Enzyme catalyzed chemical transformations provide sustainable strategies and enable synthetic approaches based on their powerful reactivity and potential for unique selectivity. However, the efficient biosynthesis of high-value biomolecules in vitro is still underexplored. As chlorophyll(ide) pigments are extensively used in the cosmetic, food, agricultural, and pharmaceutical industries, the custom-tuned synthesis of natural and unnatural chlorophyll(ide) derivatives in vitro is desirable. In this issue of ACS Central Science, Jennifer Bridwell-Rabb and co-workers employ four chlorophyll(ide) a oxygenase (CAO) homologues in cooperation with a non-native reductase to convert chlorophyllide a (Chlide a) into chlorophyllide b (Chlide b) in vitro.

Chlorophylls are a class of naturally occurring pigments and serve key roles in photosynthetic organisms; however, their synthesis in vitro is complicated due to a lack of efficient protocols for in vitro reconstitution of an enzyme and demonstration of its activity in isolation. With the increasing demand for green and sustainable chemistry, chemists are pursuing those desirable systems that exclude noble metals, harsh conditions, toxic reagents, etc. In this context, tools and approaches from nature have provided inspiration to humans, especially the power of enzyme catalysis. Given the robust ability of chlorophylls to capture solar energy and transform it into chemical energy, the development of enzyme catalyzed efficient modification of high-value chlorophylls in vitro is of critical importance.
to prohibit electrons from reacting with the Chlide a substrate, the protein, or an activated oxygen intermediate.\(^1\) The investigation of the substrate scope of Chlide a suggested the lack of a phytol chain, the presence of a central metal ion, and the electronics of the substrate may be important for achieving an efficient reaction with CAO. In contrast with the in vitro system, CAO could not convert chlorophyll a into chlorophyll b even in cell lysate.\(^6\) To deepen the fundamental understanding of chlorophyll biosynthesis and Rieske oxygenase chemistry, chlorophyllase was successfully applied to cleave chlorophyll into chlorophyllide, which facilitated subsequent detailed mechanistic investigations: (1) CAO is unable to abstract the hydrogen atom to serve as the initial step of the formylation reaction. (2) The MS experiments indicated 7-OH-Chlide a is a true intermediate of the oxygenation reaction (Figure 1c).\(^1\)

Regarding the reductase system, we anticipate that exogenous light or electricity could serve as a potentially green, Earth-abundant, and sustainable reductant to replace the reductase, which might improve the yields,\(^7\) extend the substrate scopes, and generate new selectivity. Also, CAO has an obvious preference for the Chlide a' diastereomer,\(^8\) indicating that the stereochemistry in enzyme catalysis is also crucial. We believe theoretical calculations could make great contributions in the study of the stereoselectivity of biosynthetic enzymes, owing to the more credible and predictable transition states, intermediates, and reaction pathways.\(^9\) It could provide powerful support to understand the complicated chemistry of biomolecules and stimulate the exploration of more challenging and intricate biosynthetic transformations, especially enzyme-catalyzed transformations which proceed through radical intermediates with the potential to afford stereoenriched products.

This work by Jennifer Bridwell-Rabb and co-workers enriches the knowledge of chlorophyll biosynthesis, extends the known reactivity of the Rieske oxygenase class, and provides a framework for developing the CAO-VanB system as a tool to produce non-native chlorophyll pigments.
catalytic activity of CAO will have important implications for future structural and biochemical studies on enzyme catalysis. Determination of the key amino acids involved in the transformation of Chlide $a$ into Chlide $b$, revealing clearer mechanisms and improving the yields and stereoselectivity, should be the focus of future research.

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