Structure of AlkB–AlkG shows details of alkane terminal C–H selectivity and functionalization

The cryo-EM structure of a natural AlkB–AlkG fusion from *Fontimonas thermophila* reveals the mechanistic basis for its selectivity towards, and functionalization of, alkane terminal C–H groups. AlkB contains an alkane entry tunnel and a diiron active site, and AlkG docks through electrostatic interactions and transfers electrons to the diiron center for catalysis.

This is a summary of:
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The question

Alkanes are abundant hydrocarbons in crude oils, which makes them attractive starting materials for the production of chemical feedstocks and value-added products[1]. However, they are chemically inert and difficult to functionalize selectively. The complex electron transfer system AlkBGT – which consists of the catalytic membrane-bound monooxygenase AlkB, the soluble rubredoxin AlkG, and the soluble rubredoxin reductase AlkT – was first identified in *Pseudomonas oleovorans* and uses alkanes as its sole carbon and energy source. AlkB selectively hydroxylates alkanes at the terminal C–H group with broad chain-length specificity and can be engineered to convert alkanes into alcohols, aldehydes, carboxylic acids, and epoxides[2], which provides a cost-effective and efficient way to functionalize alkanes. For the past 50 years, a branch of research has focused on understanding and engineering the AlkBGT system. However, structural understanding of the system and the basis for its alkane terminal selectivity and functionalization remained elusive.

The observation

We rationalized that determining the structure of key components in the system would provide the basis for understanding its function. To identify suitable proteins for structural analysis, we synthesized more than 20 genes, each from a different species, encoding AlkB or its AlkB–AlkG fusion (AlkBG) variants. We expressed the genes in *Escherichia coli* and purified the proteins using affinity tags. This approach yielded highly purified homogeneous *F. thermophila* AlkBG (*Ft*AlkBG). To stabilize the complex for structure determination, we screened reconstitution conditions using different detergents, lipids, and polymers, and identified the polymer PMAL-C8 as a suitable reagent for stabilizing *Ft*AlkBG. We then deployed single-particle cryo-electron microscopy (cryo-EM) to characterize the *Ft*AlkBG structure. To improve the signal-to-noise ratio of the small (48 kDa) *Ft*AlkBG particles on the micrographs, we optimized the cryo-EM data collection parameters.

We determined a structure consisting of *Ft*AlkB and *Ft*AlkG domains of the *Ft*AlkBG complex as a monomer at 2.76 Å resolution (Fig. 1a). The *Ft*AlkB structure contains six transmembrane α-helices and *Ft*AlkG sits on a positively charged surface of *Ft*AlkB. The *Ft*AlkBG system contains three iron atoms, one in *Ft*AlkG and two in *Ft*AlkB, which forms an electron transfer chain from *Ft*AlkG to *Ft*AlkB. In *Ft*AlkB, an alkane molecule is oriented by hydrophobic-tunnel-lining residues to present a terminal C–H bond towards the diiron-center active site. *Ft*AlkG acts as a shuttle to relay two electrons, one at a time, from the electron donor *Ft*AlkT to *Ft*AlkB for terminal C–H bond hydroxylation (Fig. 1b).

The implications

Biocatalysts offer a greener alternative to traditional precious-metal-based catalysis for alkane C–H bond activation and functionalization. The *Ft*AlkBG structure is distinct but analogous to the well-studied cytochrome P450 systems. It could thus serve as the basis for novel biocatalysts for the selective production of chemical feedstocks and value-added products from abundant alkanes[3].

This research provides a landmark structural view of the diiron-center structure, alkane binding, and electron transfer of AlkBG. However, many steps illustrated in Fig. 1b remain to be clarified. For example, the current structure does not reveal conformational changes associated with various stages of the reaction cycle. Furthermore, the reaction chemistry and redox states of the diiron center cannot be determined from this study. Further work will therefore focus on trapping reaction intermediates and determining their structures to elucidate details of the reaction mechanism. Site-directed mutagenesis of residues defining the alkane binding site will likely create variants that can act on new sites or produce new reaction products at the terminal site.

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EXPERT OPINION

"The described structure of AlkBG is fascinating and provides direct insights into the activation of largely inert alkanes. In this sense, the article is of broad, general interest with many potential downstream applications." **Wolf-Dieter Schubert, University of Pretoria, Pretoria, South Africa.**

FIGURE

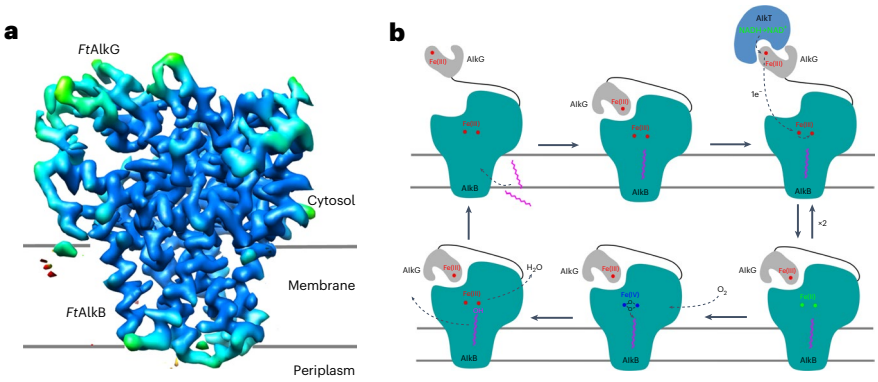

Fig. 1 | The structure of *Ft*AlkBG structure and its mechanism of alkane C–H functionalization. **a**, Cryo-EM structure of the *Ft*AlkBG complex colored with local resolutions. Blue–red colors show specific local resolutions ranging from 2.8 to 4.4 Å. The position of membrane bilayers (gray lines) is estimated. **b**, A model of alkane terminal C–H functionalization. AlkB recognizes and manipulates substrate alkanes (purple lines) to orient their terminal C–H groups to an Fe(iii)–Fe(iv) diiron center. AlkG shuttles electrons from AlkT to the diiron center in AlkB. Fe(iii)–Fe(iii) is reduced to Fe(ii)–Fe(ii) and oxygen is activated to form a high-valent iron-oxo intermediate. Hydrogen is then abstracted by the intermediate and an OH group is added, which completes the terminal C–H hydroxylation process.

BEHIND THE PAPER

The Shanklin lab has been researching the biochemistry of the AlkB enzyme since 1994 and discovered the conserved histidine residues[4] that are found in integral membrane desaturases and related enzymes, including AlkB[5]. However, challenges with this membrane protein enzyme have prevented the determination of its structure and validation of the hypothesis that the conserved histidines coordinate a diiron center. In 2016, our labs teamed up to determine the structure of AlkB. We initially focused on the crystallization of AlkB proteins and, after three years of unsuccessful attempts, focused on cryo-EM. The COVID-19 pandemic put our research on hold for a year, but in 2021, with the establishment of a cryo-EM facility at Brookhaven National Laboratory, we were able to resume the work and were able to determine the *Ft*AlkBG structure just before Christmas 2022. It was a great way to end the year, and our teams celebrated the success with a well-deserved break. **J.S. & Q.L.**

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FROM THE EDITOR

"We have found this article interesting due to the potential use of AlkB in biotechnology. The authors proposed a model for alkane C–H functionalization by this archetypal complex." **Editorial Team, *Nature Structural & Molecular Biology*.**