SIZE DOESN'T MATTER FOR CYTOCHROME P450

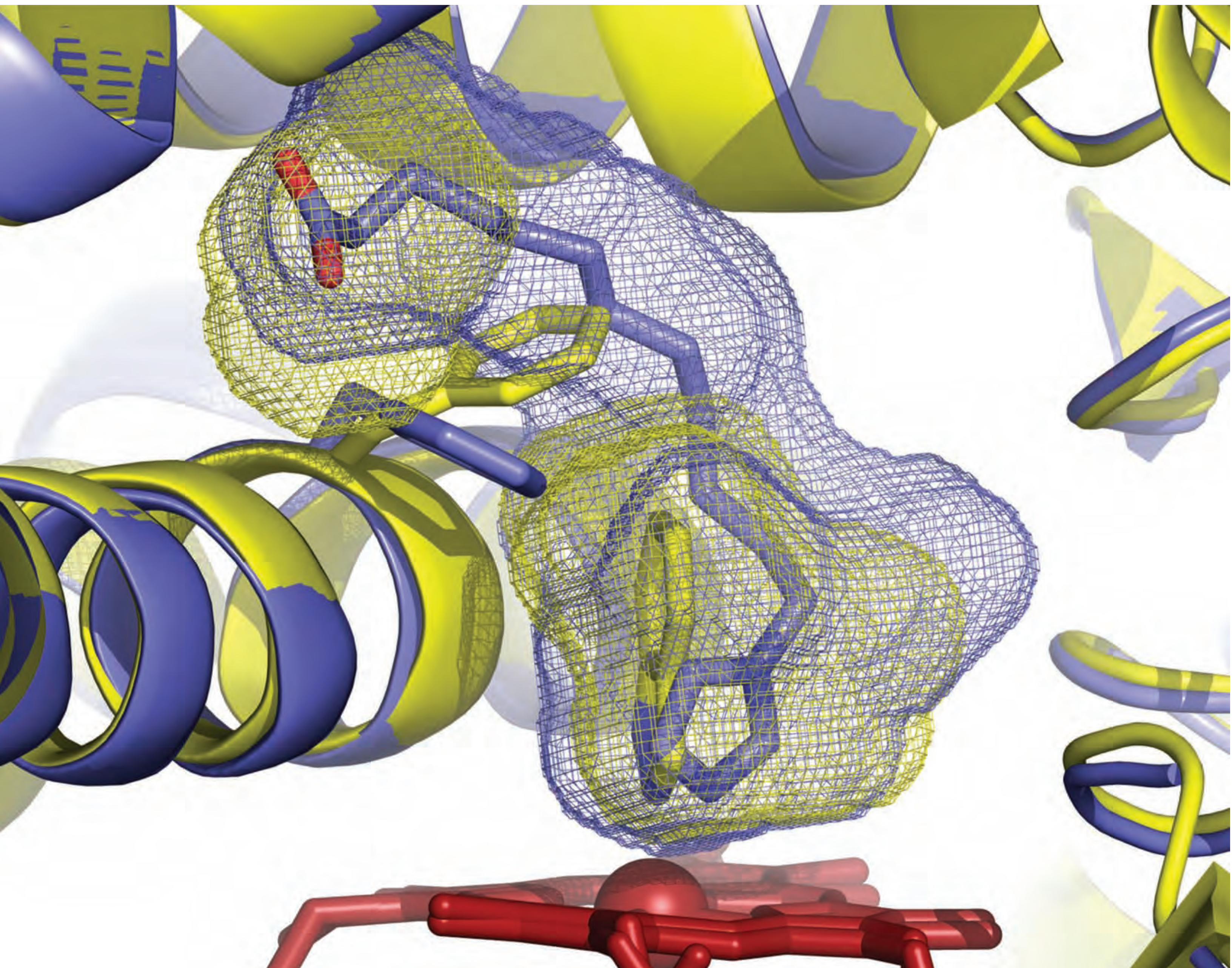

Fig. 1. When CYP2E1 (yellow ribbons) binds indazole (yellow sticks), it binds above the heme prosthetic group (red sticks) in a small enclosed active site (yellow mesh) separated from another small adjacent void (yellow mesh) by Phe298 (yellow sticks). However, when CYP2E1 binds an imidazolyl fatty acid analog (blue sticks), the overall backbone structure of the enzyme (blue ribbons) doesn't change, but Phe298 (blue sticks) rotates to merge the two indazole structure voids into one continuous cavity (blue mesh) to accommodate the fatty acid compound.

The cytochrome P450 (CYP) superfamily is responsible for the metabolism of many normal cellular compounds as well as foreign compounds such as drugs. In humans, nine members of this superfamily of enzymes are estimated to process up to 75% of pharmaceutical compounds, making them an important focus of clinical medicine. The CYP enzyme 2E1(CYP2E1) oxidizes more than 70 compounds, ranging from small-molecule drugs such as acetaminophen to larger molecules such as the long-chain fatty acid arachidonic acid, an important cellular signaling molecule. Previous work determined the structure of CYP2E1 in the presence of small-molecule substrates, but how the active site might also accommodate the long fatty acid substrates was unclear. To answer this question, a research team utilizing two U.S. Department of Energy x-ray light sources built on previous work by solving the co-crystal structures of CYP2E1 with three fatty acid analogs of varying chain length. This study provides new insights into the mechanism of action of this important class of enzymes.

One challenge faced by the researchers — from The University of Kansas and IMCA-CAT — in determining the structure of the enzyme bound to a fatty acid was to identify an appropriate fatty acid to co-crystallize with CYP2E1. Fatty acids consist of a head group of an acid at one end and a long hydrocarbon tail. The latter makes them relatively water-insoluble and difficult to work with under crystallization conditions that require millimolar concentrations of protein in an aqueous solution. In this case, addition of a carbon-nitrogen-containing ring called imidazole to the last carbon in the fatty acid tail increased the solubility, as well as causing an absorbance change that could be used to monitor ligand binding to the protein. Finally, imidazole addition encouraged the fatty acid analogs to bind in a single orientation corresponding to the major fatty acid metabolite observed experimentally. This is helpful because fatty acid ligands can bind CYP2E1 in multiple orientations, which are difficult to resolve with x-ray crystallography.

These substrate analogs, imidazole-containing fatty acid chains of 8, 10, and 12 carbons in length, were co-crystallized with CYP2E1 and their structures solved to 2.9 Å-, 2.7 Å-, and 3.1-Å resolution, respectively. The 10-carbon analog was solved at the IMCA-CAT 17-BM beamline at the APS; the 8- and 12-carbon analogs were solved at beamline 9-2 at the Stanford Synchrotron Radiation Light Source.

This work reveals the nature of a CYP2E1 active site that is dramatically expanded to accommodate these molecules. Furthermore, the binding mode is unlike that observed in other fatty acid-binding cytochrome P450 molecules. The structures all had the basic CYP fold and were overall very similar to the previous CYP2E1 structure, but the active site itself reveals dramatic changes when long chain fatty acids are bound. In the small molecule structure, the size of the active site was 190 $Å^3$, but when bound to fatty acids, the active site was more than doubled to 420 $Å^3$ for the 8-carbon compound, 440 $Å^3$ for the 10, and 473 $Å^3$ for the 12-carbon compound. This striking change in active site architecture is not the result of large scale changes in the structure of the enzyme but rather the rotation of a single amino acid, phenylalanine 298, which moves to merge the previously-identified small active site with an adjacent void between nearby helices in the protein (Fig. 1).

Studies on this enzyme have revealed a third, substantially different, active site topology when an inhibitor is bound. These remarkable changes in active site shape and volume are determined by the substrate that is present and make the prediction of drug substrate metabolism very difficult. For this reason, the researchers plan to solve additional structures of the enzyme with other substrates bound in order to understand its ability to oxidize 70+ different substrates. — *Sandy Field*

See: Patrick R. Porubsky[1], Kevin P. Battaile[2], and Emily E. Scott[1]*, "Human Cytochrome P450 2E1 Structures with Fatty Acid Analogs Reveal a Previously Unobserved Binding Mode," J. Biol. Chem. **285**(29), 22282 (July 16, 2010). DOI:10.1074/jbc.M110.109017

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17-BM • IMCA-CAT • Life sciences • Macromolecular crystallography, multi-wavelength anomalous dispersion, single-wavelength anomalous dispersion • 7.5 keV-14 keV • On-site, remote, mail-in • Accepting general users