caesium-doped phenanthrene, prepared by Yasuhiro Takabayashi and colleagues through a second synthetic route[9]. In this case, the reaction of caesium with the PAH was carried out in solution, affording highly pure crystalline samples of Cs_xPhenanthrene, with $x = 1$ or 2. Although Cs_2Phenanthrene ($x = 2$) remains a nonmagnetic insulator, analogous to the K_2PAH samples discussed above, CsPhenanthrene ($x = 1$) is shown to exhibit quantum spin-liquid behaviour. In this rare and exotic magnetic phase, no long-range magnetic order is possible and the magnetic frustration, which persists even down to the lowest measured temperatures, supports unconventional excitations with fractional quantum numbers such as spinons. The experimental realization of such a quantum phase — materials that are currently discussed as a suitable platform for performing calculations in quantum computers — is, however, very scarce. With CsPhenanthrene, Takabayashi and co-workers propose a possible new spin-liquid candidate and directly demonstrate the potential for further exploration of these materials, for which high-quality samples are now attainable.

Stepping back, this work continues a long history of materials research in organic carbon-based systems. In 1911, Herbert McCoy and William Moore had already advanced the idea that suitably prepared organic materials could support unpaired electrons, granting them conductivity combined with magnetic properties analogous to those of metals[10]. It was not until the 1950s and 60s, however, that this prediction was experimentally realized, with the discovery of semiconducting behaviour in doped PAHs. By the 1970s, the utility of doping to introduce charge-carrying electrons was fully established, through the report of metallic conductivity in polyacetylene treated with halide vapours[11] — a development that ultimately earned Alan Heeger, Alan MacDiarmid and Hideki Shirakawa the 2000 Nobel Prize in Chemistry. Around this time, Robert Haddon posited that superconductivity should also be realizable in PAH materials[12], pre-empting later discoveries in the 1980s and 90s. These culminated in the achievement of record-high superconducting transition temperatures exceeding 30 K in alkali-doped C_{60} (refs 13,14).

Over the intervening time, the development of organic electronics has been pursued in earnest, inspiring far-reaching advances from organic semiconductors to light-emitting devices, to the emergence of graphene and carbon nanotubes. The present advancements in synthetic methods, 26 years since the first inspiring reports of superconducting buckyballs, now firmly establish alkali-doped PAHs as attractive species to explore novel material science. ❐

Roser Valentí and Stephen M. Winter are at the Institut für Theoretische Physik, Goethe Universität Frankfurt, Max-von-Laue-Strasse, 1, 60438 Frankfurt, Germany.
e-mail: valenti@itp.uni-frankfurt.de

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ENZYMATIC CATALYSIS

New functional twists for P450s

Two papers provide insight into the reactivity of cytochrome P450s. A direct link between electron donation and reactivity has been shown with a selenocysteine-ligated P450 compound I, whereas a serine-ligated P450 (P411) has been engineered to catalyse an intermolecular C–H amination via nitrene transfer.

Rudi Fasan

Cytochrome P450s are a superfamily of heme-dependent enzymes that fulfil a broad range of critical functions across all domains of life[1]. In humans, liver P450s are responsible for the metabolism of drugs and detoxification of other exogenous substances to which we are exposed on a daily basis. In bacteria, these enzymes are involved in the breakdown of environmental compounds, including man-made pollutants, converting them into metabolites that are utilized by these organisms as food or as an energy source.

Across all living species — including bacteria, fungi, plants and animals — cytochrome P450s play also a key role in the biosynthesis of important compounds such as steroid and eicosanoid hormones as well as a plethora of structurally diverse natural products. Because of the breadth of functional roles and reactions performed by these enzymes, they have been the focus of intense investigations, ranging from fundamental studies to elucidate their structure and catalytic mechanisms[2], to efforts directed at exploiting them as biocatalysts for synthetic applications[3,4]. Now, two Articles, one by Michael Green[5] and co-workers and another by Frances Arnold and colleagues[6], provide new important insights and twists into the catalytic function of this fascinating class of enzymes.

A distinctive feature of cytochrome P450s is their ability to generate a short-lived and highly reactive species in the form of oxoiron(IV) porphyrin π-cation radical, also known as Compound I (Cpd I)[2]. Compound I has been compared to a 'blowtorch' capable of engaging the substrate in a variety of oxidation reactions, including mono-oxygenations (hydroxylation, epoxidation, N/S oxidation) as well as other oxidative processes[7,8]. Most notably, P450-Cpd I is able to mediate the insertion of an oxygen atom into carbon–hydrogen (C–H) bonds, including electronically unactivated C(sp^3)–H bonds. This transformation is notoriously challenging from a chemical standpoint because of the strength of the C–H chemical bond, and their ubiquitous

presence in organic molecules makes controlling the site-selectivity of such reactions very difficult. Nevertheless, some P450s can catalyse the hydroxylation of C–H bonds in a specific substrate with high efficiency and high degrees of chemo-, regio- and stereoselectivity.

It is well known that the Cpd I intermediate provides the oxidative power for mediating C–H bond cleavage and oxyfunctionalization — via a hydrogen abstraction/radical rebound mechanism — whereas the protein structure surrounding the active site provides the framework that positions the substrate above the active centre in a defined orientation, thereby providing the high site selectivity for the oxidation reaction. Nonetheless, the question of what structural features contribute to the peculiar reactivity of P450s compared to other heme-dependent enzymes has intrigued scientists for a long time. For example, histidine-ligated heme peroxidases also give rise to a Cpd-I intermediate as part of their catalytic cycle, yet this species is unable to functionalize strong aliphatic C–H bonds as P450-Cpd I does.

A highly conserved structural feature in P450s is a cysteine residue axially coordinating to the iron atom of the heme cofactor (iron protoporphyrin IX). Previous studies involving synthetic models of P450s as well as proximal ligand variants of P450s, and other hemeproteins have pointed to the importance of the axial thiolate ligand in dictating the peculiar reactivity of P450-Cpd I[9–11]. The Article by Green and co-workers now sheds new light into the correlation between electron donation from the axial ligand and the reactivity of this catalytic intermediate. In this study, Green and co-workers substituted the heme-coordinating cysteine in a thermophilic P450 (CYP119) with the structural analog selenocysteine. This was achieved by expressing the enzyme in cysteine auxotropic bacteria in the presence of selenocysteine. Due to the small size difference between the sulfur and selenium atoms (~0.1 Å), this substitution causes minimal structural perturbation in the protein and metal coordination environment. At the same time, the selenolate group is more polarizable and has greater nucleophilic character and lower redox potential than thiolate, thus furnishing an ideal system to probe the influence of electron donation from the axial ligand on the electronic properties and reactivity of the oxoferryl radical intermediate.

Selenocysteine heme-ligated P450s have been prepared and investigated before[12,13], but successful trapping and characterization of the SeP450-I intermediate has been elusive. This challenging task has now been achieved via freeze-quench techniques adopting experimental conditions applied previously for trapping Cpd I in wild-type CYP119[14]. These experiments indeed showed that selenolate-bound Cpd I occurs very transiently, accumulating and rapidly decaying within a 10–15 millisecond timescale. Characterization of the trapped intermediate via Mössbauer and electron paramagnetic resonance (EPR) spectroscopy revealed a series of spectroscopic features (larger quadrupole splitting and ^{57}Fe hyperfine coupling, smaller isomer shift, higher | J/D | ratio) that are consistent with the increased electron donation by the selenocysteine axial ligand compared to the cysteine-ligated system found in the wild-type protein. Furthermore, by monitoring reactions in the presence of a deuterated and protiated substrate via stopped-flow spectroscopy, valuable information on the differential reactivity between SeCYP119-Cpd I and the cysteine-ligated counterpart could be obtained. These experiments showed that although Cpd I forms at a comparable rate in the selenocysteine- and cysteine-ligated systems, SeCYP119-Cpd I reacts more rapidly with the substrate than CYP119-Cpd I (Fig. 1), to the point of becoming undetectable in the presence of the protiated substrate.

Together, these studies provide direct evidence showing that the increased electron donation provided by the selenolate ligand enhances the reactivity of the oxoferryl radical intermediate toward C–H bond cleavage. In turn, these findings provide another important piece of experimental evidence in support to the critical role of the heme-coordinating axial ligand in dictating the reactivity of P450 Cpd I. Further studies will be required to elucidate in more detail the kinetic and thermodynamic factors that underlie the enhanced reactivity of selenocysteine-ligated Cpd I compared to the cysteine-ligated counterpart during C–H bond cleavage and oxyfunctionalization. From an applied perspective, it will be interesting to see whether these findings can be leveraged to engineer P450s with improved catalytic properties, even though cysteine-to-selenocysteine substitutions in other P450s have resulted in small differences in reactivity[12]. A complicating factor in this regard relates to the complex catalytic cycle of P450s, which entails multiple steps as well as unproductive pathways[2], each of which could be affected by the introduction of selenocysteine as the heme-coordinating ligand.

Natural and engineered P450s hold great potential as biocatalysts for selective C–H hydroxylation and epoxidation reactions[5,6]; however, recent studies have demonstrated that the scope of these enzymes can be expanded to include other synthetically

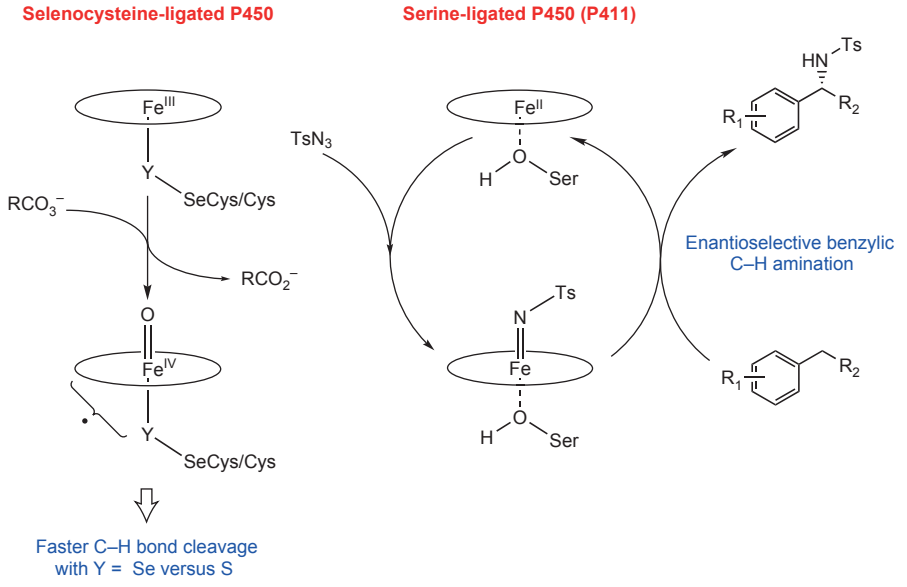

Figure 1 | Substitution of the heme-coordinating cysteine residue in P450s provides a point of entry for investigating the native reactivity of these enzymes as well as developing new chemical reactivity. Left: Trapping and characterization of a transient catalytic intermediate in a selenocysteine-ligated P450 provides new evidence for the importance of electron donation from the axial ligand on the C–H bond cleavage reactivity of this species. Right: A serine-ligated P450 was engineered to yield a biocatalyst capable of mediating the intermolecular amination of benzylic C–H bonds with high activity and selectivity.

valuable transformations such as C–H amination reactions via nitrene transfer processes[15,16]. These reactions are important because they provide a streamlined route for the synthesis of C–N bonds, which are ubiquitous in biologically active molecules, including many drugs. Furthermore, metal-catalysed nitrene transfer reactions are not found in nature and so far they have been accessible only via chemical catalysis; although synthetic catalysts have been developed for these transformations, they generally suffer from limited catalytic efficiency and/or selectivity, and/or require rare and toxic metals.

Recent studies showed that in the presence of azide-based substrates as nitrene donors, P450 variants can be engineered to catalyse the conversion of C(sp^3)–H bonds into C–N bonds via a nitrene C–H insertion mechanism[15,16]. However, so far these C–H amination reactions have been limited to intramolecular settings. Arnold and co-workers have now demonstrated that P450s can be engineered in the laboratory to mediate intermolecular C(sp^3)–H aminations with high catalytic efficiency and stereoselectivity (Fig. 1).

A key challenge in these non-native P450-catalysed nitrene transfer reactions is related to the occurrence of an off-cycle pathway that leads to the unproductive transformation of the azide substrate to a reduced by-product (for example, sulfonamides from sulfonylazide reagents)[16]. To overcome this issue, the team has first identified a P450 variant that exhibits detectable, albeit still modest activity in the desired reaction; that is, the benzylic C–H amination of 4-ethylanisole with tosyl azide. This starting point was provided by an engineered variant of the bacterial P450 BM3 containing a total of 17 mutations and bears a substitution of the heme-coordinating cysteine with serine (P411). The latter was previously found to enhance the intermolecular nitrene transfer reactivity of these enzymes with other substrates such as olefins and sulfides[17,18]. Next, the P411 variant was subjected to iterative rounds of active site mutagenesis followed by library screening, resulting in the identification of a significantly improved biocatalyst called $P411_{CHA}$, which catalyses the intermolecular C–H amination of the initial substrate as well as other related compounds with high activity (200–1,300 turnovers) as well as excellent stereoselectivity (92–99% e.e.). Careful examination of the substrate scope revealed that the scope of these $P411_{CHA}$-catalysed reactions is rather broad, although it is currently limited to electronically activated benzylic C(sp^3)–H bonds. The challenge of targeting stronger C–H bonds in biocatalytic intermolecular amination reactions thus remains one which could be addressed by future efforts in this field.

Along with other recent studies[19], the work of Arnold and co-workers nicely demonstrates how challenging C(sp^3)–H functionalization reactions can be realized by means of inherently 'green' and renewable biocatalysts incorporating earth-abundant metals. In these cases, neither the free metal cofactor nor the wild-type protein exhibit the desired reactivity, highlighting the power of combining protein engineering and directed evolution to endow natural enzymes with new catalytic functions.

The structural and functional diversity of cytochrome P450s have fascinated chemists, biochemists and biologists alike for decades. These two Articles elegantly show how exciting new functional insights as well as synthetically useful and unprecedented reactivity can be uncovered through the investigation and engineering of these enzymes. ❐

Rudi Fasan is in the Department of Chemistry, 416 Hutchison Hall, RC Box 270216, University of Rochester, Rochester, New York 14627-0216, USA.
e-mail: rfasan@ur.rochester.edu

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DNA NANOTECHNOLOGY

Bringing lipid bilayers into shape

Lipid bilayers form the thin and floppy membranes that define the boundary of compartments such as cells. Now, a method to control the shape and size of bilayers using DNA nanoscaffolds has been developed. Such designer materials advance synthetic biology and could find use in membrane research.

Stefan Howorka

Lipid bilayers play a crucial biological role by forming a dynamic boundary around cells and subcellular organelle units. Membranes are also used in biomedicine to coat bioimaging agents or drugs. In contrast to proteins or DNA, membranes lack a defined structure. Instead the semifluid membranes are composed of amphiphilic lipids that typically form layers, within which the lipid molecules are able to move freely. Although this non-covalent nature makes lipid membranes fragile, this is also a virtue — by being deformable they can also be adapted into various dynamic forms, as needed for a specific function.

In biology, the task of shaping and stabilizing membranes is carried out by the cytoskeleton, a soft scaffold composed largely of interconnected protein rods that are linked to the membrane. The cytoskeleton