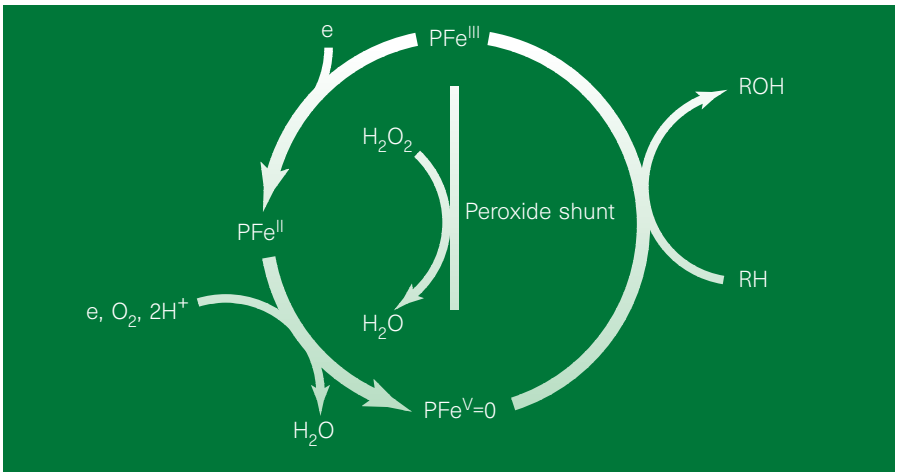

Figure 2 **Catalytic cycle of cytochrome P450. Arnold and co-workers[2] have developed more efficient enzymes using the 'peroxide shunt' pathway to bypass a complex cofactor regeneration system. In this pathway, reaction of hydrogen peroxide (H_2O_2) with the oxidized iron porphyrin complex (PFe^{III}) leads to the active oxidant (PFe^{V}=O) in a single step, instead of by reduction to PFe^{II} followed by reaction with O_2 and reducing sources (electrons and protons) from the cofactor. Reaction of the substrate (RH) with PFe^{V}=O gives the product (ROH), returning the enzyme to the resting state (PFe^{III}).**

The main task was to develop a suitable high-throughput screening method. The authors achieved this in the following novel way. The enzyme horseradish peroxidase catalyses the oxidative coupling of hydroxylated aromatics, using H_2O_2 as the oxidant, to generate coloured and fluorescent materials. They showed that expression of cytochrome $P450_{cam}$ with a variant of horseradish peroxidase (HRP1A6) in *Escherichia coli* creates a pathway for the conversion of aromatic substrates such as naphthalene into fluorescent compounds.

Approximately 200,000 mutants of cytochrome $P450_{cam}$, produced by error-prone PCR and coexpressed with HRP1A6, were screened for activity in the reaction of naphthalene with H_2O_2 using fluorescence digital imaging. A benefit of this screening method is that different hydroxylation substitution patterns lead to different colours. For instance, a combination of 1- or 2-naphthol and 2,7-dihydroxynaphthalene produces red fluorescence, whereas 2-naphthol with 1,5-dihydroxynaphthalene produces yellow fluorescence. So, the screening allows insight into the altered regiospecificities of the various mutants. Indeed, a palette of colours was observed with bacteria expressing the $P450_{cam}$ mutants (see Fig. 4 on page 672).

Three brightly fluorescing clones were selected for growth and their enhanced activity was confirmed in a whole-cell assay. Moreover, DNA sequencing revealed that all three of the improved P450 mutants share the same mutation (a lysine substituted for glutamate). There is no obvious, rational explanation for this mutation, which highlights the unique ability of the directed evolution technique to identify mutants that may not be selected by other methods. A second-generation $P450_{cam}$ library prepared by *in vitro* recombination of five improved variants yielded two clones that were roughly 20 times as active as the wild-type $P450_{cam}$ in the whole-cell assay. In short, Arnold and co-workers[2] have developed an elegant screening method that enables the directed evolution of cytochrome P450 enzymes to generate mutants that are more active, possibly more stable and bypass the need for cofactor regeneration in the natural enzyme.

The resulting enzymes are still far from being commercially viable, yet the first hurdle has been passed. The enzymes may show improved activities in other characteristic P450 transformations, such as olefin epoxidation. But there may be a limit to the stability that can be achieved with haem-dependent enzymes, owing to the inherent instability of the porphyrin structure under oxidizing conditions (a lesson we have learned from studies of peroxidases[4]). So it would be interesting to extend the methodology of Arnold and co-workers to the directed evolution of non-haem monooxygenases, some of which catalyse similar reactions to the P450 enzymes. Perhaps this will eventually lead to commercially viable monooxygenases for the regioselective and enantioselective oxidation of fine chemicals. □

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1. Sheldon, R. A. (ed.) *Metalloporphyrins in Catalytic Oxidations* (Dekker, New York, 1994).
2. Joo, H., Lin, Z. & Arnold, F. H. *Nature* **399,** 670–673 (1999).
3. Stemmer, W. P. *Nature* **370,** 389–391 (1994).
4. van Deurzen, M. P. J., van Rantwijk, F. & Sheldon, R. A. *Tetrahedron* **53,** 13183–13220 (1997).

Photonics

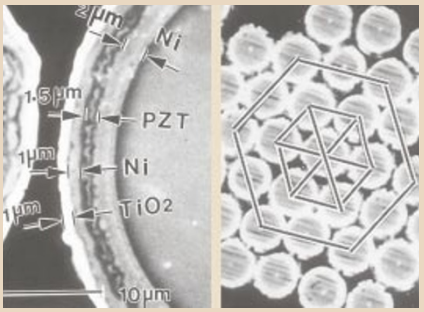
Crystals to order

Controlling the behaviour of particles suspended in a fluid is critical, whether you are trying to mix paint or separate crude oil. A group at the Hong Kong University of Science and Technology are taking the control of particle properties in solution to a new level, in the hope of creating useful photonic materials (the optical analogues of semiconductors).

Ping Sheng and his colleagues (*Phys. Rev. Lett.* 82, 4248–4251; 1999) coated micrometre-sized glass spheres with a layer of nickel, followed by PZT (lead zirconate titanate, a quartz-like ceramic), another layer of nickel and finally titanium oxide. The four coatings (left side of figure) are intended to give a large response to applied electric or magnetic fields.

The coated spheres are suspended in silicon oil. In zero applied field, the spheres are randomly dispersed. As the external electric field is increased, the particles form columns, and other changes emerge when a magnetic field is applied. By freezing the system at different field strengths and taking cross-sectional micrographs, Sheng and co-workers have snapshots of the changing structure (right side of figure).

They discover that as the magnetic field increases, the spheres change from a body-centred-tetragonal (bct) structure to a face-centred-cubic (fcc) structure. Small movements of the spheres produce the structural transition, without any long-range diffusion. Such a transition can be achieved — simply by varying the relative strengths of the external fields — because the difference in energy between the bct and fcc structures is very small.



A tunable crystal structure might offer new ways to make photonic materials that permit the passage of photons of certain energies, while excluding others. The difficulty in finding photonic crystals with the right properties (lattice dimensions from a few hundred nanometres to tens of micrometres) could be solved by these made-to-order crystals. **Sarah Tomlin**