Boyer Center. This was a real shot in the arm for the project, allowing a continuous exchange of ideas and enthusiasm — a great deal of which emanated from Paul. Enter also David Boisvert, a student with considerable experience in crystallization. Soon he and Kerstin had set up a 'wall' of trays with one particular molecule that produced nice-looking crystals under several conditions. That molecule, which we had termed 'wild type', turned out to be a variant form, carrying two mutations that had been introduced by PCR. Somehow (it's not clear in chemical terms why, even at this point), those mutations allowed Kerstin to produce an ammonium sulfate orthorhombic crystal that was the first structure solved (with one ring in the asymmetric unit).

"The day she put one of those crystals up on the Siemens area detector next door was a memorable one. There it was — 3.5 Å diffraction. We grabbed Paul, who came running in to have a look. Wow! was all any of us could think. But Paul, in a very organized way, immediately considered how to proceed. He integrated crystals of this form into a synchrotron trip — ensuing literally the next week — and gathered us all to consider with care how to proceed to collect data from this complex, where to put the detector, what the oscillations could reasonably be, what mosaicity would be acceptable, and how much time would be taken for the data set. Then he, Zbyszek, and basically Paul's entire team geared up to help collect and reduce data from this large complex. At CHESS, Kerstin put up several crystals that did little or nothing, and then at last she mounted a gem that diffracted to 2.8 Å.

"In the next week, Paul spent literally an entire afternoon educating us on MIR and the various things to try. Kerstin, working mainly with Zbyszek, but also with help from the entire group, was successful in producing an ethyl mercury derivative. And then Zbyszek, in an unbelievably short period of time, maybe 48 hours, put together a Patterson search program and solved the structure. I rushed home from a meeting in the UK to the mystical experience of sitting there on a Saturday morning in front of the graphics terminal with Paul, Kerstin, and Zbyszek looking at the molecule, still at low resolution — but there it was!

"I remember driving Paul home, ideas buzzing between us at light speed. It must have been a high point for everyone involved. Extending phases with seven-fold NCS averaging, Zbyszek produced a model from which he traced the entire chain, correctly, despite some pretty messy-looking density in the experimental map of the apical peptide-binding domain. Months of further work on refinement followed, much of it driven by Rashmi Hegde in Paul's group. Throughout it all were the many wonderful sessions of looking at this molecule and discussing it with Paul, as he pointed out features and contacts, always thinking about the functional aspects of this machine and proposing hypotheses that were immediately testable."

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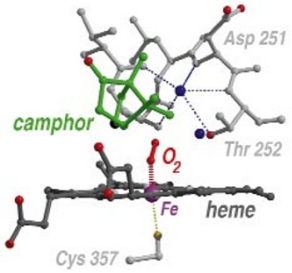
Freeze frame

Adding oxygen, say in the form of a hydroxyl group, onto a hydrocarbon is not easy. In the absence of catalytic agents, the reaction will proceed only at high temperatures, and even then, the addition of hydroxyl groups may occur at several positions simultaneously. To perform this task at physiological temperature and at specific sites, Nature has designed enzymes, such as those in the cytochrome P450 family, to catalyze a wide range of oxidation reactions of organic molecules, including those involved in steroid synthesis and drug metabolism.

At the active site of a cytochrome P450 is an iron-containing heme group that binds and activates oxygen for the oxidation reaction. Both the molecular oxygen-bound form and the subsequently activated oxygen-bound form of the enzyme are short-lived intermediates in the reaction, making it difficult to directly observe their structures. Now, by collecting X-ray diffraction data at liquid nitrogen temperature on crystals of a cytochrome P450–substrate system (the bacterial $P450_{cam}$–camphor complex) trapped at different intermediate stages of the reaction, Schlichting and coworkers (*Science*, **287**, 1615–1622) have provided the first snapshots of these intermediate species.



To obtain a stabilized intermediate for structural characterization, Schlichting and coworkers first diffused a reductant, dithionite, into the crystal of the $P450_{cam}$–camphor complex to reduce the ferric ion and prime the protein for oxygen binding. They then exposed the crystal to oxygen. Importantly, both steps were performed at low temperature thus trapping the dioxygen bound intermediate.

At the active site of the ternary $P450_{cam}$–O_2–camphor complex, one atom in the dioxygen molecule (red) is clearly observed bound to the iron (purple); the other oxygen atom is in close proximity to camphor (green) allowing van der Waal interactions between the two molecules. Two well-ordered water molecules (blue) are in place to form hydrogen bonds with the highly conserved Asp 251 and Thr 252, both of which had been implicated in the catalytic reaction. These two water molecules were absent in the structure of the ferric $P450_{cam}$–camphor complex (no oxygen bound), and could be involved in the oxygen-activation step of the reaction.

The structure of the enzyme intermediate at atomic resolution confirms many conclusions derived from spectroscopic and mutagenesis studies and provides a new reference point for future studies on the reaction mechanism of cytochrome P450. This work also demonstrates the potential of low temperature X-ray crystallography in investigations of enzyme catalysts. *Hwa-ping Feng*