been found to be effective organocatalysts. But so have many other small molecules, naturally derived or otherwise. Researchers such as David Baker at the University of Washington are looking to design enzymes with novel activity by building them around an organocatalyst active site.

Foldamers
These protein-inspired structures take the idea of unnatural enzymes and sprint with it. Why use any natural amino acids at all? Donald Hilvert at ETH Zurich has worked with Samuel Gellman at the University of Wisconsin, Madison, US, to make a polymer of β-amino acids, which have an extra carbon in their structure compared to natural α-amino acids. This polymer folds up to form a structure that catalyses the aldol reaction. Polymers made from other synthetic building blocks can also form foldamers. One potential advantage of these materials is that they could be used where natural enzymes typically don’t work, such as at high temperature.

Breaking nature’s rules

Less waste, more product
All told, the Merck–Codexis team took the enzyme through 11 rounds of evolution, making 27 individual mutations to the original enzyme. The benefits of all that work are clear. Compared to the original rhodium-catalysed route, the overall yield is improved by over 10 per cent, the total waste produced by the process falls by 19 per cent, heavy metals are eliminated and costs are reduced. ‘We are currently in the process of validating the new chemistry for use in our commercial sitagliptin manufacturing operations,’ says Janey.

Accumulating mutations (purple) through rounds of directed evolution can adapt natural enzymes for new activities

It’s examples such as this that have made industry think that the potential benefits are worth the effort. ‘This publication really caught people’s imagination,’ says Turner. ‘They not only changed the substrate specificity, they also improved the stability of the enzyme for process applications, which is obviously very attractive to industry.’

Turner has applied his own work with engineered enzymes to improve drug synthesis. Last year, he published the results of his team’s collaboration with Romano Orru at VU University of Amsterdam in the Netherlands, who specialises in multicomponent reactions. These reactions clip together three or more starting molecules in a single step, so offer rapid access to complex compounds. However, such reactions tend to be poorly stereoselective. But controlling stereochemistry is one of the key advantages of biocatalysis – and putting the two together turns out to be a very efficient way to make molecules, says Turner.

Generic drug companies, which produce low-cost copies of medicines once their patent protection expires, are certainly starting to take more notice of engineered enzymes. ‘A lot of big blockbuster drugs are coming off patent, or will do in the next few years, so the generics companies are really gearing up,’ says Turner. ‘They plan many years ahead to make sure that they have new registered routes available when the molecule does come off patent. They’ve started to get interested in biocatalysis – again, in some cases it could be a low-cost option. Once things become generic, cost is really key.’

Where no enzyme has gone before
Such approaches are all very well for tapping the kinds of chemistry that nature already uses. But what about the reactions for which there is no enzyme-catalysed equivalent? The Diels–Alder reaction, say, which is one of the most powerful approaches synthetic chemists have devised to build complex molecular structures?

The second of the pair of papers in Science shows that there is a way – but also illustrates the size of the challenge. David Baker at the University of Washington in Seattle, US, and colleagues developed a
functioning ‘Diels–Alderase’ – an enzyme that binds two substrates in the right orientation for them to undergo the Diels–Alder reaction, forming two new carbon–carbon bonds to generate a new six-membered ring.

‘We’ve worked for quite a while on the problem of computing protein structures from their sequence, and designing protein structures is the flip-side of that,’ says Baker. ‘Catalysis is one of the most impressive functions that natural proteins do, so designing new catalysts was an obvious challenge.’

The first step was to compute the shape of the transition state of the reaction, then design the ideal active site to fit around and stabilise it. At this point in the design process, the active site is simply a cluster of atoms in macromolecular systems. They searched a database of known stable protein scaffolds for ones that had the right geometry on which to graft their designed active site.

This process generated 80 potential proteins, which the team synthesised and tested. 50 of the enzymes were soluble, and of these, two showed some catalytic activity. These were further improved by site-selective mutagenesis. The resulting catalysts were highly substrate-specific, highly stereoselective – but not very fast.

‘In terms of science, the work is really fantastic,’ says Turner. ‘Not many labs can do what he can do. But in terms of putting it on the map of absolute activity, there’s still some way to go to get to the kinds of activity that enzymes derived from natural sources have.’

Hilvert, who was also involved in the project, agrees that there is still a way to go before computer-designed enzymes come close to the catalytic performance of the real thing.

‘Computational design is a very, very promising tool for the future, and a number of significant successes have been achieved in the last few years,’ he says. ‘The Diels–Alderase is not the final answer, but it shows that it should be possible to get there.’

Baker is already working on the next step. ‘Our big push now is to improve our success rate, so that instead of having to test 80 [structures] to get 2 [active enzymes], we’d like to test 80 to get 80. And we’d like to increase the activity of the designed proteins, which is usually really low.’ Rather than have to transpose their designed active site onto an existing scaffold, one thing the team is working on is designing a scaffold from scratch to hold everything exactly in place for that reaction, he adds.

In the meantime, computation will continue to play a vital role in re-engineering enzymes for industrial applications – just more along the lines of the calculations carried out by Merck and Codexis. ‘What I’m most optimistic about is the possibility of combining computational searches of sequence space with experimental evolutionary searches,’ says Hilvert. ‘The computation gives you the starting platform from which you can then evolve new activities. I think there’s enormous promise there.’

However, Hilvert also sees another compelling reason to continue to push forward in this area – to fully understand natural enzymes themselves. ‘True enzymes accelerate reactions by 6–16 orders of magnitude. When you try and mimic this you capture a fraction of it, and the question is, where do enzymes get those last few orders of magnitude, what makes them so special? I don’t think that we understand that at all. So trying to design new catalysts that rival the efficiency of natural enzymes will hopefully teach us about steps we need to take to reproduce these marvellous efficiencies.’

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References
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